UNIVERSIDAD DE BARCELONA

FACULTAD DE MEDICINA

DEPARTAMENTO DE MEDICINA.

SERVICIO DE ENFERMEDADES INFECCIOSAS. INSTITUTO DE MEDICINA Y DERMATOLOGÍA. HOSPITAL CLÍNIC.

TESIS DOCTORAL:

FACTORES DEPENDIENTES DEL MICROORGANISMO Y DEL HUÉSPED EN LA PATOGENIA DE LAS INFECCIONES URINARIAS.

Presentada por ALEJANDRO SMITHSON AMAT para optar al grado de Doctor en Medicina y Cirugía.

Directores: Jordi Vila Estapé y Francisco Lozano Soto

Barcelona, noviembre de 2008

A Mark y Pol, por ser mi tesoro más preciado

A Chus, por su infinita paciencia y por creer en mí...

ÍNDICE

1. INFORME DE LOS DIRECTORES DE LA TESIS.

Barcelona, 15 de Octubre 2008

El Dr. Jordi Vila Estapé, Catedrático del Departamento de Anatomía Patológica, Farmacología y Microbiología de la Facultad de Medicina y Jefe de Sección del Servicio de Microbiología del Hospital Clínico de Barcelona y el Dr. Francisco Lozano Soto, Profesor Titular del Departamento de Biología Celular de la Universidad de Barcelona y Consultor del Servicio de Inmunología del Hospital Clínico de Barcelona,

CERTIFICAN:

Que la tesis doctoral "**FACTORES DEPENDIENTES DEL MICROORGANISMO Y DEL HUÉSPED EN LA PATOGENIA DE LAS INFECCIONES URINARIAS**" presentada por Alejandro Smithson Amat para optar al grado de Doctor en Medicina y Cirugía ha sido realizada bajo nuestra dirección y cumple todos los requisitos establecidos para ser defendida ante el Tribunal de evaluación correspondiente.

Dr. Alejandro Smithson Amat

Doctorando

Dr. Jordi Vila Estapé

Codirector de la tesis doctoral

Dr. Francisco Lozano Soto

Codirector de la tesis doctoral

2. AGRADECIMIENTOS.

Esta tesis ha sido realizada bajo la dirección del Dr. Jordi Vila y el Dr. Francisco Lozano, sin los cuales nunca hubiese sido posible.

A Sara Soto por aceptar el reto que en su día se le planteó y que finalmente ha conducido a la realización de gran parte de los trabajos que forman parte de esta tesis.

A Alex Soriano por su amistad y sencillez, y por haberme dado la oportunidad de formarme en el Hospital Clínic, del que tanto he aprendido.

Al Dr. Josep Mª Gatell por su pragmatismo. A Josep Mensa y a José Antonio Martínez por haber dejado que aprendiera tanto a su lado y por demostrarme que la práctica de la medicina nunca está reñida con la ciencia y con la posibilidad de publicar. También agradecer al Dr. Juan Pablo Horcajada sus buenos consejos que al fin y a la postre han llevado a la consecución de esta tesis.

A Elisa García y a Mar Ortega por haberme enseñado tanto sin esperar nunca nada a cambio. A Ana Muñoz por estar allí.

A Lucia Fontes y Belén Suárez, técnicos de los laboratorios de Microbiología e Inmunología, por haberme facilitado tanto el trabajo.

A mi familia por haberme dado todo su apoyo y la mejor de las educaciones. A Rafael Perelló por su amistad incondicional.

3. ABREVIATURAS.

ITUs:	Infecciones del tracto urinario
ITURs:	Infecciones del tracto urinario recurrentes
E. coli:	*Escherichia coli*
BA:	Bacteriuria asintomática
CA:	Cistitis aguda
PA:	Prostatitis aguda
PNA:	Pielonefritis aguda
ExPEC:	*Extraintestinal pathogenic E. coli*
FV:	Factores de virulencia
LPS:	Lipopolisacárido
CNF-1:	Factor de necrosis citotóxico de tipo 1
UPEC:	*Uropathogenic E. coli*
GSL:	Glucoesfingolípididicos
PTH:	Proteína de Tamm-Horsfall
IL:	Interleucina
PMN:	Polimorfonucleares
TLR:	Receptores Toll-like
BGN	Bacilos Gram negativos
SAT-1:	Toxina autotransportadora tipo 1
PAI:	Isla de patogenicidad
MBL:	Lectina fijadora de manosa
ENA- 78:	Proteína activadora de los neutrófilos de origen epitelial
GRO-α:	Factor de crecimiento relacionado con el oncogén α
SNPs:	Polimorfismos de nucleótido único

4. RELACIÓN DE ARTÍCULOS INCLUIDOS.

La presente tesis se basa en los siguientes artículos publicados:

Artículo 1.

Título: "Biofilm formation in uropathogenic *E. coli* strains: relationship with prostatitis, urovirulence factors and antimicrobial resistance".

Autores: S.M. Soto, A. Smithson, J.A. Martínez, J.P. Horcajada, J. Mensa, J. Vila.

Publicación: J Urol 2007; 177: 365-368.

Factor de impacto: 4,053.

Artículo 2.

Título: "Implication of biofilm formation in the persistence of urinary tract infection caused by uropathogenic *E. coli*".

Autores: S.M. Soto, A. Smithson, J.P. Horcajada, J.A. Martínez, J. Mensa, J. Vila.

Publicación: Clin Microbiol Infect 2006; 12: 1021-1045.

Factor de impacto: 2,980.

Artículo 3.

Título: "Expression of interleukin-8 receptors (CXCR1 and CXCR2) in premenopausal women with recurrent urinary tract infections".

Autores: A. Smithson**,** M.R. Sarrias, J. Barceló, B. Suárez, J.P. Horcajada, S.M. Soto, A. Soriano, J. Vila, J.A. Martínez, J. Vives, J. Mensa, F. Lozano.

Publicación: Clin Diagn Lab Immunol 2005; 12: 1358-1363.

Factor de impacto: 2,511.

Artículo 4.

Título: "Association between mannose binding lectin deficiency and septic shock following *E. coli* acute pyelonephritis".

Autores: A. Smithson, A. Muñoz, B. Suárez, S.M. Soto, R. Perelló, A. Soriano, J.A. Martínez, J. Vila, J.P. Horcajada, J. Mensa, F. Lozano.

Publicación: Clin Vaccine Immunol 2007; 14: 256-261.

Factor de impacto: 1,995.

5. INTRODUCCIÓN A LAS INFECCIONES DEL TRACTO URINARIO.

5.1. Epidemiología, clasificación y etiología.

Se considera que las infecciones del tracto urinario (ITUs) adquiridas en la comunidad constituyen la infección bacteriana más común[1]. Se estima que cada año en los Estados Unidos las ITUs causan unos 7 millones de visitas a los médicos de cabecera, alrededor de 1 millón de consultas a los departamentos de urgencias y cerca 100.000 ingresos hospitalarios [2]. Alrededor de un 25 % de las mujeres con un primer episodio de infección urinaria, presentarán ITUs recurrentes (ITURs)[3]. Además, las ITUs representan la principal fuente de infección nosocomial en relación, en la mayor parte de los casos, con la presencia de catéteres urinarios [4].

Esta gran prevalencia de las ITUs permite entender el enorme impacto que tienen este grupo de infecciones bacterianas, en términos de morbilidad y de costes económicos, tanto directos como indirectos. A modo de ejemplo cabe decir que en los Estados Unidos, en el año 1997, los costes directos e indirectos derivados de las ITUs adquiridas en la comunidad supusieron unos 1,6 billones de dólares, cifra que ascendió hasta los 2 billones de dólares cuando también se tuvieron en consideración las ITUs de origen nosocomial[5].

Las ITUs se han dividido clásicamente en complicadas o no complicadas, según existan anomalías anatómicas o funcionales de la vía urinaria, antecedentes de instrumentación reciente o infección urinaria en las semanas previas, circunstancias todas ellas que pueden influir en la distribución de los gérmenes causales, en la respuesta al tratamiento y en la evolución final de la infección[6].

En la **tabla 1** se describen las anomalías que se asocian a las ITUs complicadas[5].

Tabla 1. Alteraciones asociadas a las ITUs complicadas.

Causas obstructivas
Hipertrofia prostática
Tumores
Litiasis
Estenosis ureterales
Divertículos
Quistes renales
Cuerpos extraños
Sondaje urinario
Tubo nefrostomía
Stent ureteral
Causas metabólicas
Diabetes mellitus
Insuficiencia renal
Trasplante renal
Espongiosis medular renal
Causas funcionales
Vejiga neurógena
Reflujo vesicoureteral
Otras
Instrumentación de la vía urinaria
Derivación urinaria ileal
Infección urinaria previa
Tratamiento antibiótico reciente

Modificado de Nicolle LE, 2001[6].

Mientras que las ITUs no complicadas acostumbran a producirse en huéspedes sanos, las ITUs complicadas suelen darse en huéspedes con algún tipo proceso subyacente, siendo la obstrucción al flujo urinario el factor de riesgo que con mayor frecuencia conduce a la aparición de una ITU complicada[6].

Otro rasgo diferencial de las ITUs complicadas es que suelen estar causadas por microorganismos con una mayor tasa de resistencia a los antibióticos, particularmente a las quinolonas, siendo en general cepas menos virulentas que

las causantes de ITUs no complicadas, por lo que suelen precisar de la coexistencia de factores del huésped que favorezcan la infección[7].

Escherichia coli (E. coli) es el microorganismo responsable de la mayor parte (>80%) de las ITUs no complicadas. Le sigue en frecuencia *Staphylococcus saprohyticus*, particularmente en mujeres jóvenes, *Klebsiella pneumoniae*, *Proteus mirabilis* y *Enterococcus faecalis*. Aunque *E. coli* sigue siendo el microorganismo causal más frecuente de las ITUs complicadas, las cepas de *E. coli* implicadas en ellas son con frecuencia más resistentes y menos virulentas que las cepas de *E. coli* causantes de ITUs no complicadas[7,8]. En la **tabla 2** se resumen los gérmenes implicados con mayor frecuencia en las ITUs complicadas.

Tabla 2. Gérmenes causales de las ITUs complicadas.

Gérmenes	**Prevalencia**
Gram negativos	
Escherichia coli	21-54%
Klebsiella pneumoniae	1.9-17%
Enterobacter sp.	1.9-9.6%
Citrobacter sp.	4.7-6.1%
Proteus mirabilis	0.6-9.6%
Providencia sp.	18%
Pseudomona aeruginosa	2-19%
Otras	6.1-20%
Gram positivos	
Enterococcus sp.	6.1-23%
Estreptococos del grupo B	1.2-3.5%
Estafilococos coagulasa-negativos	1.4-3.7%
Staphylococcus aureus	0.9-2%
Hongos	
Candida sp.	0-5%

Tomado de Nicolle LE, 2001[6].

Las ITUs se pueden también clasificar en función de la localización de la infección en el tracto urinario. Así, con el término de infección del tracto urinario inferior se englobarían la bacteriuria asintomática (BA), la cistitis aguda (CA), y la prostatitis aguda (PA). La pielonefritis aguda (PNA) se clasificaría como una infección del tracto urinario superior. Las infecciones urinarias asociadas a catéteres urinarios suelen considerarse como capítulo aparte porque, aunque en la mayor parte de los casos la infección se localiza en la vía urinaria inferior, ocasionalmente se ve afectado el parénquima renal.

El tracto urinario es un medio ambiente hostil para la bacteria y, excepto la uretra distal, es normalmente estéril. La mayor parte de las ITUs están causadas por la colonización ascendente por parte de microorganismos de origen entérico que suelen, en primer lugar, colonizar el introito vaginal y el área periuretral para a continuación ascender hasta la vejiga urinaria y eventualmente hasta la próstata o el riñón. Con mucha menor frecuencia, la vía de propagación de la infección es hematógena. Entre los microorganismos que tienden a invadir la vía urinaria por vía hematógena destacan *Staphylococcus aureus* y *Candida*[9].

5.2. Concepto de *E. coli* patógeno extraintestinal frente a uropatógeno.

Las cepas de *E. coli* se agrupan en cuatro grupos filogenéticos, A, B1, B2 y D que se han identificado en función de la variación alélica de los genes codificantes de determinadas enzimas[10]. La técnica más ampliamente utilizada para la determinación del grupo filogenético es el "*multilocus enzyme electophoresis"*[11], existiendo actualmente técnicas de reacción en cadena de la polimerasa múltiple que permite su determinación de forma rápida y sencilla[12].

Basándose en criterios genéticos y clínicos, las cepas de *E. coli* se clasifican en 3 grupos: cepas comensales, cepas patógenas intestinales y cepas patógenas extraintestinales, denominadas ExPEC ("*extraintestinal pathogenic E. coli*"). Las infecciones que producen estas últimas pueden afectar a prácticamente todos los órganos y localizaciones, excepto el tracto intestinal. Las cepas causantes de infecciones extraintestinales, como las ITUs, y las septicemias y meningitis neonatales, pertenecen en su mayoría, al grupo B2, y con menor frecuencia al grupo D. Estas cepas poseen genes que codifican factores extraintestinales de virulencia mientras que las cepas comensales pertenecen en su mayoría a los grupos filogenéticos A y B1[13-15]. La barrera entre comensalismo y virulencia es el resultado de un complejo equilibrio entre la presencia y expresión de determinados factores de virulencia (FV) por parte del microorganismo y el estado de los mecanismos defensivos del huésped. En general, estas cepas comensales, adaptadas a convivir con el huésped, no producen enfermedad intestinal y sólo causan enfermedad extraintestinal cuando existen factores favorecedores como la presencia de sondaje urinario o de patologías que causen estados de inmunodeficiencia en el huésped. Las cepas de los grupos B2 y D poseen más genes de virulencia que las cepas de los grupos A y B1[16].

Por tanto se puede afirmar que la patogenicidad difiere entre los distintos grupos filogenéticos. Además el grado de patogenicidad o virulencia entre las cepas de un mismo grupo filogenético también puede ser diferente. En este sentido, Moreno *et al*[17] estudiaron la cantidad de FV presente en cepas de *E. coli* pertenecientes a los dos grupos filogenéticos considerados como comensales (A y B1) causantes de infecciones extraintestinales, y la carga de FV presente en cepas de *E. coli* pertenecientes a los grupos considerados

como patógenos (B2 y D). Demostraron como algunas de las cepas pertenecientes a los grupos menos patogénicos habían adquirido FV frecuentemente asociados al grupo B2 y que eran capaces de producir infecciones en pacientes no inmunodeprimidos.

La capacidad de las cepas de ExPEC de producir infecciones extraintestinales se debe a la adquisición de genes que codifican diversos FV, lo que hace posible que causen infecciones en focos distintos al intestino, tanto en pacientes sin enfermedades subyacentes como en aquellos inmunodeprimidos. La mayor parte de estos genes de virulencia son distintos a los que están implicados en las infecciones intestinales[18]. Entre los FV presentes en la mayoría de las cepas de ExPEC encontramos factores implicados en la adherencia de los microorganismos a las células del huésped (por ej., fimbrias tipo 1 y fimbrias tipo P), factores que permiten evitar o sobrevivir los sistemas defensivos del huésped (por ej, la cápsula y el lipopolisacárido (LPS)), mecanismos de adquisición de nutrientes (sideróforos) y toxinas (por ej, la hemolisina y el factor citotóxico necrotizante tipo 1 (CNF-1)), que pueden variar entre los diferentes síndromes o tipos de infección[19].

La concentración de genes de virulencia en determinados linajes evolutivos de ExPEC ha dado como resultado el concepto de clones sindrómicos específicos: uropatógenos ("*uropathogenic E. coli"* o UPEC), asociados a sepsis, a meningitis, etc. Desafortunadamente, estos clones específicos de determinados síndromes, aunque válidos, representan un concepto demasiado limitado, ya que implica que ciertos *E. coli* poseen FV que los capacita para causar únicamente infección en un determinado lugar anatómico. Sin embargo, ningún FV es capaz de conferir esta propiedad. Además los ExPEC tienden a acumular

diversos FV, lo que les dota de múltiples perfiles que posibilitan la existencia de varias alternativas posibles para causar una infección en una determinada localización.

Por todo ello, se ha propuesto sustituir el término UPEC, que se utiliza para describir cepas de *E. coli* capaces de causar ITUs pero que también causan infecciones extraintestinales no urinarias, por la designación más inclusiva de ExPEC[20].

5.3. Patogenia de las infecciones del tracto urinario.

Aunque las diferentes formas de ITUs comparten unos mecanismos patogénicos y una etiología similar, cada una de las diferentes formas de ITU son el resultado de un complejo proceso, resultado del equilibrio que se establece entre una serie de FV dependientes del microorganismo y de una serie de condiciones propias del huésped.

5.3.1. Factores dependientes del microorganismo.

5.3.1.1. Factores de virulencia.

La virulencia de un microorganismo condiciona en gran medida su potencial para establecer una infección. Tanto los ligandos o adhesinas como las toxinas que emplean las cepas de UPEC para invadir los tejidos se denominan FV. En la **figura 1** se resume la participación de los diferentes FV en la patogenia de las ITUs. Los FV son fundamentales para sobrepasar las defensas normales del huésped. De forma inversa, en huéspedes comprometidos, los FV bacterianos tienen una menor relevancia. En esta última situación, los factores de huésped son críticos, lo que constituye el paradigma de la ITU complicada.

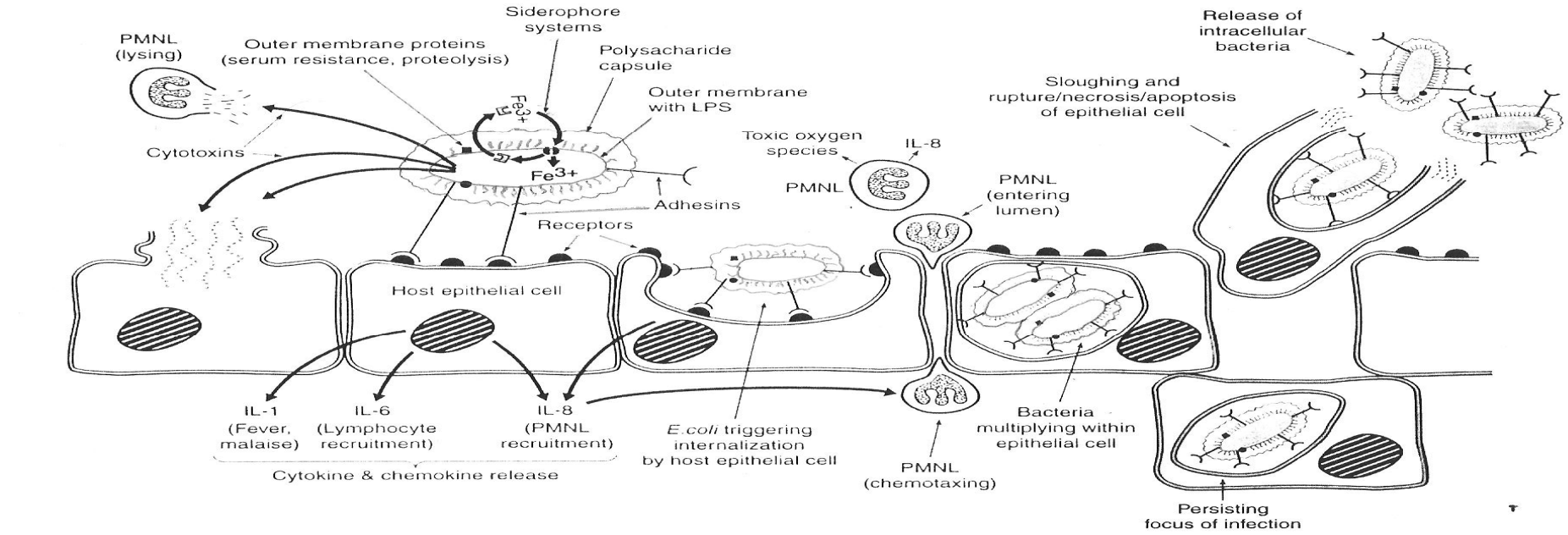


Figura 1. Factores de virulencia en ExPEC. Destacan, por su importancia patogénica, las adhesinas fimbriadas, las citotoxinas, el LPS, la cápsula, los sistemas de adquisición de hierro (sideróforos) y las proteínas de la membrana externa. Las interacciones bacterianas con las células de huésped desencadenan la producción de citocinas, la aparición de un infiltrado inflamatorio, básicamente constituido por polimorfonucleares, y la internalización de las bacterias hacia el interior de las células epiteliales. Las bacterias internalizadas se pueden multiplicar intracelularmente e inducir la necrosis o la apoptosis de las células del huésped, o pueden persistir como un foco quiescente de infección en la vejiga urinaria. Tomado de Johnson JR. Microbial virulence determinants and the pathogenesis of urinary tract infection. Infect Dis Clin North Am 2003; 17: 261-278.

En una cepa de UPEC pueden coexistir diversos FV siendo más virulenta cuantos más FV concurran en ella. En este sentido, tal y como describió Johson *et al*[21], algunos FV, como las fimbrias tipo P, la aerobactina y la hemolisina, son más frecuentes en cepas de *E. coli* procedentes de pacientes con PNA o urosepsis que en las cepas de *E. coli* de origen fecal.

a. Adhesinas.

Como ya se ha dicho, el mecanismo habitual de producción de las ITUs es el ascenso de los microorganismos desde la zona periuretral hasta la vejiga urinaria en el caso de las CA, hasta el parénquima prostático en el caso de las PA y hasta la pelvis renal, a través de los uréteres, en el caso de las PNA[9]. Por tanto, el paso inicial en la patogénesis de las ITUs implica la adherencia del microorganismo patógeno al epitelio del tracto urinario, lo que permite a la bacteria permanecer en el tracto urinario a pesar del efecto de arrastre del flujo urinario. Además las adhesinas, al permitir un estrecho contacto entre los microorganismos y el epitelio urinario del huésped, permiten exponer al urotelio a altas concentraciones de productos tóxicos o inflamatorios de origen bacteriano, lo que incrementa la actividad de estos componentes sobre el epitelio urinario del huésped[22]. Las fimbrias o pili de los microorganismos se consideran los principales ligandos responsables de esta adhesión, aunque también existen adhesinas no fimbriales.

Las fimbrias son apéndices filamentosos que parten de la superficie de la bacteria. En *E. coli* la mayoría de estructuras adherentes son fimbrias proteicas que se unen a receptores específicos situados en las membranas de las células epiteliales. Los diferentes tipos de adhesinas fimbriales comparten una

estructura general similar, en forma de filamentos heteropoliméricos constituidos por subunidades proteicas que conforman el cuerpo de la fimbria. Ya sea en el extremo o bien intercaladas a lo largo de estos filamentos se encuentran unas estructuras moleculares diferenciadas llamadas adhesinas que se unen a receptores específicos del huésped.

Las cepas de ExPEC poseen genes que codifican para varias clases de órganos de adhesión, entre las que se incluyen las fimbrias tipo 1 y las fimbrias tipo P, los dos principales tipos de fimbrias. Ambos tipos de fimbrias reconocen a receptores glucoconjugados diferentes: las fimbrias tipo 1 se unen a los residuos de α-D-manosa presentes en los receptores glicoproteicos (fimbrias sensibles a la manosa) mientras que las fimbrias tipo P se unen a los residuos de galactosa presentes en los receptores glucoesfingolípidicos (GSL) (fimbrias resistentes a la manosa).

Las fimbrias tipo 1 son las más universales de las adhesinas, estando presentes en la práctica totalidad de las cepas de *E. coli* y en más de un 80% de las cepas de los UPEC[23]. Tal y como se muestra en la **figura 2**, el cuerpo de la fimbria esta constituida por las subunidades proteicas FimA unidas a una estructura distal compuesta por dos proteínas adaptadoras, FimG y FimF, y finalmente la adhesina FimH en su extremo distal[24]. La expresión y el ensamblaje de las fimbrias tipo 1 precisa de 9 genes que están presentes en el operón de las fimbrias tipo 1 (**figura 2**).

En la vejiga urinaria, las fimbrias tipo 1 se unen a los residuos de manosa de la uroplaquina Ia y Ib, que son glucoproteínas que se disponen en la superficie del epitelio de la vejiga urinaria[25]. Dicha unión se bloquea en presencia de la proteína de Tamm Horsfall (PTH), uromucoide rico en manosa secretado por las

células epiteliales de las vías urinarias y, eventualmente, en presencia de inmunoglobulina A (Ig A). La PTH actúa como un mecanismo de defensa inespecífico, ya que evita la unión de *E. coli* a la uroplaquina[26]. De forma experimental Balish *et al*[27] demostraron como, tras lesionarse la capa formada por la PTH, gran número de *E.coli* quedaban adheridos al epitelio vesical, proceso que podría representar el punto de inflexión en el que la colonización se convierte en infección y explicar la evolución a brotes observadas en las ITURs.

El papel de las fimbrias de tipo 1 es controvertido, aunque actualmente se cree que desempeñan un papel fundamental en el inicio del proceso patogénico que conduce a las ITUs. Numerosos estudios han sugerido que las fimbrias tipo 1, juegan un papel en la cistitis bacteriana. Utilizando un modelo murino de cistitis, Hultgren *et al*[28] evidenciaron como la colonización vesical por aislados clínicos de *E. coli* está favorecida por aquellas condiciones de crecimiento que inducen la expresión de fimbrias tipo 1, mientras que aquellas condiciones que favorecen la expresión exclusiva de fimbrias P son insuficientes para promover dicha colonización.

Como ya se ha dicho, la FimH es la adhesina de las fimbrias tipo 1 que se encarga de la unión a los residuos de manosa de la uroplaquina. La unión de la FimH a la uroplaquina puede variar en relación a la presencia de cambios menores estructurales en el extremo N-terminal de la proteína. Estos cambios conducen a modificaciones significativas en la capacidad de reconocimiento del receptor.

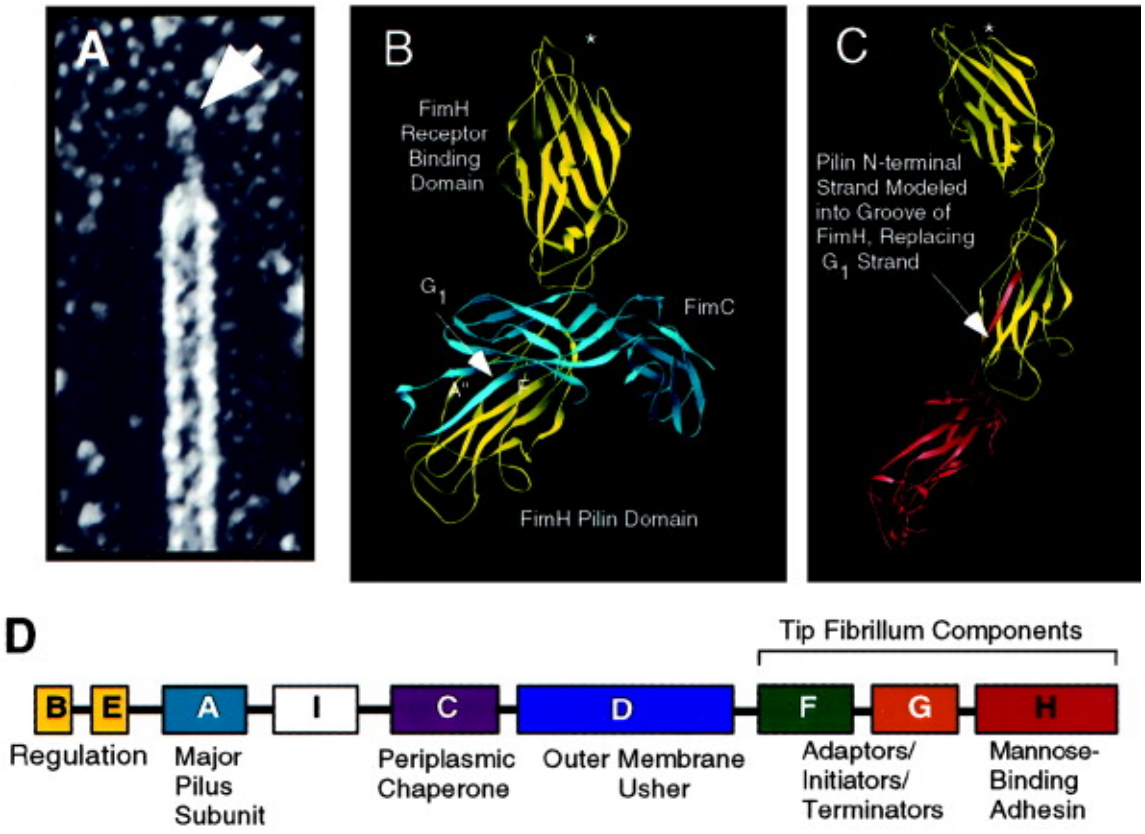


Figura 2. Estructura y genética de las fimbrias tipo 1. *A, B,* y *C,* Microscopia de alta resolución de una fimbria tipo 1. Las flechas indican la localización de la adhesina Fim H. *D,* Organización genética del operón de las fimbrias tipo 1. El gen *fim*A codifica la subunidad mayor de la fimbria mientras que los genes *fim*F, *fim*G y la *fimH*, codifican las proteínas que forman el extremo de la fimbria. Modificado de Schilling JD, *et al*, 2001[24].

En este sentido cabe decir que un 80% de los aislados fecales de *E. coli*, la Fim H se une únicamente a receptores trimanosa, mientras que un 70% de las adhesinas FimH procedentes de aislados de ITUs poseen cambios menores, comparado con sus homólogos fecales, que incrementa su capacidad para reconocer receptores con monomanosa. Por tanto la presencia de alelos mutantes confieren un mayor tropismo por el uroepitelio y aumenta de forma notable la capacidad de *E. coli* para colonizar el tracto urinario[29]. Schembri *et al*[30] demostraron la capacidad de ciertas variantes de FimH de promover la formación de biopelícula sobre superficies abióticas bajo ciertas condiciones

hemodinámicas de flujo, lo que no se produce entre las variantes de FimH procedentes de la flora comensal.

Tal y como se muestra en la **figura 3**, la unión de la FimH con su receptor uroepitelial parece ser la señal que activa la cascada defensiva del huésped que incluye la muerte programada y la exfoliación de las células del epitelio vesical así como la síntesis y liberación de los mediadores de la respuesta inflamatoria, fundamentalmente interleucina (IL)-1, IL-6 y IL-8[31]. Como veremos, el patrón de producción de citocinas inducido por las fimbrias tipo 1 es distinto del que causan las fimbrias tipo P, lo que probablemente permite al huésped ajustar el tipo de respuesta inflamatoria en función del microorganismo causal de la infección.

La FimH, además de mediar la adherencia bacteriana, tras unirse con la uroplaquina presente en el epitelio vesical, induce la activación de la fosfatidilinositol 3 cinasa (PI 3-Kinase) y la consiguiente fosforilación de la cinasa de adhesión focal (FAK) del huésped. La fosforilación de la FAK y la formación de complejos entre la FAK y la PI 3-Kinase, y entre la α-actinina y la vinculina conduce a una reordenación del citoesqueleto de actina, de forma que, tal y como se muestra en la **figura 3**, éste rodea y posteriormente internaliza las cepas de UPEC que poseen dichas adhesinas. Por tanto se puede decir que la adhesina FimH de las fimbrias tipo 1 actúa como una invasina propiamente dicha[32].

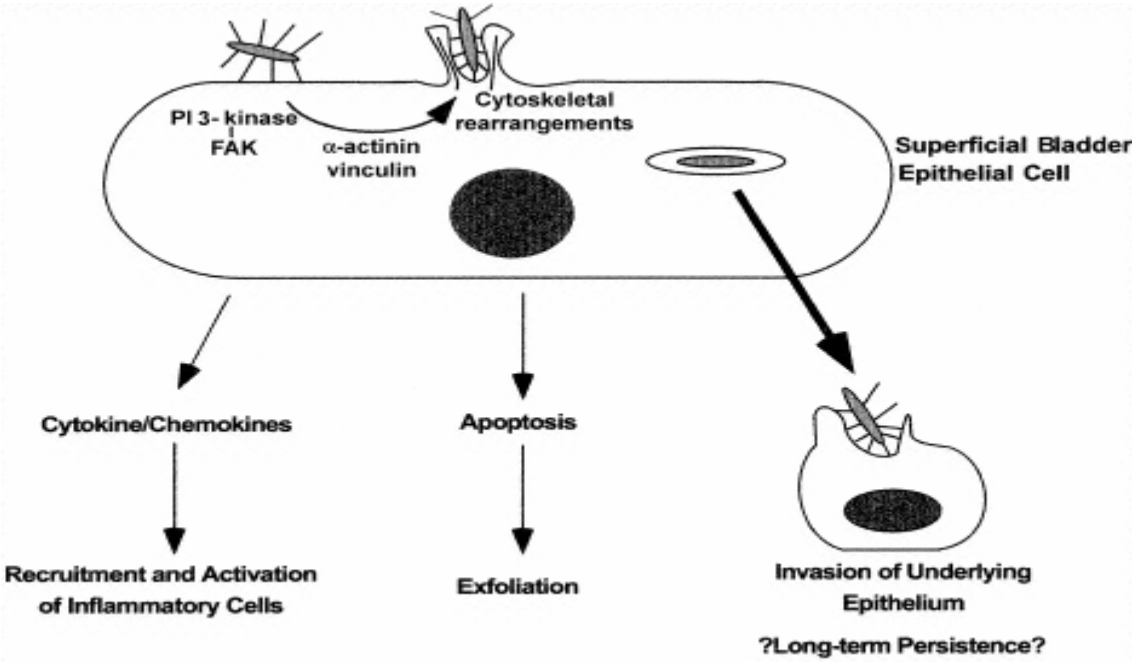


Figura 3. Mecanismos y consecuencias de la invasión bacteriana mediada por las fimbrias tipo 1. Estas fimbrias facilitan la adherencia de las cepas de UPEC a la superficie del epitelio vesical. La interacción entre la FimH y las células del epitelio vesical induce una cascada de señales en el huésped, incluyendo la formación de complejos proteicos entre la FAK y la PI 3-Kinase y la α-actinina y la vinculina, que conduce a la formación de cambios en el citoesqueleto y la internalización de las bacterias adheridas. La invasión de las células epiteliales vesicales induce la producción de citocinas proinflamatorias y el consiguiente reclutamiento de células inflamatorias así como la apoptosis de las células epiteliales y su exfoliación. Con el fin de prevenir la eliminación de la bacteria tras la exfoliación de las células epiteliales vesicales y evitar las defensas del huésped, las cepas de UPEC invaden el epitelio vesical, facilitando de esta manera la persistencia de la bacteria en la mucosa vesical. Tomado de Schilling JD, *et al*, 2001[24].

Tal y como han demostrado Mulvey MA *et al*[33] y Anderson *et al*[34], tras el proceso de invasión de las células epiteliales de la vejiga urinaria, las cepas de UPEC pueden multiplicarse intracelularmente, eludiendo los mecanismos defensivos del huésped, invadiendo las capas profundas del tejido vesical y replicándose en el interior de sus células. De esta forma se crean biopelículas o "pods", que contienen bacterias bañadas en una matriz rica en polisacáridos, rodeados por una envoltura de uroplaquina que forman focos masivos de *E. coli* que constituyen un reservorio quiescente crónico en la vejiga urinaria. Por tanto,

la vejiga urinaria se sumaría a la vagina y al intestino como reservorio de cepas de UPEC y, por tanto, como fuente de futuras ITUs.

Langerman *et al*[35] evidenciaron en un modelo murino como la presencia de mutaciones en el gen de la *fimH* suprimía totalmente esta cascada patogénica y como la vacunación con adhesina FimH protegía frente al desarrollo de colonización del epitelio vesical y, por tanto, frente al desarrollo de infección.

Mientras que el papel de las fimbrias de tipo 1 parece tener especial relevancia en la vejiga urinaria el de otras fimbrias, como las tipo P y las adhesinas Dr, como posteriormente abordaremos, parecen ser críticas para el desarrollo de infección en el parénquima renal[36,37].

A diferencia de las adhesinas sensibles a la manosa, las adhesinas de *E. coli* resistentes a la manosa constituyen un grupo muy heterogéneo. Las fimbrias tipo P constituyen el grupo mejor conocido de fimbrias resistente a la manosa. Estas fimbrias se unen a los receptores GSL de las células uroepiteliales. La especifidad de receptor viene definida por la porción oligosacárida del GSL y, particularmente, por el disacárido Galα1-4Galβ. Este oligosacárido está presente en los antígenos humanos del grupo sanguíneo P, de donde las fimbrias P adoptan su nombre, así como en la superficie de las células del epitelio urinario, desde la vejiga urinaria hasta los túbulos renales. Todo ello facilita la ascensión de aquellas cepas de *E. coli* dotados de fimbrias P hasta la pelvis renal, por lo que se consideran un elemento crítico en el desarrollo de infección de tracto urinario superior[38]. Las fimbrias P están presentes en cerca del 80% de las cepas de *E. coli* aisladas de PNA, en un 40-50% de las cepas causantes de CA y en cerca de un 20% de las cepas causantes de BA o que forman parte de la flora fecal de portadores sanos[39].

Tal y como se muestra en la **figura 4**, la estructura de las fimbrias P está constituida por una subunidad mayor, llamada PapA que, en un proceso de polimerización, da lugar a la fibrilina, proteína estructural de las fimbrias P. En el extremo de la fibrilina se localiza la PapG, que es la adhesina de las fimbrias P. Los genes implicados en este proceso están codificadas por el operón *pap* que contiene los diferentes genes (genes *pap* A, B, C, D, E, F, G, H, I) que codifican para las diferentes proteínas que conforman la fimbria P[40]. El gen *pap*A codifica para la subunidad mayor mientras que los genes *papE*, *papF* y *papG* codifican el complejo que forma la adhesina. La adhesina es la proteína que primero es transportada al exterior y, posteriormente, se forma el cuerpo de la fimbria por adición secuencial de la subunidad mayor.

Las fimbrias P presentan 3 variantes moleculares de *PapG* (I, II, y II) que son codificadas por los correspondientes alelos (*papG* alelo I, *papG* alelo II, *papG* alelo III) que determinan la especificad de la adhesina PapG. Probablemente las 3 variantes ejercen funciones patogénicas distintas. Se ha visto que las adhesinas G de clase II, codificadas por el gen *pap*G_{IA2}, son más comunes entre las cepas de UPEC implicadas en PNA y en las formas bacteriémicas de ITUs mientras que las adhesinas G de clase III (fimbrias Prs), codificadas por el gen *prs*G_{J96}, son más frecuentes entre los aislados procedentes de PA. Finalmente las adhesinas G de clase I, codificadas por el gen *pap*G_{J96}, son mucho más infrecuentes y precisan de la presencia de globotriaosilceramida como epítope del receptor[41-43].

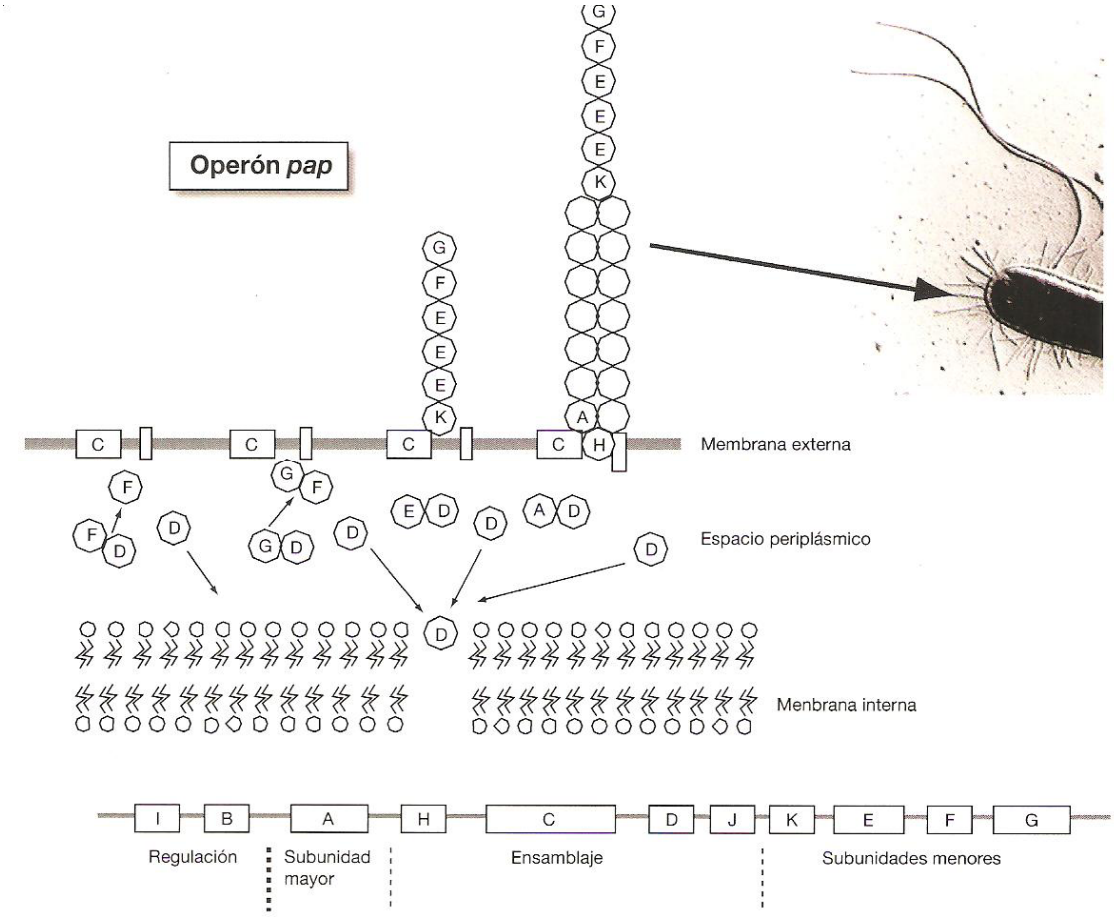


Figura 4. Genes del operón *pap*. Síntesis y estructura de las fimbrias P. Tomado de Vila J, Soriano A, Mensa J. Bases moleculares de la adherencia microbiana sobre los materiales protésicos. Papel de las biocapas en las infecciones asociadas a los materiales protésicos. Enferm Infecc Microbiol Clin 2008; 26: 48-54.

Como evidenció Otto *et al*[45], el huésped puede ejercer una presión selectiva sobre los genotipos de la *papG* de forma que aquellos episodios de ITUs febriles que ocurren en huéspedes no comprometidos suelen estar causadas por cepas de *E. coli pap*G_{IA2} mientras que en aquellos que ocurren en pacientes con enfermedades de base suelen estar implicados una mezcla de cepas *pap*$G_{IA2,}$ *prs*G_{J96} y cepas *pap*$^{-}$. Finalmente, Godaly *et al*[45] demostraron como el subtipo de fimbrias P también influye en la intensidad de la respuesta inflamatoria de forma que las adhesinas G de clase II inducen una mayor producción de citocinas por parte del urotelio que las adhesinas G de clase III.

Las fimbrias tipo P tienen la capacidad de activar en el epitelio urinario del huésped la aparición de una respuesta inflamatoria con producción de citocinas, principalmente de IL-8, y de otras moléculas como la proteína C reactiva que conducen a la activación de los polimorfonucleares (PMN) y la consiguiente eliminación de la bacteria[46]. En este sentido, un estudio realizado por nuestro grupo demostró como pacientes afectas de PNA causadas por cepas de *E. coli* que expresaban PapG presentaban niveles de proteína C reactiva en sangre, más altos que aquellas pacientes con PNA causadas por cepas de *E. coli* Pap G negativas[47]. Las fimbrias tipo P son capaces de inducir esta respuesta inflamatoria a través de la ceramida y del reclutamiento de los llamados receptores *Toll-like* (TLR)-4, componentes de la inmunidad innata, que actúan como correceptores para la transducción de la señal[48]. El receptor GSL de las fimbrias P carece de un dominio transmembrana, anclándose en la superficie externa de la bicapa lipídica por medio de la ceramida. Tras unirse con sus receptores del uroepitelio, las fimbrias P inducen la hidrólisis del receptor, por medio de la activación de esfingomielinasas, con la consiguiente liberación de la ceramida, que actúa como señal intermediaria entre las fimbrias P y los TLR-4, induciendo la activación de estos últimos[49]. La **figura 5** ilustra los mecanismos de liberación de la ceramida.

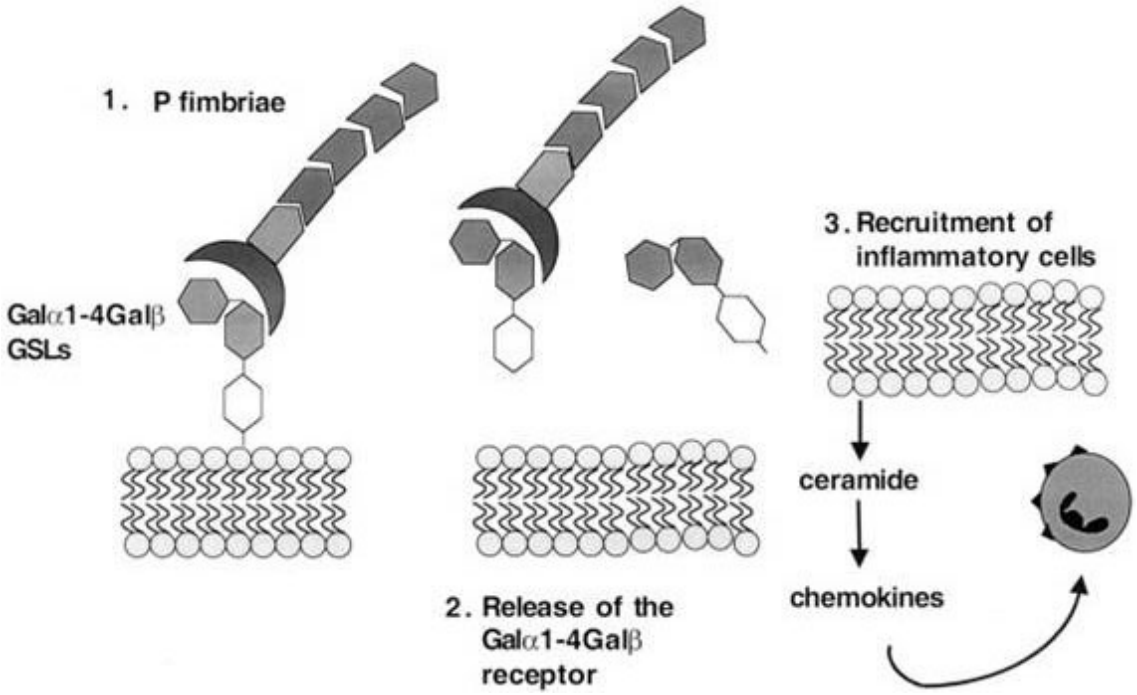


Figura 5. Mecanismos de liberación de la ceramida en respuesta a las fimbrias P de *E. coli* de clase II. 1. La bacteria se libera de su unión al urotelio tras la hidrólisis del receptor; 2, los oligosacáridos hidrolizados actúan como receptores solubles que se unen a las fimbrias y evitan que se vuelvan a unir al urotelio hasta que nuevos receptores son expresados en la superficie celular; 3, la ceramida liberada, induce a la célula a producir mediadores de la inflamación que reclutan PMN al lugar de la infección, donde ejercen su efecto antibacteriano. Modificado de Hedlund M, Duan RD, Nilsson A, Svensson M, Karpman D, Svanborg C. Fimbriae, transmembrane signaling, and cell activation. J Infect Dis 2001; 183: S47-S50.

Los TLR-4 reconocen el LPS presente en la pared bacteriana de los bacilos Gram negativos (BGN) que, a su vez, representa el principal factor pro-inflamatorio de las infecciones causadas por estas bacterias. Después de su liberación por parte de la bacteria, el LPS se une a la proteína soluble fijadora de LPS o *"lypopolisaccharide binding protein"* (LBP) y este complejo es transferido al CD 14 presente en los macrófagos. El CD14 presente en la membrana celular está anclada a ella pero carece de un dominio transmembrana, por lo que precisa de los TLR-4 como correceptores para la transducción de la señal. A pesar de que la mayor parte de los

microorganismos uropatógenos son BGN, el urotelio responde pobremente al LPS soluble[50]. Tal y como han evidenciado Samuelsson *et al*[51], el uroepitelio expresa TLR-4 en toda su extensión pero no CD14 lo que explicaría porque el urotelio no responde ni al LPS soluble ni a cepas avirulentas de *E. coli*, como las que forman parte de la flora comensal. Como se muestra en la **figura 6**, en el tracto urinario, las fimbrias P y los receptores GSL reclutan y activan los TLR-4, de forma similar a como lo hacen los CD14 a nivel de los macrófagos[52].

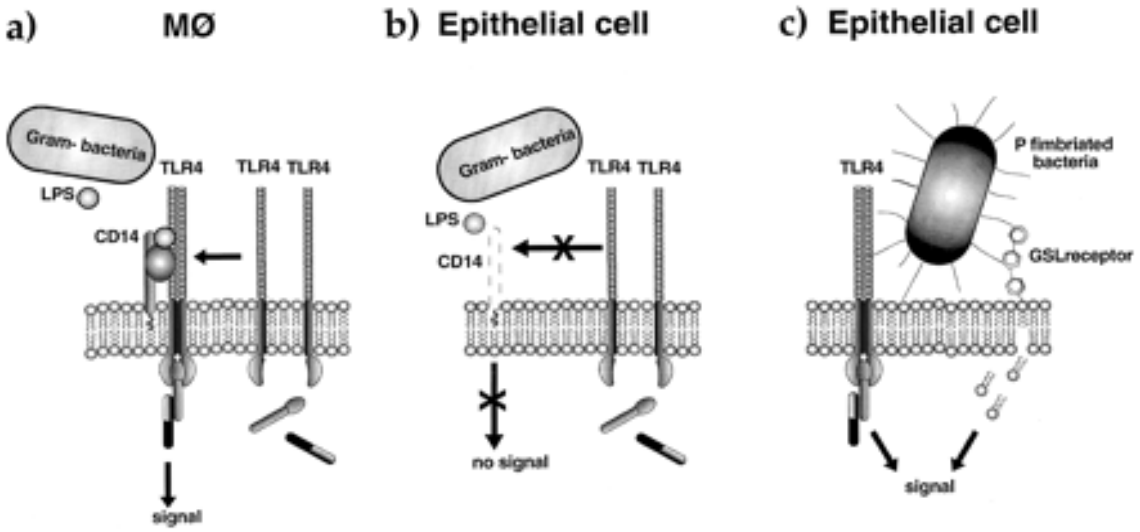


Figura 6. La activación de los TLR-4 a nivel de los macrófagos (MØ) y de las células epiteliales implica diferentes receptores primarios. A) El LPS de los BGN activa los MØ vía CD14. El LPS unido al LBP es transferido al CD14 y posteriormente se recluta al TLR-4 como correceptor para la transducción de la señal. B) El uroepitelio humano carece de CD14, y las células uroepiteliales responden con dificultad al LPS soluble. Aún así, las cepas de UPEC son capaces desencadenar una respuesta de citocinas, proceso que resulta incrementado por las fimbrias P. Las bacterias afimbriadas son menos virulentas y no inducen la activación celular y, por tanto, tampoco la liberación de citocinas. C) Las fimbrias P primero se unen su receptor GSL y reclutan TLR-4 para la producción de una señal transmembrana y la subsiguiente activación celular. De esta manera superan la refractariedad al LPS de las células uroepiteliales, e inducen una respuesta de citocinas. Tomado de Godaly G, *et al*, 2001[52].

Las fimbrias tipo 1 también son capaces de inducir una respuesta inflamatoria por parte del urotelio, diferente de la producida por las fimbrias P, aunque

ambas parecen estar mediadas por los TLR-4. Recientemente Fischer *et al*[53] han demostrado que estas diferencias podrían ser debidas a la participación de diferentes proteínas adaptadoras.

Además de las fimbrias tipo P, existen dos grupos adicionales de adhesinas resistentes a la manosa que también participan en el proceso de adhesión bacteriana a las superficies epiteliales: la familia de adhesinas afimbriadas (AFA) Dr[54] y la familia de las S/F1C[55]. La familia de adhesinas AFA Dr se han implicado en las pielonefritis gestacionales[56] mientras que las fimbrias S/F1C se han relacionado con las meningitis neonatales, aunque existen algunas evidencias experimentales que sugieren su participación en la patogénesis de las ITUs[57].

b. Toxinas.

Además de las adhesinas, las cepas de UPEC secretan proteínas específicas (toxinas) cuyo papel en la patogénesis de las ITUs es aún controvertido. Ejemplos de dichas toxinas incluyen la hemolisina, la toxina autotransportadora tipo 1 (SAT-1), el CNF-1, que actúan favoreciendo la invasión tisular, y la aerobactina que es un sideróforo[58,59].

Las hemolisinas son proteínas citotóxicas con capacidad para producir lesión en un amplio abanico de tipos celulares como por ejemplo las células tubulares renales. La α hemolisina es el tipo de hemolisina habitualmente producida por las cepas de *E. coli* causantes de ITUs. La producción de hemolisina, que se ha asociado con la presencia de infección extraintestinal, probablemente otorga a *E. coli* una ventaja selectiva al favorecer la captación del hierro liberado por

los hematíes lisados que puede ser usado por los sideróforos[60], la destrucción de las células fagocíticas[61] y la aparición de enfermedad invasiva[62].

La SAT-1 es una toxina proteolítica cuya presencia se ha asociado con cepas pielonefríticas de *E. coli*, y que parece tener un efecto tóxico frente a líneas celulares de origen renal y vesical[63]. Recientemente Guignot *et al*[64] han demostrado que el poder patógeno de las SAT-1 podría estar en relación con la producción de lesión a nivel de las uniones intercelulares o "*tight junctions"*.

c. Sideróforos.

E. coli precisa hierro para su metabolismo aeróbico y para los procesos de crecimiento y multiplicación bacteriana. Los ExPEC poseen múltiple mecanismos diseñados para recaptar hierro del huésped, siendo los más importantes los sistemas de receptor sideróforo-sideróforo y los de captación del grupo hemo[65] (ver **figura 1**).

d. Cápsula.

La mayor parte de las ExPEC poseen una cápsula de polisacáridos (antígeno K) que difiere de la cápsula que poseen las cepas comensales. Esta cápsula interfiere en los procesos de fagocitosis y protegen al microorganismo frente a la opsonización y la lisis mediada por el complemento[66].

e. Lipopolisacárido.

Aunque el LPS es un componente estructural de todas las cepas de *E. coli*, independientemente de su potencial virulento, se puede considerar un FV debido a sus efectos tóxicos en el huésped y a la respuesta inflamatoria que

induce. Como ya hemos visto, el LPS liberado desde la membrana externa de bacterias viables o tras la lisis bacteriana, interactúa con los TLR-4 y con otros receptores situados a nivel del epitelio y las células inmunes del huésped iniciando una cascada de transducción de señales que induce la síntesis y liberación de citocinas y otros mediadores inflamatorios[52,67].

f. Combinación y regulación de los factores de virulencia.

Las cepas de ExPEC típicamente expresan múltiples FV. El número y tipo de FV de los que dispongan los microorganismos uropatógenos determinará, al menos en parte, las distintas formas de ITUs (por ejemplo CA frente a PNA), la gravedad y el grupo de pacientes afectos. Además, y como se ha comentado, *E. coli* requiere un mayor número de FV para causar ITUs no complicadas o sintomáticas que para causar ITUs complicadas o asintomáticas[6].

En relación con los FV cabe decir que las cepas de *E. coli* aisladas de pacientes con CA tienen menos FV que las cepas *E. coli* procedentes de pacientes con PNA. A este respecto, tal y como publicaron Ruiz *et al*[43], los aislados de *E. coli* procedentes de pacientes con CA presentan una menor frecuencia de hemolisina y de CNF-1 que los aislados procedentes de PNA y estos últimos menos que las cepas procedentes de pacientes con PA. En este estudio también se evidenció una mayor prevalencia de aerobactina entre los aislados de *E. coli* procedentes de PNA y PA respecto aquellos causantes de CA y del gen de la *papG III* (fimbrias Prs) entre las cepas procedentes de PA[43]. Estos resultados demuestran que las cepas de *E. coli* causantes de PA son, en general, más virulentas (tienen más FV) que las cepas de *E. coli* aisladas de PNA y particularmente de las aisladas de CA.

El patrón de resistencia a las quinolonas de *E. coli* también determina el tipo de ITU y el huésped susceptible. Velasco *et al*[7] demostraron como las cepas de UPEC resistentes a quinolonas se aíslan con mayor frecuencia en pacientes con ITUs no invasivas (CA), en las ITUs aparecidas tras manipulación del tracto urinario o en las que se dan en pacientes con anomalías estructurales de la vía urinaria, que en pacientes con PNA o PA. Esto sugiere que los microorganismos causantes de PNA o de PA precisar ser más virulentos para poder producir la infección, aunque cuando llegan a producir enfermedad urinaria invasiva no existen diferencias en la incidencia de bacteriemia. También se ha demostrado como el gen de la *hemolisina*, del *CNF-1* y del *SAT-1* así como la expresión de las fimbrias tipo 1 son menos prevalentes tanto en las cepas de *E. coli* resistentes a quinolonas causantes de CA como en aquellas causantes de PNA[68], independientemente de su pertenencia al grupo filogenético B2[69]. En el estudio de Vila *et al*[68] se observó una buena correlación entre la ausencia del gen de la *hemolisina* y del *CNF-1* y la ausencia de su expresión demostrando como esta última era debida a la ausencia del gen. Cabe recordar que ambos genes se localizan en la misma isla de patogenicidad (PAI)[70]. Soto *et al*[71], demostraron como la exposición a concentraciones subinhibitorias de ciprofloxacino era capaz de inducir o provocar una pérdida total o parcial de los genes de la *hemolisina* y del *CNF-1*, localizados en la PAI-I, a través de la activación del sistema SOS, lo que podría contribuir a la pérdida de FV.

Además de la presencia o ausencia de un determinado FV, el patrón de expresión variable y la diversidad funcional de un FV será también determinante para definir la virulencia de una cepa. Así por ejemplo, el operón

fim, que codifica las fimbrias tipo 1, está presente en la mayoría de las cepas comensales y patógenas de *E. coli*. A pesar de esta ubicuidad, hay diferencias significativas con respecto a la regulación de este operón entre las cepas patógenas y las comensales. La expresión de las fimbrias tipo 1 es fase dependiente, con bacterias que pasan de un estado fimbriado a otro sin fimbrias y viceversa. Esta expresión está bajo el control de un promotor situado inmediatamente adyacente al gen *fimA* y que es potencialmente invertible[72]. Cuando dicho elemento está con la orientación "ON", el promotor permite la transcripción de los genes de las fimbria tipo 1. Como se muestra en la **figura 7**, cuando dicho elemento está con la orientación "OFF", la transcripción de los elementos estructurales no es posible y la expresión de los genes de las fimbrias tipo 1 permanece bloqueada. Estudios experimentales han demostrado como el porcentaje de aislados procedentes de CA que presentan el promotor en posición ON es mucho más elevado que en cepas procedentes de PNA, lo que sugiere que los aislados de CA y los de PNA expresan fimbrias tipo 1 de forma diferente durante el transcurso de una ITU[73].

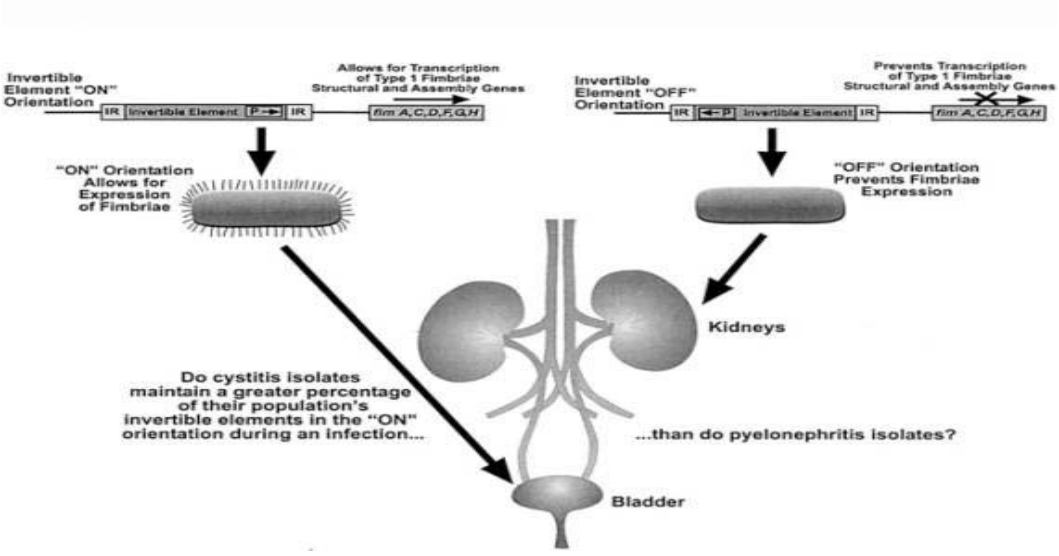


Figura 7. Control de la transcripción de los genes de las fimbrias tipo 1. Modificado de Guyer DM, *et al*, 2001[73].

5.3.1.2. Islas de patogenicidad.

Los genes que codifican los diferentes FV no se encuentran aislados en el cromosoma bacteriano sino que se agrupan en fragmentos de ADN conocidos como PAIs. Estas PAIs son elementos genéticos de gran tamaño que se pueden localizar en diferentes lugares de la bacteria ya sea en plásmidos, bacteriófagos o en el cromosoma bacteriano. Estos grandes bloques de ADN son móviles lo que facilita la diseminación simultánea de múltiples FV entre diferentes cepas de *E. coli*[74,75].

Cada PAI puede codificar para distintos FV, aunque algunos FV pueden coincidir en más de una PAI. Asimismo, una cepa de *E. coli* puede albergar diversas PAIs. En este sentido, las cepas de UPEC poseen PAIs y genomas que son un 20% mayores en relación a las cepas no patógenas fecales de *E. coli*[76]. En un estudio de Sabate *et al*[77], sólo un 42% de las cepas de *E. coli* aisladas de heces de personas sanas presentaban PAIs (con una media de 1 PAI por aislamiento), en contraste con el 91% de las cepas de *E. coli* causantes de ITUs (con una media de 3 PAIs). En este mismo estudio se observó como las cepas de *E. coli* pertenecientes al grupo filogenético B2, independientemente de si se habían aislado en heces de personas sanas o en la orina de pacientes con ITUs, presentaban una media de 3,9 PAIs en contraste con las cepas de *E. coli* del grupo D que presentaban una media de 0,9, las del grupo A con una media de 0,4 y las del grupo B1 con una media 0,3. Como ya se ha comentado, las quinolonas son capaces de inducir la pérdida de PAIs y, por tanto, de FV[71].

5.3.1.3. Papel de la biopelícula en la infección urinaria.

Las bacterias generalmente se encuentran adheridas a las superficies y de forma más infrecuente como células aisladas (planctónicas). Con frecuencia estas bacterias adheridas forman comunidades sesiles denominadas biopelículas. Las biopelículas se definen como acúmulos de microorganismos envueltos por una matriz que está compuesta por moléculas sintetizadas por el microorganismo y otras procedentes del huésped, formando comunidades estructuradas adheridas a las superficies.

Las biopelículas permiten a los microorganismos sobrevivir en condiciones ambientales adversas incluyendo las que el sistema inmune y los antibióticos producen. Por tanto su presencia es causa de infecciones bacterianas crónicas y recurrentes como es el caso de las periodontitis, las otitis medias, las infecciones del tracto biliar, las endocarditis y las infecciones que se producen sobre material protésico[78]. La formación de biopelículas desempeña un importante papel en la supervivencia bacteriana en las superficies de la mucosa vaginal, oral e intestinal. En estas localizaciones, la biocapa formada por la flora comensal evita la colonización por parte de microorganismos patógenos. Sin embargo, los microorganismos también pueden formar biocapas sobre cualquier dispositivo inerte insertado a un paciente (por ejemplo catéteres, prótesis articulares o valvulares) o sobre aquellas superficies mucosas con defectos en los mecanismos de aclaramiento necesarios para mantener su esterilidad (por ej. mucosa bronquial en pacientes con enfermedad pulmonar obstructiva crónica o fibrosis quística).

En el campo de las ITUs está plenamente demostrado el papel de las biopelículas en la patogenia de las infecciones urinarias asociadas al uso de

sondas urinarias[79]. Parece que la capacidad de *E. coli* para formar biopelículas podría estar en gran medida determinado por la naturaleza de la superficie inerte. Recientemente Ferreries *et al*[80] han demostrado como las cepas de *E. coli* causantes de BA producen biopelícula con mayor facilidad sobre polestireno y sobre cristal mientras que las cepas de *E. coli* causantes de ITUs invasivas producen biopelícula con mayor facilidad sobre catéteres de silicona.

La descripción por parte de Sheikh *et al*[81] de la formación de biopelículas sobre mucosas por parte de *E. coli* enteroagregativo nos debe hacer considerar la existencia de biopelículas como un posible factor patogénico implicado en las ITUs no asociadas a sondas urinarias. De hecho, la formación de biopelículas ya había sido sugerida tanto en el caso de la prostatitis crónica bacteriana[82] como sobre los cálculos de estruvita[83]. Nickel *et al* demostraron, en un modelo animal, como las bacterias se podían adherir al urotelio formando finas capas de biopelícula, antes de invadir el tejido renal causando pielonefritis[84], y que estas biopelículas se erradicaban con antibióticos con mayor facilidad que aquellas biopelículas asociadas a catéteres[83]. Sin embargo, la producción de biopelícula por parte de cepas de UPEC causantes de cistitis, pielonefritis y prostatitis simples o recurrentes no asociadas a cuerpos extraños no ha sido bien evaluada, de forma que su importancia en la patogenia y manejo de estas entidades está aún por determinar. Recientemente Hancock *et al*[85] han evidenciado que las cepas de *E. coli* productoras de BA, producen biopelícula con mayor facilidad que las cepas de *E. coli* causantes de ITUs sintomáticas. Se ha sugerido que las cepas causantes de ITURs podrían quedar acantonadas en reservorios como el colon o la vagina durante periodos de tiempo prolongados y desde estos reservorios causarían recurrencias[86]. Tal y como

Soto *et al*[87] presentaron en el XI congreso de la SEIMC, en un estudio en el que simultáneamente se evaluaban aislamientos de *E. coli* procedentes de ITUs y cultivos de frotis vaginal, el 50 % de los aislamientos de *E. coli* procedentes de la orina pertenecían al mismo clon que las cepas *E. coli* aisladas en el cultivo del frotis vaginal, lo que refuerza la idea del papel de la vagina como reservorio de *E. coli*.

Tal como se expone en la **figura 8**, la formación de biopelícula por parte de *E. coli* es un fenómeno complejo en el que participan una gran variedad de compuestos extracelulares (fundamentalmente ácido colánico), así como varias organelas (flagelos, fimbrias tipo 1 y curli) y proteínas de superficie (antígeno 43) que tienen un papel fundamental en las fases iniciales de la formación de la biopelícula[88].

Cuando la bacteria alcanza la piel o las mucosas, debe disponer de mecanismos de adherencia para poder colonizarla. Este aspecto es de especial relevancia en aquellas áreas como la boca, el intestino y las vías urinarias donde las mucosas están sometidas a un flujo continuo de líquidos que tiende a arrastrar a las bacterias no adheridas. En éstas áreas sólo las bacterias con capacidad para fijarse a las superficies permanecerán en ellas. En el caso de *E. coli*, las fimbrias tipo 1 se han identificado como las estructuras básicas que median en el proceso de adherencia o fijación[89]. En condiciones de crecimiento estático, las fimbrias tipo 1 permiten una interacción estable entre la bacteria y diversas superficies, incluyendo poliestireno, PVC, policarbonato y cristal borosilicato.

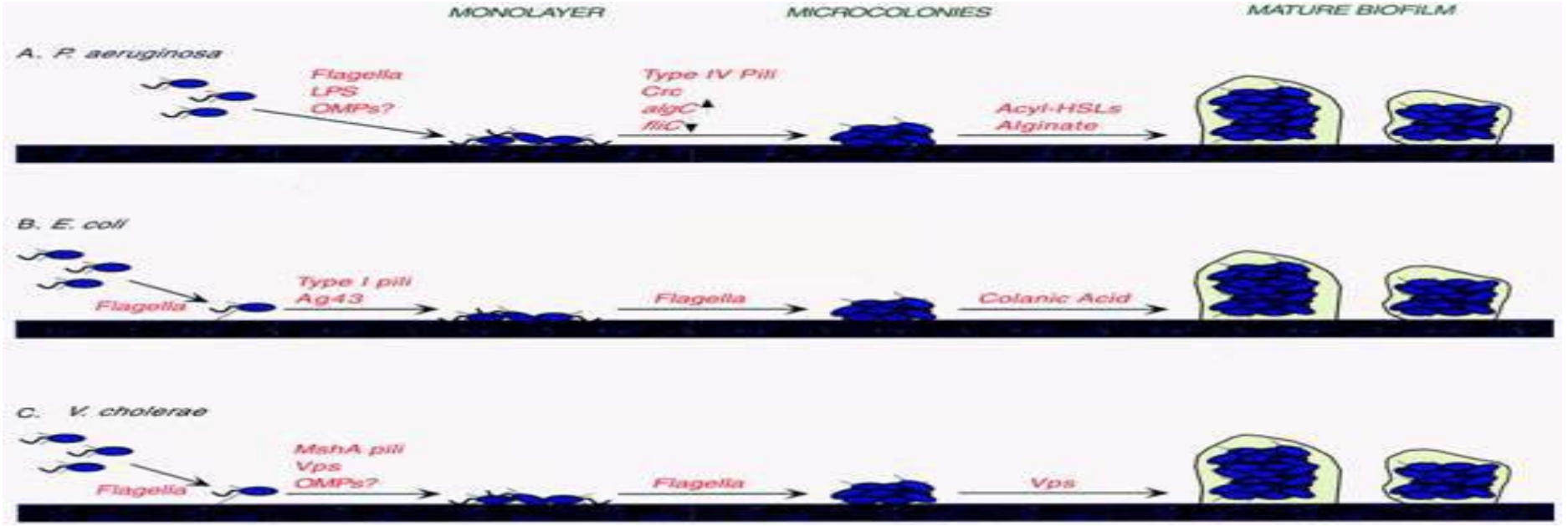


Figura 8. Producción de biopelícula por BGN (*P. aeruginosa, E. coli,* y *V. cholerae)*. A) En *P. aeruginosa,* los flagelos son necesarios para aproximar la bacteria a la superficie, y el LPS media las interacciones iniciales, con un posible papel adicional de las proteínas externas de la membrana bacteriana (OMPs). Una vez la bacteria se encuentra en la superficie formando una monocapa, la motilidad de las fimbrias tipo IV permite agregarse y formar microcolonias. La síntesis de dichas fimbrias está regulada por señales nutricionales (Crc). En las fases iniciales de la formación de la biopelícula se observa un incremento en la expresión de los genes implicados en la biosíntesis de alginato y una disminución en la expresión flagelar. La producción de una biopelícula madura precisa de la síntesis de señales moleculares intercelulares (acyl-HSLs). Probablemente el alginato también juega un papel estructural en este proceso. B) En *E. coli*, la movilidad flagelar es importante tanto para acercarse como para moverse a lo largo de la superficie. Las interacciones entre las bacterias y las superficies precisan de las fimbrias tipo 1 y de una proteína externa de la membrana bacteriana, el antígeno 43. Finalmente un exopolisacárido, el ácido colánico, es preciso para el desarrollo de la arquitectura normal de una biopelícula de *E. coli*. C) *V. cholerae*, como *E. coli*, utiliza sus flagelos para acercarse y mobilizarse a través de la superficie. La unión a la superficie precisa de los pili MshA y de probablemente otras OMPs. Una serie de exopolisacáridos estabilizan este proceso de adhesión. La formación de una biopelícula madura precisa también de la síntesis de algún exopolisacárido como el Vps. Modificado de Davey ED, *et al*, 2000[88].

La adherencia estable es, por tanto, un prerrequisito para la formación de la biopelícula sobre estas superficies. Sin embargo no se conoce aún la relación exacta entre la producción de biopelícula y la expresión de otros FV en el caso de ExPEC. Un hecho bien conocido y estudiado por nuestro grupo es que las cepas de *E. coli* procedentes de distintas formas clínicas de ITUs presentan diferencias en cuanto a su dotación de FV[43]. Esto sugiere que podrían existir diferencias en la capacidad de producción de biopelícula por estas cepas. Además, como nuestro grupo también ha demostrado, la adquisición de resistencia a quinolonas por parte de cepas de UPEC se asocia a una pérdida de FV, de forma que estas cepas son menos invasivas[7,68,69]. El mismo fenómeno podía acontecer respecto a la producción de biopelícula.

En este sentido, diversos estudios han demostrado como las bacterias que constituyen las biopelículas son capaces de intercambiarse material genético vehiculizado por plásmidos. Es más, la tasa de transmisión horizontal de plásmidos es mucho mayor en las bacterias que forman las biopelículas que en los cultivos en medios líquidos de los mismos microorganismos[90]. Recientemente Reisner *et al*[91] han descrito la existencia de un plásmido conjugativo en *E. coli* que induce la formación y expansión de las biopelículas.

Una característica crucial de las biopelículas es la extraordinaria resistencia a los antibióticos que presentan las bacterias que las constituyen. Existen varias razones que lo explican. En primer lugar, la matriz extracelular de exopolisacáridos que constituyen la biopelícula actúa de barrera que impide el paso o inactiva algunos antibióticos como los betalactámicos o los aminoglucósidos. En segundo lugar, las bacterias embebidas en la biopelícula presentan un ritmo de crecimiento disminuido o nulo en comparación con las

bacterias aisladas o planctónicas, de forma que la penetración y actuación de los antibióticos en ellas resulta menos eficaz[78]. En tercer lugar, el medio que rodea a las bacterias en la biopelícula puede llegar a crear un ambiente químicamente hostil para la acción de los antibióticos (cambios en el pH, anaerobiosis, etc). Además se ha sugerido que las bacterias de las biopelículas expresan un fenotipo diferente que les confiere resistencia a los antibióticos, un estado similar al que presentan las esporas[92]. De esta forma, la concentración mínima inhibitoria de los antibióticos puede llegar a multiplicarse por miles para conseguir una reducción suficiente de la población bacteriana.

Actualmente se están estudiando diversas formas de contrarrestar estos mecanismos de resistencia y así poder contar con esquemas terapéuticos eficaces frente a las biopelículas bacterianas. Uno de los mecanismos planteados es el de frenar la producción de la matriz extracelular mediante diversas substancias. Así, las fluoroquinolonas, los macrólidos y algunos probióticos, como el zumo de arándanos, han demostrado ser capaces de reducir la producción de biopelícula en diversos modelos[93,94]. Las quinolonas y los macrólidos a dosis altas pueden eliminar la producción de biopelícula por parte de algunos microorganismos. Sin embargo, las dosis necesarias de algunos de estos fármacos para erradicar una biopelícula madura pueden llegar a ser tóxicas por lo que se han ensayado combinaciones de antibióticos como ofloxacino con fosfomicina o ambas con claritromicina con resultados favorables[95].

A medida que se avance en el conocimiento de las bases genéticas de la producción y mantenimiento de las biopelículas surgirán nuevos esquemas terapéuticos.

5.3.2. Factores dependientes del huésped.

Con la excepción de la mucosa uretral, el tracto urinario normal es resistente a la colonización bacteriana y, gracias a una serie de mecanismos defensivos, es capaz de eliminar eficazmente aquellos microorganismos que consiguen acceder a la vía urinaria. Tal y como se expone en la **tabla 3** esto se consigue gracias a la existencia de un variado repertorio de mecanismos defensivos en el huésped[96].

La orina constituye la primera línea defensa del tracto urinario gracias a la existencia de una serie de características propias que actúan dificultando la supervivencia de los microorganismos, principalmente la elevada osmolaridad y concentración de urea y la acidez urinaria. Más allá de las propiedades químicas de la orina, los mecanismos defensivos más importantes del huésped son la existencia de un flujo unidireccional de orina y de un movimiento peristáltico, desde el riñón a la vejiga urinaria, ambos críticos para mantener estéril el tracto urinario. La orina contiene además inhibidores de la adherencia bacteriana entre los que destaca la PTH, glicoproteína producida por las células tubulares del asa ascendente de Henle que se encuentra en suspensión en la orina[26] y mucopolisacáridos que se disponen en la superficie de las células del epitelio vesical[96]. Asimismo, el uroepitelio es capaz de producir péptidos con acción antimicrobiana, entre las que destaca las defensinas y la catelicidina, y lactoferrina que compite con los sideróforos de los microorganismos por el hierro, nutriente esencial para el crecimiento bacteriano. Por último la flora vaginal normal, constituida por lactobacilos, forma una eficaz barrera frente a la colonización por gérmenes uropatógenos[96].

Tabla 3. Mecanismos defensivos antibacterianos del huésped.

Orina (pH, osmolaridad, ácidos orgánicos)
Flujo urinario y micción
Mucosa del tracto urinario (actividad bactericida, citocinas)
Inhibidores urinarios de la adhesión bacteriana
Proteína de Tamm-Horsfall
Mucopolisacáridos vesicales
Oligosacáridos de bajo peso molecular
Inmunoglobulina A
Péptidos antimicrobianos (defensinas y catelicidina)
Lactoferrina
Respuesta inflamatoria
Polimorfonucleares
Citocinas
Sistema Inmune
Innato
Adquirido (Inmunidad humoral y celular)
Miscelánea
Secreciones prostáticas
Flora vaginal

Modificado de Sobel JD, 1997[96].

Si a pesar de todo el microorganismo consigue superar esta primera línea defensiva y entra en contacto con el uroepitelio, el sistema inmune innato es capaz de poner en marcha una respuesta inmediata de carácter no específico. Esta respuesta se desarrolla gracias a la existencia de los llamados "*pattern recognition receptors"* (PRRs) que son receptores capaces de reconocer patrones moleculares altamente preservados presentes en los patógenos ("*pathogen-associated molecular patterns"* o PAMPs). Los TLR-4 constituyen el principal tipo de PRRs presente en la superficie luminal del epitelio del tracto

urinario[51]. Tal y como se muestra en la **figura 6** los TLR-4, tras entrar en contacto con el LPS principal PAMPs de la pared bacteriana de los BGN, actúan como correceptores para la transducción de la señal con la consiguiente respuesta inflamatoria por parte del uroepitelio[48].

Tras entrar en contacto con el tracto urinario, los microorganismos inducen la aparición de una respuesta inflamatoria que se desarrolla, tal y como que se esquematiza en la **figura 9**, en dos fases. En una primera fase (Fase o *Step* 1) los microorganismos adheridos inducen una respuesta por parte de las células del uroepitelio vía TLR-4 y otros PRRs con la consiguiente liberación de citocinas que reclutan PMN hacia el lugar de la infección. En una segunda fase (Fase o *Step* 2) los PMN migran atravesando el uroepitelio hacia la luz del tracto urinario, donde fagocitan a las bacterias uropatógenas[97].

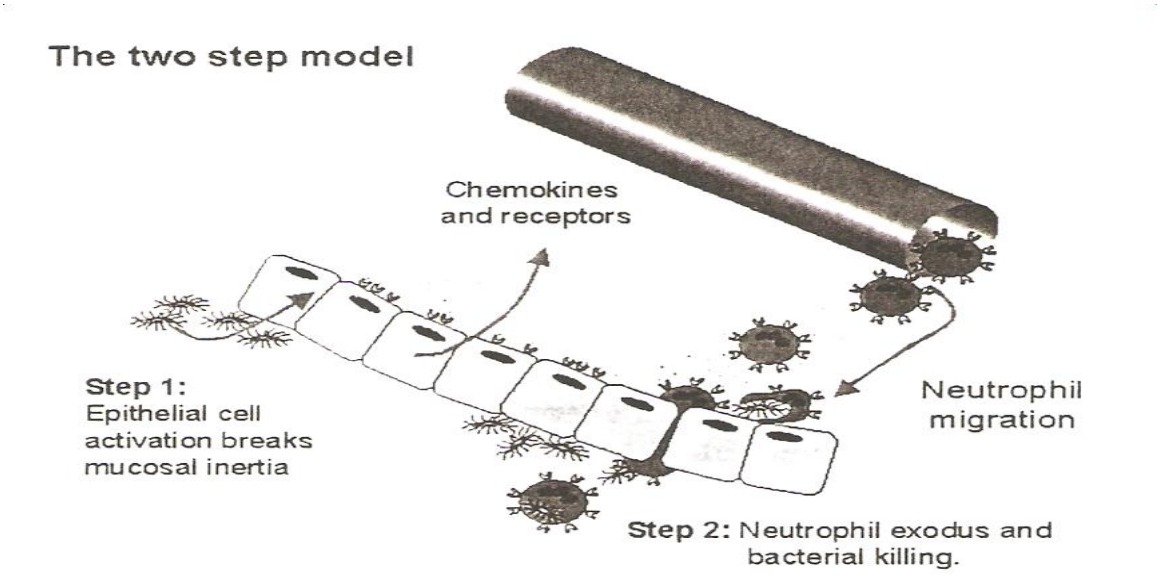


Figura 9. Modelo en dos fases. La adhesión bacteriana activa a las células uroepiteliales (Fase o *"Step"* 1) con la producción de citocinas que reclutan PMN al lugar de la infección. Durante este éxodo a través de la mucosa, los PMN eliminan las bacterias (Fase o *"Step"* 2). Tomado de Svanborg C, *et al*, 2001[97].

Como ya se ha explicado, las cepas de UPEC, a través de las fimbrias P y también de las fimbrias tipo 1, son capaces de desencadenar a nivel de las células epiteliales del tracto urinario una respuesta inflamatoria en la que se producen y liberan un variado elenco de citocinas detectables en sangre y orina, entre las que destaca la IL-1, IL-6 y IL-8. La IL-8 y el resto de miembros de la familia de citocinas CXC, que incluye la proteína activadora de los neutrófilos de origen epitelial (ENA)-78 y el factor de crecimiento relacionado con el oncogén (GRO)-α, son potentes factores quimiotácticos de los PMN[98,99] que además tienen la capacidad de inducir la expresión de los receptores de la IL-8[100]. El uroepitelio, además de sintetizar citocinas que participan en el reclutamiento de PMN, también expresa moléculas de adhesión implicadas en la trasmigración de los PMN[101].

En las infecciones urinarias, la respuesta inmunitaria dependiente de los PMN es mucho más importante que la respuesta inmunitaria específica (celular y humoral) por lo que se considera que es uno de los principales mecanismos defensivos del huésped frente a las ITUs, especialmente en el caso de las PNA[100]. Aunque los PMN se encuentran en la orina de la práctica totalidad de los pacientes con ITUs, parece que este no es el lugar donde ejercerían su función principal sino que fundamentalmente actuarían limitando la invasión tisular.

En los humanos, la IL-8 y el resto de quimiocinas CXC median su actividad biológica a través de dos receptores, el CXCR1 y el CXCR2. Estos receptores se expresan a nivel de la superficie de los PMN y son codificados por dos genes localizados a nivel del 2q35[102]. Estos dos receptores comparten el 78% de su secuencia de aminoácidos aunque se unen a la IL-8 con diferente afinidad.

Mientras que el CXCR1 es muy específico de la IL-8, el CXCR2 es más promiscuo y se une a la IL-8 así como a otras citocinas CXC que contengan la secuencia aminoterminal ácido glutámico-leucina-arginina (ELR). Las quimiocinas CXC ELR positivas incluyen la IL-8, la ENA-78 y el GRO-α, en humanos así como a la proteína macrofágica inflamatoria (MIP-2), el homólogo murino de la IL-8, y la citocina inductora quimiotáctica de los neutrófilos (KC) en ratones[103,104].

La importancia de los receptores de la IL-8 en el proceso de migración transepitelial de los neutrófilos ha sido bien demostrado en un modelo experimental de ITU por Frendeus B *et al*[100]. Tal y como se muestra en la **figura 10**, aquellos ratones a los que se les bloquea la expresión del receptor homólogo de la IL-8 se muestran incapaces de eliminar una infección por *E. coli*.

Los ratones no poseen CXCR1, y el CXCR2 media exclusivamente la repuesta de los PMN a las citocinas ELR-positivas, principalmente a través de la MIP-2 y la KC[105]. Los estos ratones a los que se les bloquea la expresión del receptor homólogo de la IL-8, los PMN se muestran incapaces de atravesar el epitelio, se acumulan en el tejido subepitelial, causando cicatrices renales y desarrollando enfermedad renal terminal[106,107]. Evidencias adicionales de la importancia de la IL-8 en el proceso de migración de los PMN se derivan de la observación por parte de Olszyna *et al*[108] de la ausencia de reclutamiento tras la administración de un anticuerpo frente al receptor homólogo de la IL-8 en el modelo murino de ITU.

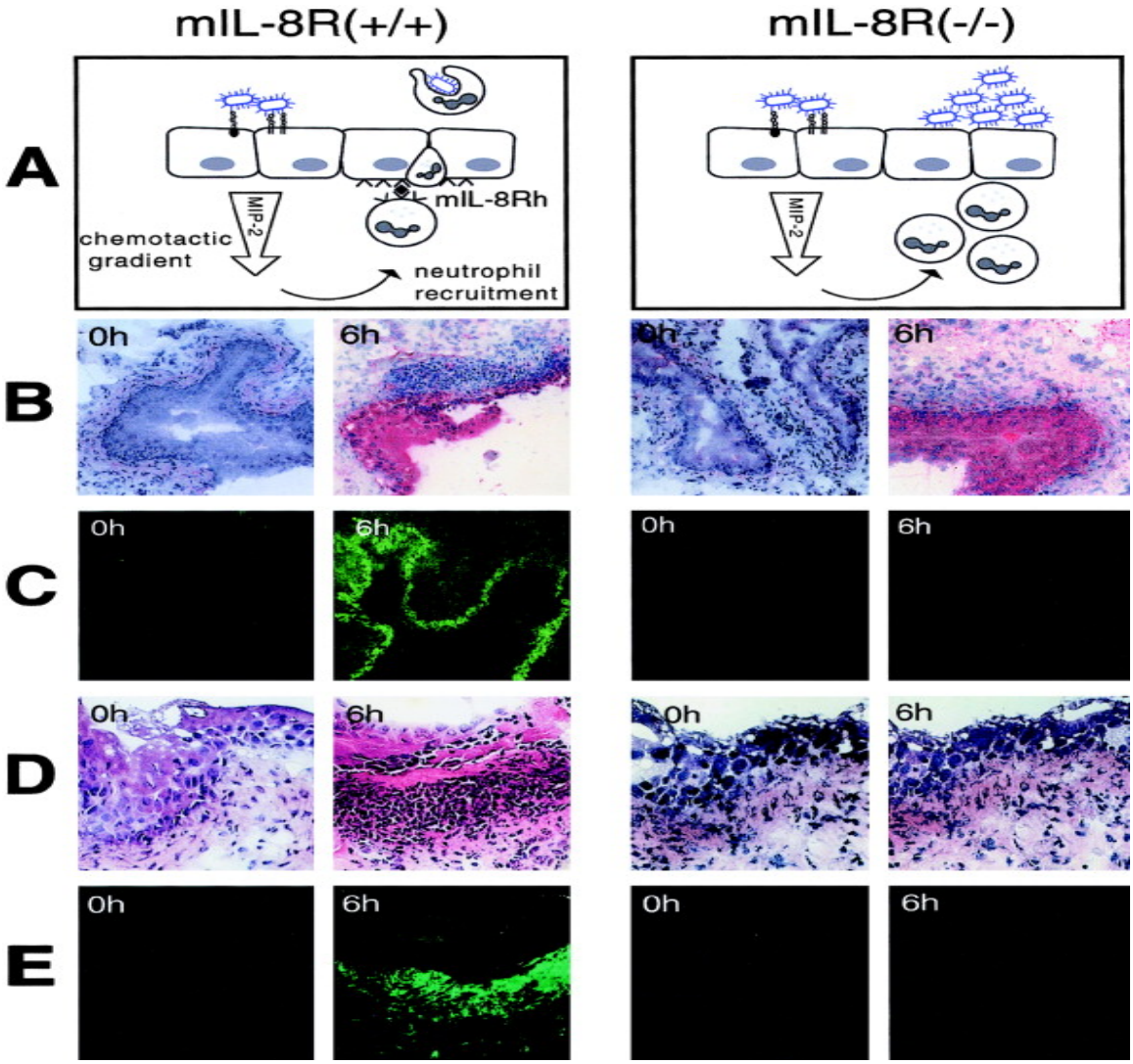


Figura 10. Interacciones entre los neutrófilos (PMN)-células epiteliales en ratones control (mIL-8R(+/+)) (panel de la izquierda) y en ratones en los que se les ha bloqueado la expresión del receptor de la IL-8 (mIL-8R (-/-)) (panel de la derecha). *A,* Esquema de la migración de los PMN a través del uroepitelio infectado. En huéspedes sanos, los PMN migran hacia y a través de la barrera epitelial a través de la interacción de la IL-8 y su receptor. En los ratones mIL-8R(-/-) los PMN son incapaces de atravesar el epitelio y se acumulan en el tejido subepitelial. *B,* La producción de citocinas por parte del epitelio en respuesta a la infección está intacta en los ratones mIL-8R(-/-), comparada con la respuesta que se produce en los ratones control. Secciones del riñón (6 h después de la infección) teñidas mediante el uso de un anticuerpo frente a la proteína macrofágica inflamatoria-2 (MIP-2). *C,* La infección induce la expresión del receptor homólogo murino de la IL-8 en ratones control pero no en los ratones mIL-8R(-/-). Secciones del riñón (6 h después de la infección) mediante el uso de anticuerpos frente al receptor de la IL-8. *D y E,* Diferencias entre el reclutamiento de PMN en los ratones mIL-8R(-/-) y en los ratones sanos. La infección en los ratones sanos produce un rápido flujo de PMN hacia la mucosa de la vejiga urinaria y los PMN se observan cruzando el epitelio hacia la luz del tracto urinario. En los ratones mIL-8R(-/-), el flujo de PMN está retrasado, se muestran incapaces de atravesar el epitelio y eventualmente se acumulan en el tejido subepitelial. Hematoxilina-eosina *(D)* y tinción mediante la utilización de anticuerpos frente a los PMN RB6-8C5 *(E)*. Modificado de Frendeus B, *et al* 1999[100].

En los últimos años se ha evidenciado que el receptor de la IL-8, CXCR1, es un factor con variabilidad genética que podría influir en desarrollo de ITUs. Frendeus *et al*[109] observaron como niños con predisposición a presentar PNA de repetición presentaban niveles bajos de expresión de CXCR1, pero no de CXCR2, en la superficie de los PMN comparado con un grupo de niños control y que este defecto de expresión podría estar en relación con la existencia de polimorfismo en la región del promotor del gen del *CXCR1*.

La lectina fijadora de manosa o "*mannose-binding lectin"* (MBL) es otro ejemplo de PRRs que forma parte de la inmunidad innata. Se trata de una lectina circulante de tipo C, de síntesis principalmente hepática, que se une con gran afinidad a los residuos de manosa, fucosa, glucosa y N acetil-D-glucosamina presentes en la superficie de diversos patógenos, incluyendo *E. coli*[110,111]. Además de actuar como una opsonina para la fagocitosis de numerosos microorganismos patógenos, es capaz de activar el complemento utilizando proteasas asociadas a la MBL, principalmente la MASP-2[112].

El gen *MBL2* (MBL1 es un pseudogén), está localizado en el cromosoma 10q11.2-q21[113]. Se conocen tres polimorfismos de nucleótido único (SNPs) a nivel de los codones 52 (alelo D), 54 (alelo B), y 57 (alelo C) del exón 1 del gen *MBL2*, que originan substituciones de aminoácidos que interfieren en la oligomerización de los monómeros de MBL en multímeros reduciendo, por tanto, los niveles de MBL[114,115]. Además de estas variantes alélicas estructurales, tres SNPs en la región del promotor del gen *MBL2* a nivel de -550 (H/L), +4 (P/Q) y, particularmente -221 (Y/X), influyen en la tasa de transcripción y también se asocian a bajas concentraciones séricas de MBL[116] (**Figura 11**).

Los SNPs del exón 1 establecen una fuerte relación de desequilibrio con los SNPs situados en el promotor, lo que da lugar a los siete haplotipos más comunes (HYPA, LYQA, LYPA, LXPA, LYPB, LYQC y HYPD) que presentan una variación considerable en su frecuencia entre diferentes grupos étnicos[117,118]. El haplotipo HY induce altas concentraciones de MBL mientras que las mutaciones en el exón 1 (variantes O) y los haplotipos LX causan concentraciones reducidas de MBL[119]. Teniendo en cuenta lo anterior, los pacientes pueden ser clasificados como pertenecientes a los grupos de expresión de MBL alta (HYA/HYA, HYA/LYA, HYA/LXA, LYA/LYA y LYA/LXA), intermedia (LXA/LXA, HYA/O y LYA/O) o baja (LXA/O y O/O)[118]. Aunque el déficit de MBL parece predisponer a infecciones severas[120], particularmente durante la infancia[121] y en pacientes que reciben quimioterapia [122] así como en adultos con enfermedades concomitantes[123,124], la asociación entre el déficit de MBL y las formas severas de ITUs no ha sido, hasta la fecha, evaluada.

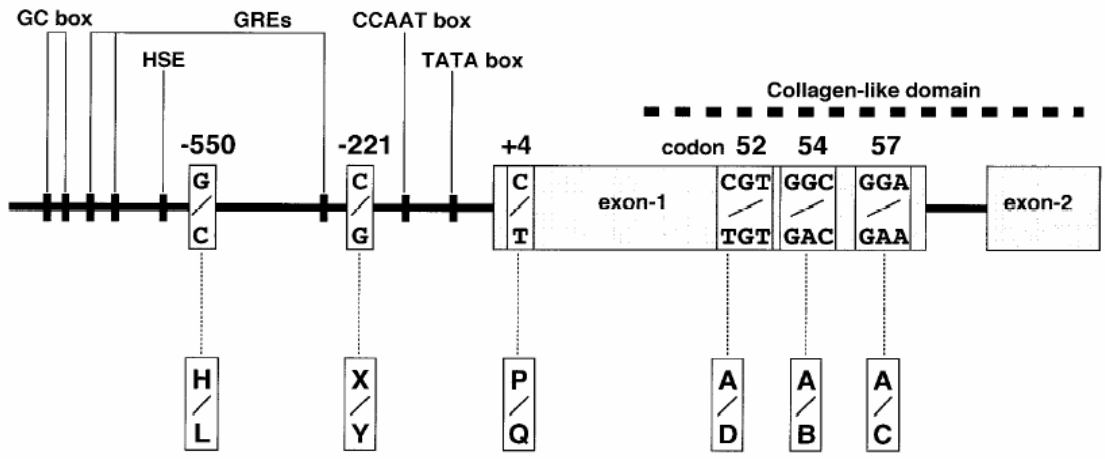


Figura 11. Representación esquemática del gen *MBL2.* A nivel del promotor el SNPs -221 (Y/X) y a nivel del exón 1 las variantes alélicas a nivel de los codones 52 (alelo D), 54 (alelo B), y 57 (alelo C) (variantes O) se asocian a niveles disminuidos de MBL. HSE: heat shock protein inducible element. GRE: Glucocorticoid-responsive element.

5.4. Formas clínicas de infección urinaria.

5.4.1. Bacteriuria asintomática.

La BA se define por la presencia de > 10^5 UFC/mm^3 del mismo microorganismo en dos muestras de orina consecutivas, en ausencia de signos y síntomas clínicos de ITU[125]. Aunque la BA precede a toda forma de ITU, su presencia no siempre conduce a la aparición de infección sintomática. Las mujeres gestantes constituyen un grupo donde la BA adquiere una especial importancia tanto por su prevalencia (entre un 4 a un 7% de las mujeres gestantes) como por sus posibles consecuencias. En ausencia de tratamiento antibiótico, un tercio de las mujeres gestantes con BA desarrollaran una PNA. Además la presencia de BA en la mujer gestante se asocia con un riesgo incrementado de parto prematuro y de presentar un recién nacido de bajo peso. Por ello la BA en la mujer gestante constituye una de las indicaciones de tratamiento antibiótico[126].

La BA es también una forma muy prevalente de ITU en el anciano[127], en los individuos portadores de sondas urinarias[128] y en los pacientes con lesiones medulares[129], y en los diabéticos[130]. En estos grupos de pacientes su presencia no condiciona una mayor mortalidad ya que no suele seguirse de una ITU sintomática y por lo tanto no estaría indicado ni su cribaje ni su tratamiento[125].

Las cepas de *E. coli* causantes de BA expresan menos FV que las cepas causantes de ITUs sintomáticas, particularmente aquellos FV relacionados con la adherencia bacteriana[40]. Paradójicamente, muchas de las cepas que causan BA poseen muchos de los genes de virulencia típicos de las cepas de UPEC. Recientemente Zdziarski *et al*[131] han sugerido que algunas de las cepas causantes de BA podrían originarse de cepas virulentas, tras procesos de atenuación de los genes de virulencia, mientras que otras cepas serían

avirulentas y se asemejarían más a las cepas comensales. Además, Hancock *et al*[85] han demostrado como las cepas de *E. coli* causantes de BA producen biopelícula con mayor facilidad que aquellas cepas de *E. coli* causantes de ITUs sintomáticas.

Se cree además que no sólo la mayor o menor cantidad de FV concurren en la aparición de la BA sino también la presencia de determinados factores del huésped. En este sentido Ragnarsdottir *et al*[132] han demostrado que niños con BA presentaban una menor expresión de TLR-4. Esta menor expresión de TLR-4 protegería al tracto urinario de las consecuencias de la inflamación y promovería el desarrollo de un estado de portador asintomático. Dado que en este estudio no se encontraron mutaciones en los genes del *TLR-4* sino sólo un descenso en los niveles de RNA mensajero se ha postulado que el defecto en la expresión del TLR-4 estaría en relación a alteraciones en los mecanismos de regulación de dichos genes.

5.4.2. Cistitis aguda.

Con este término se define la inflamación aguda, difusa y superficial de la mucosa vesical que, en la mayor parte de los casos, tiene un origen infeccioso. Se presenta principalmente en mujeres, sin enfermedades de base y sin anomalías funcionales o estructurales del tracto urinario, por lo que la mayoría de los casos se consideran ITUs no complicadas. La CA es un proceso muy frecuente entre mujeres sexualmente activas. Se ha estimado que dichas mujeres tienen aproximadamente 0,5 episodios de cistitis por persona y año con un impacto considerable dado que cada episodio supone una media de 6 días de síntomas y de unos 2 días de actividad restringida[133].

En relación a los FV, sabemos que las cepas de *E. coli* causantes de CA tienen menos FV que los aislados de *E. coli* procedentes de pacientes con PNA o con PA, con una menor frecuencia de α-hemolisina, CNF-1 y aerobactina[43].

Aunque todas las mujeres tienen riesgo de desarrollar una CA, se han definido una serie de condiciones que incrementan este riesgo y que se desarrollarán en el apartado dedicado a las ITURs.

5.4.3. Infecciones del tracto urinario recurrentes.

Las ITURs en general se definen como la aparición de 3 episodios de ITUs en los últimos 12 meses o de 2 episodios en los últimos 6 meses. Para entender la magnitud del problema cabe decir que las ITURs afectan a un 5-10% de las mujeres adultas y que aproximadamente un 25% de las mujeres con un primer episodio de CA desarrollarán ITURs[134]. La mayor parte de la ITURs afectan a mujeres premenopáusicas sanas sin alteraciones anatómicas o funcionales del tracto urinario[135].

E. coli es el patógeno más frecuentemente aislado en los episodios de ITURs. Clásicamente, las ITURs se han clasificado como reinfecciones cuando el microorganismo responsable es distinto del que ocasionó el episodio inicial, y como recidivas (responsables del 20% de las ITURs), cuando el microorganismo que causó la infección original es de nuevo aislado en la orina. Las cepas de *E. coli* asociadas a ITURs son con frecuencia, fenotípica y genotípicamente, idénticas que las cepas causantes de la primera ITU[13,137]. Este hecho junto con la capacidad de *E. coli* de invadir el uroepitelio[32] y formar reservorios vesicales[33,34] sugeriría que muchas ITURs podrían estar causadas por el resurgimiento de las cepas de UPEC desde estos reservorios

quiescentes. Aunque Johnson JR *et al*[138] han sugerido que algunos FV, como el *papA*, el *papG* alelo II, el *iha (“iron-regulated gene homologue adhesin”*) y el *iutA* (receptor de la aerobactina) se encuentran con mayor frecuencia entre las cepas de *E. coli* causantes de recidivas, parece que en la patogenia de las ITURs priman los factores propios del huésped.

En relación a los factores dependientes del huésped, la susceptibilidad del individuo para desarrollar infecciones urinarias, tal y como se expone en la **tabla 4**, depende una serie de factores genéticos, anatómico-funcionales y conductuales. Esta clasificación tiene un sentido práctico, dado que los factores genéticos no son abordables, los anatómico-funcionales son susceptibles de ser corregidos, al menos en parte, y los conductuales pueden ser fácilmente modificables.

Tabla 4. Factores dependientes del huésped en las RUTIs.

Genéticos	**Anatómico-funcionales**	**Conductuales**
- Estatus no secretor del Antígeno Lewis - Grupo sanguíneo P_1 - Elevada densidad de receptores epiteliales de adhesión - Baja expresión de CXCR1 y/o TLR-4 - Historia materna de ITUs - Historia de ITUs en la infancia	- Anomalías urológicas congénitas o adquiridas - Reflujo vesicoureteral - Embarazo - Incontinencia urinaria - Déficit de estrógenos (menopausia) - Historia de ITUs tras la adolescencia - Diabetes mellitus - Urolitiasis - Sonda urinaria o manipulación urológica - Distancia uretral-anal	- Coito - Uso de espermicidas y/o diafragma como anticonceptivo - Uso reciente de ciertos antibióticos

Modificado de Horcajada JP, Smithson A. Acute pyelonephritis in adults: an update. Rev Med Microbiol 2003; 14: 119-127.

a. Factores genéticos.

Numerosos estudios han sugerido la existencia de factores de riesgo de tipo genético para explicar la mayor susceptibilidad a ITURs que se da en ciertas mujeres premenopáusicas con vías urinarias normales. La mayor frecuencia de ITURs en mujeres con antecedentes maternos de ITUs o en aquellas con historia de ITUs antes de los 15 años son datos que sugieren la existencia de factores de riesgo de tipo genético[139].

Es un hecho conocido que las mujeres con ITURs presentan una mayor adhesión vaginal y periuretral a los uropatógenos que las mujeres sin ITURs[140-142]. Esto sugiere la existencia de diferencias en la densidad o expresión de los

receptores para los microorganismos uropatógenos a nivel de la mucosa del tracto urinario lo que podría estar mediado, al menos en parte, genéticamente. En este sentido se ha demostrado que aquellas mujeres con fenotipo no secretor del grupo sanguíneo de Lewis presentan un mayor riesgo de ITURs[143]. El gen que determina el estado secretor codifica para una de las glucosiltransferasas que intervienen en la composición carbohidratada de los GSL, de forma que las mujeres no secretoras expresan dos GSL en las membranas celulares que son lugares de unión para los UPEC. Por tanto las mujeres no secretoras presentan una mayor densidad de receptores para las adhesinas de *E. coli*. Además las mujeres no secretoras tienen menos receptores solubles en sus secreciones mucosas y, por tanto, una menor capacidad de inhibición competitiva de la colonización mediada por estas adhesinas[144].

También se ha demostrado que aquellas mujeres con fenotipo P_1 tienen mayor riesgo de ITURs, en particular de PNA recurrente[145,146] y que determinados antígenos HLA son más frecuentes en mujeres con ITURs[147]. Como ya se ha comentado ampliamente, hay evidencias de que tanto los CXCR1 como los TLR-4 serían ser factores adicionales con variabilidad genética que podrían influir en el desarrollo de ITUs[52,109,132]. Finalmente, en un único estudio, Condron *et al* [148] encontraron defectos en la función bactericida de los PMN en mujeres con ITURs aunque se desconoce la posible base genética de esta disfunción.

b. Factores anatómico funcionales.

Tanto en el hombre como en las mujeres, la obstrucción al flujo urinario a cualquier nivel anatómico del tracto urinario ya sea tanto por causas congénitas como adquiridas, constituye el factor de riesgo más importante para el desarrollo de ITUs y por consiguiente de ITURs. Se ha demostrado como factores anatómicos o funcionales que afectan al vaciado de la vejiga urinaria, fundamentalmente el cistocele y la incontinencia urinaria, son las alteraciones que con mayor frecuencia se asocian a ITURs en las mujeres postmenopáusicas[149] mientras que, como veremos, los factores conductuales son más importantes en las ITURs que aparecen de las mujeres premenopáusicas.

Se ha sugerido que las ITURs que ocurren en las mujeres postmenopáusicas no asociadas con patología urológica podrían estar relacionadas con la presencia de niveles bajos de estrógenos vaginales, lo que parece condicionar un descenso en la concentración vaginal de glucógeno y secundariamente de *Lactobacillus* spp. Esta alteración de la ecología vaginal produciría un incremento del pH vaginal, que favorecería la colonización vaginal por *Enterobacteriaceae*[149]. En este sentido Raz *et al*[150] demostraron como la administración tópica de estrógenos vaginales reducía la incidencia de ITURs en este grupo de mujeres.

La diabetes mellitus es otro factor que se ha asociado con una mayor susceptibilidad a ITURs. Aunque la presencia de glucosa en la orina facilita el crecimiento bacteriano, el factor que parece favorecer la mayor susceptibilidad a ITURs es la disfunción vesical que con frecuencia presentan estos pacientes con la consiguiente dificultad para el vaciamiento vesical[151].

La anatomía pelviana también parece jugar un papel en las ITURs de las mujeres jóvenes. En un único estudio, Hooton *et al*[152] observaron como las pacientes con ITURs tenían una menor distancia entre la uretra y el ano respecto aquellas mujeres jóvenes sin ITURs. Sin embargo en este estudio no se encontraron diferencias ni en la longitud de la uretra, ni en la presencia de residuo postmiccional ni en los patrones de flujo urinario. En cualquier caso parece que estas pequeñas diferencias tendrían una importancia menor que los factores conductuales.

c. Factores conductuales.

Se considera que los factores de riesgo conductuales son de especial relevancia en las ITURs que se dan en las mujeres premenopáusicas. En un estudio de Scholes *et al*[139] la actividad sexual y el uso de espermicidas, especialmente en combinación con el diafragma, fueron los factores que más claramente se asociaron con las ITURs de las mujeres jóvenes sanas. El incremento del riesgo asociado con la actividad sexual se relaciona por un lado con el efecto mecánico, que parece favorecer la entrada de uropatógenos a la vejiga urinaria, y por otro con el método anticonceptivo utilizado. En este sentido, la utilización de espermicidas se ha asociado con un mayor riesgo de ITURs, por el efecto deletéreo que ejercen sobre la microflora vaginal favoreciendo la colonización por UPEC[153].

También el uso reciente de antibióticos anaerobicidas, particularmente de B-lactámicos, parece que podría facilitar la aparición de recurrencias debido al efecto que ejercen sobre la microflora vaginal, favoreciendo la colonización vaginal por enterobacterias[154].

5.4.4. Pielonefritis aguda.

La infección de la vía urinaria superior o PNA se define como aquella que afecta a la pelvis y al parénquima renal. Anualmente en los Estados Unidos, se diagnostican unos 250.000 episodios de pielonefritis aguda en adultos, que precisan alrededor de 200.000 ingresos hospitalarios. La tasa de mortalidad estimada en mujeres con PNA es de unos 7,3 casos por cada 1000 ingresos hospitalarios[155], siendo algo más alta en determinados subgrupos como en los diabéticos, las mujeres gestantes y los pacientes ancianos y/o encamados[156].

La mayor parte de los factores de riesgo identificados para cistitis simple o recurrente también lo son para PNA[157]. En relación a los FV de las cepas de nefritogénicas de UPEC, se ha evidenciado una mayor frecuencia de fimbrias P, adhesinas G de clase II así como de aerobactina, CNF-1 y α-hemolisina en relación a la frecuencia encontrada en las cepas de *E. coli* aisladas de CA[40,41,43].

Entre los factores genéticos del huésped que se han asociado a PNA destaca la mayor prevalencia de fenotipo P_1 en mujeres con PNA recurrente[145,146] y la mayor predisposición a PNA recurrente observada en niños con niveles bajos de CXCR1[100,109]. Por último Ishitoya *et al*[158], demostraron como la presencia de un status no secretor se asociaba con PNA no complicada, especialmente en mujeres premenopáusicas.

5.4.5. Prostatitis aguda.

La PA se define como la infección bacteriana aguda de la glándula prostática, generalmente por cepas de UPEC. Las prostatitis constituyen la infección urinaria parenquimatosa más habitual en el varón entre la segunda y cuarta

décadas de la vida. En Estados Unidos las prostatitis generan alrededor de 2 millones de consultas médicas al año[159], aunque probablemente su incidencia real es mayor. En esta línea nuestro grupo de trabajo ha demostrado, por medio de la realización de gammagrafías marcadas con indio 111, como gran parte de las ITUs febriles con tacto rectal no doloroso y puño percusión lumbar negativa son en realidad PA[160].

Las cepas de *E. coli* causantes de PA son especialmente virulentas en cuanto a su dotación de FV, con una elevada prevalencia de CNF-1, α-hemolisina y aerobactina respecto a las cepas de *E. coli* causantes de otras formas de ITUs. Asimismo se ha descrito una mayor frecuencia del gen de la *papGIII* (fimbrias Prs) entre los aislados de *E. coli* procedentes de enfermos con PA[43]. Por último, la producción de biopelícula se ha implicado en la patogénesis de la prostatitis crónica bacteriana[82] aunque se desconoce su papel en la PA bacteriana y como podría influir su presencia en la duración de los tratamientos antibióticos.

5.4.6. Infección urinaria en el paciente sondado.

Las ITUs en pacientes portadores de sonda urinaria son las infecciones nosocomiales más frecuentes tanto en hospitales como en centros de larga estancia[161]. Parece que las cepas de *E. coli* aisladas de pacientes con sonda urinaria y BA son menos virulentas (tienen menos FV) que las cepas de *E. coli* procedentes de pacientes con ITUs adquiridas en la comunidad no relacionadas con el uso de sondas urinarias[162]. Por otra parte, las cepas de UPEC resistentes a quinolonas se aíslan con mayor frecuencia en las ITUs que se producen en los pacientes sondados[7]. Está bien establecida la implicación de la biopelícula en las ITUs asociadas al uso de sondas urinarias[79] y como la

capacidad del microorganismo para formar biopelícula viene determinada por la naturaleza de la superficie de la sonda[80].

6. JUSTIFICACIÓN, HIPÓTESIS Y OBJETIVOS GENERALES DE LA TESIS.

A pesar de los importantes avances que en los últimos años se han producido en el estudio de los mecanismos patogénicos subyacentes a las ITUs, quedan aún numerosas incógnitas por desvelar. Esta tesis pretende seguir avanzando en el conocimiento de la patogenia subyacente a este grupo de infecciones para, de esta forma, comprender mejor algunas de las problemáticas más habituales asociadas a las ITUs. Con ello quizá en un futuro no muy lejano podamos, a modo de ejemplo, implementar estrategias terapéuticas individualizadas, en función de las características propias del microorganismo causal y las del huésped afecto.

Las ITUs son el resultado de la interacción entre la mayor o menor virulencia de un microorganismo patógeno, *E. coli* en general, y una bateria de mecanismos defensivos del huésped. La virulencia de *E. coli* viene definida por los llamados FV entre los que destacan las adhesinas y las toxinas. Las fimbrias tipo 1 y las fimbrias P representan los dos principales tipos de fimbrias que expresan las cepas de UPEC. Gran parte de los artículos que en los últimos años se han publicado sobre la patogenia de las ITUs se han centrado en el estudio de estos dos tipos de fimbrias. Entre las funciones que se han atribuido a las fimbrias tipo 1 destacan la de activar la cascada defensiva del huésped y la de inducir la internalización de las cepas de UPEC que poseen dichas fimbrias, lo que conduce a la formación de reservorios quiescentes de *E. coli* en la vejiga urinaria. Las fimbrias tipo 1 también participan en las fases iniciales de la formación de las biopelículas. Por su parte, las fimbrias tipo P tienen la capacidad de inducir en el huésped la aparición de una respuesta inflamatoria

local con la producción de numerosas citocinas y quimiocinas, fundamentalmente IL-6 y IL-8, que conducen a la activación de los PMN y la consiguiente eliminación de las bacterias.

Las biopelículas representan uno de los campos de investigación más prometedores en el estudio de las ITUs. Está plenamente demostrado el papel de las biopelículas en la patogenia de las ITUs asociadas al uso de sondas urinarias. Sin embargo, la producción de biopelículas por parte de cepas de UPEC causantes de CA, PNA y PA, simples o recurrentes, no asociadas a cuerpos extraños no ha sido bien evaluada, de forma que su importancia en la patogenia y manejo de estas entidades está aún por determinar.

En relación al huésped, cada vez existen más evidencias en favor de la existencia de factores de predisposición individual a las ITUs. El receptor de la IL-8 CXCR1 es uno de factores cuya variabilidad genética parece favorecer el desarrollo de PA de repetición en niños aunque su influencia en las mujeres adultas con ITURs no ha sido aún evaluada. La MBL constituye otro importante elemento de la inmunidad innata que tiene un importante papel en la defensa del organismo frente a las infecciones. Numerosos estudios han demostrado como el déficit de MBL se asocia a una mayor frecuencia y gravedad de ciertas infecciones bacterianas. Hasta la fecha no se ha evaluado la existencia de una relación entre el déficit de MBL y la gravedad de las PNA.

6.1. Estudio 1: Formación de biopelícula por cepas uropatógenas de *E. coli*: relación con prostatitis, factores de virulencia y resistencia a antibióticos.

A diferencia de las cepas comensales, las cepas de UPEC poseen numerosos FV que les permiten, en primer lugar, colonizar las mucosas para posteriormente lesionar e invadir los tejidos del huésped. La descripción por parte de Sheikh *et al*[81] de la capacidad por parte de *E. coli* enteroagregativo de formar biopelículas sobre mucosas nos ha hecho considerar su implicación como un posible factor patogénico implicado en las ITUs no asociadas a sondas urinarias. En el caso de demostrarse su participación, la formación de biopelícula podría ser considerado como un nuevo FV que permitiría a las cepas de UPEC permanecer en el tracto genito-urinario y que dificultaría su erradicación. De hecho, se ha demostrado la formación de biopelículas por parte de cepas de *E. coli* causantes de BA[85] y de ITUs asociadas a al uso de sondas urinarias[79]. Además existen evidencias de su participación en la prostatitis crónica bacteriana[82], aunque la formación de biopelículas por parte de cepas de UPEC causantes de CA, PNA y PA está aún por determinar. Por otra parte, en los últimos años se ha observado un incremento progresivo en la tasa de resistencia a las quinolonas fenómeno que se asocia a la pérdida de FV[7,68,69]. Se desconoce si existe relación entre la adquisición de resistencia a las quinolonas y la formación de biopelícula.

Por lo tanto, las hipótesis que plantea este estudio son:

1. La formación de biopelículas juega un papel en la patogenia de las ITUs, principalmente en el caso de las PA.
2. Existe una relación entre la formación de biopelícula y la presencia de

ciertos FV y de resistencia a quinolonas.

Los objetivos concretos a evaluar son:

1. Analizar la formación de biopelículas por parte de cepas de UPEC causantes de las diferentes formas de ITUs (CA, PNA, PA).
2. Evaluar la relación entre la formación *in vitro* de biopelícula y la presencia de diversos factores de urovirulencia y la resistencia a ácido nalidíxico por parte de cepas de UPEC y de cepas de *E. coli* de origen fecal.

6.2. Estudio 2: Implicación de la formación de biopelículas en la persistencia de infección del tracto urinario causada por *E. coli*.

Un 25% de las mujeres con un primer episodio de CA presentarán ITURs, que se catalogan como recidivas cuando están causadas por la misma cepa del primer episodio y como reinfecciones cuando está causada por una cepa distinta[134]. Las biopelículas son fuentes conocidas de infecciones recurrentes, particularmente sobre material protésico[78]. La descripción de la formación de biopelículas sobre mucosas por parte de *E. coli* enteroagregativo[81] y por parte de cepas de UPEC causantes de BA[85] nos ha hecho considerar su posible implicación en la patogenia de las ITURs. Además, tal y como ha quedado demostrado por Mulvey MA *et al*[33] y Anderson *et al*[34], las cepas de UPEC son capaces de invadir el uroepitelio de la vejiga urinaria y multiplicarse intracelularmente formando biopelículas o *"pods"* que constituyen reservorios crónicos en la vejiga urinaria, fuente potencial de futuras ITUs.

La hipótesis de la que parte este estudio es:

1. La formación de biopelículas juega un papel en la patogenia de las ITURs, principalmente de las recidivas.

Los objetivos a evaluar son:

1. Analizar los factores de urovirulencia presentes en las cepas de *E. coli* causantes de recidivas y de reinfecciones.
2. Analizar la formación de biopelículas por parte de cepas de UPEC causantes de ITURs (recidivas y reinfecciones).

6.3. Estudio 3: Expresión de receptores de interleucina 8 (CXCR1 y CXCR2) en mujeres premenopáusicas con infección urinaria recurrente.

Las ITURs son muy frecuentes en mujeres jóvenes sanas que habitualmente poseen vías urinarias anatómica y funcionalmente normales[135]. Como ya hemos visto, los PMN constituyen uno de los principales mecanismos defensivos del huésped frente a las ITUs[100]. Para desarrollar su función, los PMN deben migrar desde el torrente sanguíneo hasta el tracto urinario donde ejercen su función. Este proceso de migración está mediado por un variado elenco de quimiocinas entre las que destaca la IL-8[98,99]. La IL-8 media su actividad a través de sus dos receptores, el CXCR1 y el CXCR2. Frendeus *et al*[100] demostraron la implicación de estos receptores en las ITUs tras evidenciar como niños con PNA de repetición presentaban niveles de expresión disminuidos de CXCR1, pero no de CXCR2, en la superficie de los PMN lo que podría explicar su mayor susceptibilidad a PNA. Además se ha propuesto que esta disminución en los niveles de expresión de CXCR1 podría estar en relación a la presencia de SNPs en el gen *CXCR1*[109]. La confirmación de la existencia de defectos en la expresión o función de estos receptores proporcionaría nuevas pruebas en favor de la existencia de factores de índole genético que conferirían una mayor susceptibilidad frente a las ITUs. Hasta la fecha la expresión de CXCR1 y de CXCR2 en adultos con ITURs no ha sido valorada.

Las hipótesis que plantea este estudio son:

1. La existencia de defectos en la expresión de superficie de los receptores de la IL-8, especialmente del CXCR1, juega un papel en la patogénesis

de las ITURs en las mujeres adultas premenopáusicas.

2. Los defectos en la expresión de superficie del CXCR1 están en relación con la existencia de polimorfismos a nivel del promotor y/o del exón 1 del gen *CXCR1*.

Los objetivos concretos a evaluar son:

1. Analizar la expresión, a nivel de la superficie de los PMN, de CXCR1 y de CXCR2 en un grupo de mujeres premenopáusicas con ITURs sin anomalías anatómicas de las vías urinarias ni enfermedades subyacentes y en un grupo de mujeres control sin antecedentes de ITUs previas.
2. Evaluar la existencia de SNPs a nivel del promotor y del exón 1 del gen *CXCR1* en los mismos grupos anteriores.

6.4. Estudio 4: Asociación entre el déficit de lectina fijadora de manosa y shock séptico tras pielonefritis aguda causada por *E. coli.*

Existen evidencias que hablan en favor de la implicación de factores individuales de carácter genético que explican, al menos en parte, la mayor susceptibilidad que ciertas mujeres presentan a infecciones urinarias recurrentes, particularmente a CA aunque también a PNA[145-147]. Además de los receptores de la IL-8 (CXCR1), existen indicios sobre la implicación de otros elementos de la inmunidad innata en la mayor susceptibilidad a ITUs, por ejemplo de los TLR-4 en el caso de la BA[132]. La MBL es otro componente de la inmunidad innata que actúa como opsonina y que además es capaz de activar el complemento a través de las MASP-2[112]. El déficit de MBL parece incrementar el riesgo de infecciones severas tanto en la infancia como en la edad adulta, particularmente si existen enfermedades concomitantes[120-124]. *In vitro* la MBL se une a los residuos de manosa, fucosa, glucosa y N acetil-D-glucosamina presentes en la superficie de diversos patógenos, incluyendo *E. coli*[110,111]. Se desconoce si la presencia de polimorfismos del gen *MBL2*, responsables de descensos en las concentraciones séricas de MBL, se asocian a una mayor gravedad (shock séptico) de las PNA causadas por *E. coli*.

La hipótesis que plantea este estudio es:

1. La existencia de polimorfismos del gen *MBL2*, causantes de un descenso en los niveles séricos de MBL, se asocian a una mayor gravedad de las PNA y a su posible evolución a shock séptico.

Los objetivos concretos a analizar son:

1. Comparar los genotipos y haplotipos del gen *MBL2* en pacientes con PNA por *E. coli* en relación a un grupo de controles sanos.
2. Analizar el riesgo de shock séptico y bacteriemia en pacientes con PNA por *E. coli* en relación a la presencia de SNPs del gen *MBL2*, asociados a una dismunición en los niveles de MBL circulante.

7. PUBLICACIONES ORIGINALES.

ARTICULO 1

BIOFILM FORMATION IN UROPATHOGENIC *ESCHERICHIA COLI* STRAINS: RELATIONSHIP WITH PROSTATITIS, UROVIRULENCE FACTORS AND ANTIMICROBIAL RESISTANCE

FORMACIÓN DE BIOPELÍCULA POR CEPAS UROPATÓGENAS DE *ESCHERICHIA COLI*: RELACIÓN CON PROSTATITIS, FACTORES DE VIRULENCIA Y RESISTENCIA A ANTIBIÓTICOS

S.M. Soto, A. Smithson, J.A. Martínez, J.P. Horcajada, J. Mensa, J. Vila.

The Journal of Urology 2007; 177: 365-368.

RESUMEN

E. coli es el germen que con mayor frecuencia causa infecciones del tracto urinario. La producción de biopelícula permite a las cepas de *E. coli* persistir en el tracto genito-urinario. En este estudio se ha evaluado la posible relación entre las diferentes formas de infección del tracto urinario, y la formación *in vitro* de biopelícula, la presencia de factores de virulencia y la resistencia a ácido nalidíxico. Se ha analizado la producción *in vitro* de biopelícula, el grupo filogenético así como la presencia de distintos factores de virulencia y resistencia al ácido nalidíxico en un total de 151 cepas de *E. coli* procedentes de pacientes con cistitis (44 cepas), pielonefritis (75), y prostatitis (32). Hemos encontrado una mayor producción *in vitro* de biopelícula y de hemolisina entre las cepas de *E. coli* causantes de prostatitis (*P* = 0.03 y 0.0002, respectivamente). A pesar de ello, sólo la presencia de hemolisina se ha asociado de forma independiente con prostatitis. Por otra parte, las cepas de *E. coli* productoras de biopelícula expresan con mayor frecuencia hemolisina y fimbrias tipo 1. Aunque la hemolisina es el principal factor de virulencia por el que *E. coli* causa prostatitis aguda, la asociación de hemolisina con la formación de biopelícula podría incrementar la capacidad de las cepas de *E. coli* de persistir en la próstata.

Biofilm Formation in Uropathogenic Escherichia coli Strains: Relationship With Prostatitis, Urovirulence Factors and Antimicrobial Resistance

S. M. Soto,* A. Smithson, J. A. Martinez, J. P. Horcajada, J. Mensa and J. Vila†

From the Microbiology and Infectious Diseases (AS, JAM, JPH, JM) Departments, Hospital Clinic, Institut d'Investigacions Biomèdiques August Pi i Sunyer, School of Medicine, University of Barcelona, Barcelona, Spain

Purpose: Escherichia coli strains are the most frequent cause of urinary tract infections. Biofilm formation allows the strains to persist a long time in the genitourinary tract and interfere with bacterial eradication. We determined the possible relationships between the different urinary tract infections, and in vitro biofilm formation, the presence of urovirulence factors and nalidixic acid resistance.

Materials and Methods: A total of 151 E. coli strains collected from patients with cystitis (44 strains), pyelonephritis (75) and prostatitis (32) were analyzed for in vitro biofilm formation, the phylogenetic group, the presence of several urovirulence factors and resistance to nalidixic acid.

Results: E. coli strains causing prostatitis produced biofilm in vitro more frequently than those causing other urinary tract infections and had a higher frequency of hemolysin ($p = 0.03$ and 0.0002, respectively). However, only hemolysin was independently associated with prostatitis. On the other hand, strains forming biofilm presented a significantly higher frequency of hemolysin and type 1 fimbriae expression.

Conclusions: Although hemolysin is the main virulence factor by which E. coli causes acute prostatitis, the association between hemolysin and biofilm formation may result in increased ability of E. coli strains to persist in the prostate.

Key Words: urinary tract, urinary tract infections, prostatitis, Escherichia coli, biofilms

Uropathogenic Escherichia coli strains are the most frequent cause of UTIs. In comparison to commensal strains UPEC has several virulence factors that allow it to colonize host mucosal surfaces, injure and invade host tissues, overcome host defense mechanisms and incite a host inflammatory response. Biofilm formation may be considered another pathogenic determinant, which allows the strains to persist a long time in the genitourinary tract and interfere with bacterial eradication. Biofilms are currently defined as structured bacterial communities embedded in a self-produced exopolysaccharide matrix adherent to any abiotic or biological surface.[1] Actually biofilms are probably the usual living condition of bacteria in natural environments and they are, indeed, regularly involved in infections associated with biomaterials such as catheters or prostheses. In these clinical processes biofilm formation is the main culprit of the characteristic persistence of infection despite appropriate antibiotic therapy. In addition, the number of UPEC strains showing quinolone resistance has increased in recent years and there is evidence that it is associated with a loss of invasive capacity,[2,3] although to our knowledge the relationship of quinolone resistance with biofilm formation has not been previously explored.

We determined the capacity of clinical E. coli strains to form biofilm structures in patients with different UTIs. We also determined the possible relationship between in vitro biofilm formation, the presence of urovirulence factors and nalidixic acid resistance. In addition, we compared these features with those of E. coli isolated from human feces.

MATERIALS AND METHODS

Bacterial Strains

A total of 151 E. coli strains collected from 44 patients with cystitis, 75 with pyelonephritis and 32 with acute PT were analyzed at our institution. All patients with cystitis or pyelonephritis were female with a mean ± SD age of 47.8 ± 22.2 years. Patients with prostatitis had a mean age of 59.5 ± 16 years. In addition, a collection of 30 rectal isolates obtained at hospital admission from critically ill patients who had not previously been hospitalized, had no significant comorbidity and had not received antibiotics during the last month was also analyzed.[4] These patients had a mean age of 58.2 ± 18 years and 18 were male. Rectal isolates and those from patients with pyelonephritis and prostatitis were submitted to the Clinical Microbiology Laboratory, Hospital Clinic of Barcelona. Isolates from patients with cystitis were recov-

Submitted for publication December 5, 2005.

Institutional experimentation guidelines were followed in the performance of this clinical research.

Based on work supported by Grants FIS 02/0327 and FIS 02/0453 from the Ministry of Health, Spain and 2005 SGR00444 from the Departament d'Universitats, Recerca i Societat de la Informació de la Generalitat de Catalunya, Spain (JV).

* Financial interest and/or other relationship with the Sistema Nacional de Salud, Fondo de Investigación Sanitaria of the Spanish Health and Consumption Ministry (CP05/00140).

† Correspondence: Department of Microbiology, Hospital Clinic of Barcelona, Villarroel 170, 08036-Barcelona, Spain (telephone: +34-93-227-54-00; FAX: +34-93-227-93-22; e-mail: jvila@ub.edu).

DOI:10.1016/j.juro.2006.08.081

ered from urine samples submitted to the Clinical Microbiology Laboratory, Manso Primary Care Center, Barcelona.

Cystitis was clinically defined as a syndrome involving dysuria, frequency and urgency, whereas acute pyelonephritis was defined as a syndrome characterized by fever (armpit temperature greater than 38C), flank pain and/or lumbar tenderness with or without cystitis symptoms. Prostatitis was defined by the presence of fever, pyuria and prostatic tenderness detected by gentle digital rectal exploration.

All urinary tract infection episodes were community acquired and uncomplicated, which means that no patient had an underlying comorbidity, apparent urological abnormality or an urethral catheter in place. Patients with pyelonephritis or prostatitis remained in the hospital at least 24 hours.

Antimicrobial Susceptibility

Nalidixic acid susceptibility was determined by a microdilution method according to the National Committee of Laboratory Standards.[5] E. coli American Type Culture Collection 25922 and 35218 served as control strains.

PCR

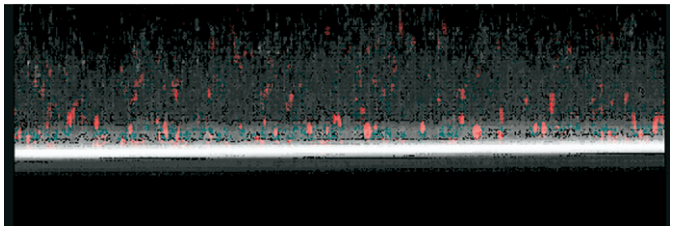
The E. coli phylogenetic group was determined with a 3 locus PCR based method.[6] The presence of the virulence factors hemolysin (*hly*A gene), aerobactin (*aer* gene), type 1 fimbriae (*fim*A gene), P-fimbriae (*pap*A and *prs* genes) and yersiniabactin (*fyu* gene) was analyzed by PCR using gene specific primers.[3] Type 1 fimbriae expression was determined by agglutination with a Saccharomyces cerevisiae strain.

In Vitro Biofilm Assay

Biofilm assay was done using M63 minimal glucose medium.[7] Strains were grown overnight in Luria-Bertani medium at 37C without shaking. An aliquot (1.25 μl) of overnight culture was subcultured in 125 μl M63 medium with 1% Luria-Bertani medium in each well of a polystyrene microtiter plate and incubated at 30C overnight without shaking. Subsequently 1.25 μl of each culture were subcultured again in 125 μl M63 medium in another polystyrene microtiter plate and incubated as cited. After 24 hours the culture was removed from the plate and the biofilm was stained with 175 μl violet crystal for 1 minute, washed with $1 \times$ phosphate buffered saline and air dried for about 1 hour. Colorant was solubilized in dimethyl sulfoxide to measure absorbance at a λ of 550 nm in an Anthos Reader 2001 automatic spectrophotometer (Innogenetics®). The result was considered positive when absorbance was greater than 4-fold the value obtained in the well containing bacteria-free medium. All assays were performed in duplicate using positive and negative controls. Biofilm formation was also assessed in a small group of selected E. coli strains by confocal microscopy (see figure).

Statistical Analysis

The association of acute prostatitis with biofilm formation, virulence factors, nalidixic acid susceptibility and phylogenetic group was assessed using the chi-square test with the Yates correction. Logistic regression was used to assess the microbial determinants independently associated with strains causing acute prostatitis vs those involved with other clinical UTIs and with in vitro biofilm formation.



Confocal image shows E. coli biofilm

RESULTS

Biofilm Formation in Uropathogenic E. coli

A total of 151 UPEC strains causing cystitis, pyelonephritis and acute prostatitis were analyzed for biofilm formation. The presence of other virulence factors was also analyzed. A total of 69 isolates (46%) were considered positive for in vitro biofilm formation. Biofilm production was noted in 19 (43%), 30 (40%) and 20 strains (63%) from patients with cystitis, pyelonephritis and acute prostatitis, respectively (table 1). The ability to form biofilm was significantly more frequent in acute prostatitis strains than in those involved in pyelonephritis and it showed a trend to be significantly more frequent than in cystitis strains ($p = 0.03$ and 0.09, respectively). No differences were observed between cystitis and pyelonephritis strains regarding biofilm formation ($p = 0.73$). After pooling cystitis and pyelonephritis strains together the characteristics significantly associated with strains causing acute prostatitis on univariate analysis were biofilm formation (20 of 32 or 63% vs 49 of 119 or 41%, $p = 0.03$) and hemolysin (20 of 32 or 63% vs 35 of 119 or 29%, $p = 0.0006$, table 1). However, logistic regression selected hemolysin as the only factor independently associated with acute prostatitis (OR 4, 95% CI 1.76–9.12).

In the series studied 88 isolates (58%) belonged to phylogenetic group B2, 35 (23%) belonged to group A, 21 (14%) belonged to group D and only 7 (5%) belonged to phylogenetic group B1. The percent of UPEC strains belonging to phylogenetic group A producing biofilm was lower than that of the other phylogenetic groups (10 of 35 or 29% vs 59 of 116 or 51%, $p = 0.02$). The highest percent of strains producing biofilm was observed in phylogenetic group B2 vs the other phylogenetic groups (48 of 88 or 55% vs 21 of 63 or 33%, $p = 0.009$). No association of biofilm production with phylogenetic groups B1 and D was found ($p = 0.87$ and 0.45, respectively).

The *fim*A gene was the virulence factor most frequently detected in UPEC (91% of the strains) but it was expressed only by 78% of the strains. Yersiniabactin (*fyu*A gene), aerobactin (*aer* gene), P-fimbriae (*pap*A gene) and hemolysin (*hly*A gene) were present in 72%, 60%, 42% and 36% of the isolates, respectively. Only hemolysin and type 1 fimbriae expression were significantly associated with biofilm producing E. coli strains (table 2).

A total of 44 UPEC strains (27%) were resistant to nalidixic acid. Strains forming biofilm were significantly less resistant to nalidixic acid than those negative for biofilm formation (table 2).

When clinical UTI type, phylogenetic background, urovirulence factors and nalidixic acid resistance were introduced in a logistic model, the only factors that remained significantly associated with in vitro biofilm formation were

TABLE 1. *Characteristics of E. coli strains causing PT, cystitis and pyelonephritis*

	No. Strains (%)		
	PT/Cystitis	PT/Pyelonephritis	Cystitis/Pyelonephritis
Overall	32/44	32/75	44/75
Virulence factors:			
Biofilm pos	20 (63)/19 (43)	20 (63)/30 (40)	19 (43)/30 (40)
p Value	0.09	0.03	0.73
*hly*A	20 (63)/7 (16)	20 (63)/28 (37)	7 (16)/28 (37)
p Value	0.00002	0.01	0.01
aer	18 (56)/31 (70)	18 (56)/41 (55)	31 (70)/41 (55)
p Value	0.2	0.88	0.08
*fim*A	30 (94)/43 (98)	30 (94)/64 (85)	43 (98)/64 (85)
p Value	0.38	0.06	0.02
*fyu*A	25 (78)/30 (68)	25 (78)/53 (71)	30 (68)/53 (71)
p Value	0.33	0.42	0.77
*pap*A	14 (44)/11 (25)	14 (44)/39 (52)	11 (25)/39 (52)
p Value	0.08	0.43	0.003
prs	16 (50)/17 (39)	16 (50)/37 (49)	17 (39)/37 (49)
p Value	0.32	0.92	0.56
fim Expression	25 (78)/36 (82)	25 (78)/58 (77)	36 (82)/58 (77)
p Value	0.68	0.92	0.56
Nal-R	6 (19)/17 (39)	6 (19)/21 (28)	17 (39)/21 (28)
p Value	0.06	0.34	0.25

the presence of hemolysin (OR 3.73, 95% CI 1.82–7.67) and type 1 fimbriae expression (OR 2.76, 95% CI 1.12–6.79).

Comparison Between UPEC Causing Acute Prostatitis and Fecal E. Coli Strains

The E. coli control group collected from feces underwent the same analysis as urine strains. Table 3 lists the differences in the virulence factor profiles between these 2 groups of isolates. E. coli strains causing acute prostatitis showed hemolysin, aerobactin, yersiniabactin, P-fimbriae and type 1 fimbriae expression significantly more frequently than those collected from feces ($p = 0.00001$, 0.03, 0.05, 0.01, 0.0002 and <0.00001, respectively). In addition, acute prostatitis strains also showed significantly greater ability to form in vitro biofilm ($p = 0.0002$). The phylogenetic distribution was different in the 2 groups of strains with B1 as the predominant phylogenetic group (37%), followed by A (33%), D (23%) and B2 (7%) in the fecal strains group.

DISCUSSION

The current study shows that biofilm production is one of the several putative virulence determinants possessed by UPEC that causes acute prostatitis and it is associated with hemolysin and type 1 fimbriae expression. Even if not directly involved in invasiveness, which is a property apparently linked to hemolysis, biofilm formation may still result in the increased ability of strains causing acute prostatitis to persist in the prostatic secretory system and lead to the recurrent UTIs characteristic of chronic bacterial prostatitis.

UTIs are a major public health concern in developed countries and they also represent one of the most common hospital acquired infections. Most uncomplicated UTIs are caused by E. coli, accounting for up to 90% of community acquired and approximately 50% of nosocomial UTIs.[3] Recurrence is a common problem in UTIs, even in patients without anatomical abnormalities or an indwelling bladder catheter. Persistence of the same E. coli strain in the urinary tract may be the cause of recurrent prostatitis. In fact, it was shown that after an episode of acute prostatitis cultures of expressed prostatic secretions are still positive 3 months after the end of a 6-week course of therapy in a third of men.[8] This may be related to the capacity of bacteria to form biofilm structures. Biofilm can promote persistence in the urinary tract and on biomaterial surfaces by protecting bacteria from the clearing out effect of hydrodynamic forces and the killing activity of host defense mechanisms and antibiotics.[9] Biofilm endows bacteria with several advantages,

TABLE 2. *Characteristics of E. coli strains that do and do not form biofilm*

	No. Biofilm Pos (%)	No. Biofilm Neg (%)	p Value
Virulence Factors:	69	82	
*hly*A	36 (52)	19 (23)	0.0002
aer	38 (55)	52 (63)	0.29
*fim*A	65 (94)	72 (88)	0.17
*fyu*A	49 (71)	59 (72)	0.89
*pap*A	34 (49)	30 (38)	0.11
prs	36 (52)	34 (41)	0.18
fim Expression	60 (87)	59 (72)	0.02
Nal-R	13 (19)	31 (38)	0.01
Phylogenetic group:			
A	10 (14)	25 (30)	0.02
B1	3 (4)	4 (5)	0.87
B2	48 (70)	40 (49)	0.009
D	8 (12)	13 (16)	0.45

TABLE 3. *Characteristics of PT and fecal E. coli isolates*

	No. PT Isolates (%)	No. Fecal Isolates (%)	p Value
Virulence factors:	32	30	
Biofilm	20 (63)	5 (17)	0.0002
*hly*A	20 (63)	3 (10)	0.00001
aer	18 (56)	9 (30)	0.03
*fim*A	30 (94)	23 (77)	0.05
*fyu*A	25 (78)	14 (47)	0.01
*pap*A	14 (44)	1 (3)	0.0002
prs	16 (50)	14 (47)	0.79
fim Expression	25 (78)	1 (3)	<0.00001
Nal-R	6 (19)	8 (27)	0.45
Phylogenetic group:			
A	5 (16)	10 (33)	0.10
B1	3 (9)	11 (37)	0.01
B2	19 (59)	2 (7)	0.00001
D	5 (16)	7 (23)	0.44

such as the acquisition of antibiotic tolerance, expression of several virulence factors, and increased resistance against phagocytosis and other host defense mechanisms. The study of factors contributing to biofilm formation may be important to conceive new therapeutic solutions to treat these infections. Wu et al suggested that the inhibition of bacterial attachment to a uroepithelial surface, which is a crucial initial event involving precise interactions between a group of bacterial adhesive molecules called adhesins and their cognate urinary tract receptors, could be interesting to avoid biofilm formation.[10]

In our study a higher percent of biofilm producing E. coli strains was observed in those involved in acute prostatitis than in those causing cystitis or pyelonephritis. However, the presence of hemolysin was the main confounder of this association because hemolysin positive strains, which were strongly linked to acute prostatitis, also showed a higher frequency of in vitro biofilm formation. These biofilm producing E. coli strains showed significantly a greater type 1 fimbriae expression than nonbiofilm producing strains. Type 1 fimbriae are considered an important factor in the first steps (adhesion to the host epithelial cells) of biofilm formation. These data are in accordance with the results of other groups,[3,11–14] who compared isolates causing prostatitis, cystitis and pyelonephritis, and found that isolates from patients with prostatitis were significantly more likely to express hemolysin than those causing complicated UTI. Mitsumori et al found hemolysin in 65% of 107 prostatitis strains studied,[15] which is a percent similar to that found in our study (63%). Our data confirm that the tropism and invasiveness of E. coli strains for the prostate rely mainly on hemolysin, but also offer a possible explanation for the persistence of such strains in the prostatic secretory system by their increased ability to form biofilm.

Our data also agree with previous studies showing that isolates collected from urine had a higher number of virulence factors and greater capacity to form in vitro biofilm than those collected from feces.[11] A reason for this biofilm ability could be the difference in type 1 fimbriae expression, which is lower in E. coli isolates from feces ($p < 0.00001$). In fact, current data indicate that in UPEC type 1 fimbriae expression is independently associated with biofilm formation. It has been previously reported that most E. coli isolates collected from feces belong to phylogenetic groups A and B1 with phylogenetic groups B2 and D being most frequently isolated in urine. Differences in the phylogenetic background of these 2 groups of isolates from urine and feces indicate that the prostate was not colonized by commensal bacteria from the intestinal tract in most cases.

It is currently recommended that treatment for bacterial acute prostatitis should be continued up to 6 weeks to prevent relapse. This approach may be justified based on the high frequency of biofilm formation in E. coli strains causing prostatitis. An interesting question deserving further investigation is whether the detection of biofilm production could be useful for selecting which patients may require prolonged or short therapeutic regimens.

Abbreviations and Acronyms

Nal-R	=	nalidixic acid resistant strains
PCR	=	polymerase chain reaction
PT	=	prostatitis
UPEC	=	uropathogenic Escherichia coli
UTI	=	urinary tract infection

REFERENCES

1. Costerton JW, Lewandowski Z, Caldurell DE, Korber DR and Lappin-Scott HM: Microbial biofilms. Annu Rev Microbiol 1995; **49:** 711.
2. Velasco M, Horcajada JP, Mensa J, Moreno-Martínez A, Vila J, Martínez JA et al: Decreased invasive capacity of quinolone-resistant Escherichia coli in patients with urinary tract infections. Clin Infect Dis 2001; **33:** 1682.
3. Vila J, Simon K, Ruíz J, Horcajada JP, Velasco M, Barranco M et al: Are quinolone-resistant uropathogenic Escherichia coli less virulent? J Infect Dis 2002; **186:** 1039.
4. Martinez JA, Nicolas JM, Marco F, Horcajada JP, Garcia-Segarra G, Trilla A et al: Comparison of antimicrobial cycling and mixing strategies in two medical intensive care units. Crit Care Med 2006; **34:** 329.
5. Performance Standards for Antimicrobial Susceptibility Testing—9th Informational Supplement: Approved Standard M100-S9. Wayne, Pennsylvania: National Committee for Clinical Laboratory Standards 1999.
6. Clermont O, Bonacorsi S and Bingen E: Rapid and simple determination of the Escherichia coli phylogenetic group. App Environ Microbiol 2000; **66:** 4555.
7. Danese PN, Pratt LA, Dove SL and Kolter R: The outer membrane protein, antigen-43, mediates cell-to-cell interactions within Escherichia coli biofilms. Mol Microbiol 2000; **37:** 424.
8. Kravchick S, Cytron S, Agulansky L and Ben-Dor D: Acute prostatitis in middle-aged men: a prospective study. BJU Int 2004; **93:** 93.
9. Hanna A, Berg M, Stout V and Razatos A: Role of capsular colanic acid in adhesion of uropathogenic Escherichia coli. App Environ Microbiol 2003; **69:** 4474.
10. Wu XR, Sun TT and Medina JJ: In vitro binding of type-1 fimbriated Escherichia coli to uroplakins Ia and Ib: relation to urinary tract infection. Proc Natl Acad Sci U S A 1996; **93:** 9631.
11. Andreu A, Stapleton AE, Fennell C, Lockman HA, Xercavins M, Fernandez F et al: Urovirulence determinants in Escherichia coli strains causing prostatitis. J Infect Dis 1997; **176:** 464.
12. Terai A, Yamamoto S, Mitsumori K, Okada Y, Kurazono H, Takeda Y et al: Escherichia coli virulence factors and serotypes in acute bacterial prostatitis. Int J Urol 1997; **4:** 289.
13. Ruiz J, Simon K, Horcajada JP, Velasco M, Barranco M, Roig G et al: Differences in virulence factors among clinical isolates of Escherichia coli causing cystitis and pyelonephritis in women and prostatitis in men. J Clin Microbiol 2002; **40:** 4445.
14. Johnson JR, Kuskowski MA, Gajewski A, Soto S, Horcajada JP, Jimenez de Anta MT et al: Extended virulence genotypes and phylogenetic background of Escherichia coli isolates from patients with cystitis, pyelonephritis, or prostatitis. J Infect Dis 2005; **191:** 46.
15. Mitsumori K, Terai A, Yamamoto S, Ishitoya S and Yoshida O: Virulence characteristics of Escherichia coli in acute bacterial prostatitis. J Infect Dis 1999; **180:** 1378.

ARTICULO 2

IMPLICATION OF BIOFILM FORMATION IN THE PERSISTENCE OF URINARY TRACT INFECTION CAUSED BY UROPATHOGENIC *ESCHERICHIA COLI*

IMPLICACIÓN DE LA FORMACIÓN DE BIOPELÍCULAS EN LA PERSISTENCIA DE INFECCIÓN DEL TRACTO URINARIO CAUSADA POR *ESCHERICHIA COLI*

S.M. Soto, A. Smithson, J.P. Horcajada, J.A. Martínez, J.P. Mensa, J. Vila.

Clinical Microbiology and Infection 2006; 12:1034-1036.

RESUMEN

E. coli es el germen más frecuentemente implicado en las infecciones del tracto urinario (ITUs). Las ITUs agudas causadas por cepas uropatógenas de *E. coli* pueden producir infecciones recurrentes, que se pueden definir como reinfecciones o recidivas. En este estudio se han analizado cepas de *E. coli* causantes de recidivas (n = 27) y de reinfecciones (n = 53). Hemos encontrado una producción *in vitro* de biopelícula, yersiniobactina y aerobactina significativamente más frecuente entre las cepas causantes de recidivas. El análisis de la producción de biopelícula podría ser una herramienta útil para seleccionar aquellas pacientes que requieran un tratamiento encaminado a erradicar cepas persistentes de *E. coli* productoras de biopelícula a fin de prevenir la aparición de recidivas.

RESEARCH NOTE

Implication of biofilm formation in the persistence of urinary tract infection caused by uropathogenic *Escherichia coli*

S. M. Soto[1], A. Smithson[2], J. P. Horcajada[2], J. A. Martinez[2], J. P. Mensa[2] and J. Vila[1]

[1]Microbiology Department and [2]Infectious Diseases Department, Hospital Clinic, Barcelona, Spain

ABSTRACT

Escherichia coli is the most frequent microorganism involved in urinary tract infection (UTI). Acute UTI caused by uropathogenic *E. coli* (UPEC) can lead to recurrent infection, which can be defined as either re-infection or relapse. *E. coli* strains causing relapse (n = 27) and re-infection (n = 53) were analysed. In-vitro production of biofilm, yersiniabactin and aerobactin was significantly more frequent among strains causing relapse. Biofilm assays may be helpful in selecting patients who require a therapeutic approach to eradicate persistent biofilm-forming *E. coli* strains and prevent subsequent relapses.

Keywords Aerobactin, biofilm formation, *Escherichia coli*, relapse, urinary tract infection, yersiniabactin

Original Submission: 22 February 2006; **Revised Submission:** 3 May 2006; **Accepted:** 4 May 2006

Clin Microbiol Infect 2006; 12: 1034–1036
10.1111/j.1469-0691.2006.01543.x

Escherichia coli is the most frequent cause of urinary tract infection (UTI). Uropathogenic *E. coli* (UPEC) strains have a number of virulence factors that increase their ability to colonise and persist in the urogenital tract [1]. Acute UTI caused by UPEC can lead to recurrent infection, which is defined as 're-infection' when it involves a strain other than that causing the original infection, or as 'relapse' when it is caused by the same strain as that involved in the original UTI. Approximately 25% of women with an episode of acute cystitis later develop recurrent UTI, which represents a substantial burden to the healthcare system. Consequently, studies are underway to elucidate the factors predisposing to recurrent UTI in order to develop effective methods of prevention and therapy [2]. In the present study, patients were followed prospectively for several months in order to determine the nature of any recurrence. The *E. coli* strains isolated were analysed to determine any possible relationships among relapse/re-infection, biofilm formation and the presence of virulence factors.

In total, 43 ambulatory female patients aged >18 years were included in the study following an index episode of UTI (cystitis or acute pyelonephritis), irrespective of any history of recurrent UTI. Women with renal or hepatic insufficiency, and those receiving immunosuppressive therapy, were excluded. The patients were followed clinically for at least 6 months, with urine cultures every month. Urine samples were analysed in the Clinical Microbiology Laboratory of the Hospital Clinic, Barcelona, Spain. Eighty urine samples positive for *E. coli* were included in this study. Clinical variables recorded were: presence of urinary incontinence; diabetes mellitus; indwelling urethral catheter; renal insufficiency and menopause; history of renal colic; urinary tract abnormalities; previous UTI or urinary instrumentation; and exposure to antibiotics in the 3-month period before the index infection. Urinary tract abnormalities included bladder diverticuli, cystocele, congenital malformations, stones and renal cyst, as well as functional disorders such as neurogenic bladder and vesicoureteral reflux.

The UPEC isolates collected from each patient were analysed by repetitive extragenic palindromic (REP)-PCR [3] and pulsed-field gel electrophoresis of chromosomal DNA digested with *Xba*I [4] to distinguish between re-infection and relapse. Isolates with the same REP-PCR and pulsed-field gel electrophoresis fingerprint patterns were considered to be the same strain. Virulence factors were detected by PCR using gene-specific primers [5] for haemolysin (*hlyA*), cytotoxic necrotising factor-1 (*cnf1*), toxin autotransporter (*sat*), type 1 fimbriae (*fimA*), yersiniabactin (*fyuA*), aerobactin (*aer*), S-fimbriae (*sfaS*), P-fimbriae (*papA*, C, G, EF and *prs*) and Ag43 (*flu*). Detection of biofilm production was based on a protocol described

Corresponding author and reprint requests: J. Vila, Department of Microbiology, Hospital Clinic of Barcelona, Villarroel 170, 08036 Barcelona, Spain
E-mail: jvila@ub.edu

previously [6]. The strains were grown overnight in Luria–Bertani broth [7] at 37°C without shaking. A 1.25-μL aliquot of an overnight culture was then subcultured in 125 μL of M63 medium [7] containing Luria–Bertani broth 1% v/v in a well of a polystyrene microtitre plate and incubated at 30°C overnight without shaking. A 1.25-μL of each culture was then subcultured in 125 μL of M63 medium in a new polystyrene microtitre plate, and re-incubated as described above. After 24 h, the culture was removed from the plate and the biofilm was stained with 175 μL of crystal violet for 1 min, washed with phosphate-buffered saline, and air-dried for *c.* 1 h. The retained stain was solubilised in dimethylsulphoxide and the absorbance was measured at 550 nm. A strain was considered to be positive for biofilm production when the absorbance was greater than four-fold the value for a control well without bacteria.

Proportions and means were compared using the chi-square test and *t*-test. Logistic regression was used to identify factors associated independently with relapse or re-infection. Two logistic models were constructed: the first, not including clinical characteristics, in which the entire collection of *E. coli* strains was considered; and the second, including clinical characteristics, in which only the isolate causing the index episode of UTI was considered.

During the study period, 80 unrelated *E. coli* strains were collected from 43 females with recurrent UTI. Twenty-four patients had the same *E. coli* strain involved in at least one recurrent episode, while 19 patients were infected by different *E. coli* strains in each episode. Hence, 27 *E. coli* strains were considered to cause relapse, and 53 were categorised as causing re-infection. The mean age of the patients was 48.3 ± 20.5 (range 19–89) years, and 19 (44%) were post-menopausal. Twenty-seven (63%) patients had at least one episode of symptomatic UTI before enrolment in the study, six (14%) had a history of renal colic, two (5%) had been subject to urinary instrumentation, 16 (37%) had a urinary tract abnormality, 22 (51%) had some degree of incontinence, and four (9%) suffered from diabetes mellitus. Thirty-three (77%) patients had been exposed to antibiotics within 3 months of the index UTI, but none had renal insufficiency or an indwelling urethral catheter.

The prevalence of in-vitro biofilm formation and virulence factors among strains causing relapse and re-infection is shown in Table 1.

Table 1. Distribution of virulence characteristics among *Escherichia coli* isolates causing relapse or re-infection

Characteristic	Relapse (n = 27) No. (%)	Re-infection (n = 53) No. (%)	p
Biofilm-positive	20 (74)	22 (42)	0.005
flu	15 (56)	32 (60)	0.67
hlyA	7 (26)	19 (36)	0.37
cnf1	5 (19)	13 (25)	0.54
sat1	8 (30)	13 (25)	0.62
fimA	24 (89)	47 (89)	0.97
fyu	20 (74)	27 (51)	0.04
aer	20 (74)	27 (51)	0.04
sfaS	9 (33)	11 (21)	0.21
papA	10 (37)	21 (40)	0.8
papC	13 (48)	24 (45)	0.8
papG	7 (26)	17 (32)	0.6
papEF	14 (52)	22 (42)	0.37
prs	7 (26)	8 (15)	0.3

Three characteristics were significantly more frequent among strains causing relapses, namely in-vitro biofilm formation (p 0.005), the presence of a yersiniabactin (*fyu*) gene and the presence of an aerobactin (*aer*) gene (both p 0.04). Logistic regression selected only biofilm formation (OR 4.96, 95% CI 1.65–14.9) and the presence of a yersiniabactin gene (OR 3.6, 95% CI 1.18–11) as factors that were associated independently with strains involved in relapse.

When the analysis was restricted to the strains involved in the index episode of UTI, again only in-vitro biofilm production (OR 11.4, 95% CI 2–64.6) and the presence of a yersiniabactin gene (OR 6.37, 95% CI 1.05–38.7) were associated independently with relapse. In this respect, none of the patient characteristics seemed to be important.

Recurrent UTIs are common among young, healthy women, despite the fact that they generally have anatomically and physiologically normal urinary tracts [8]. The data from the present study indicate that the only factors associated consistently with UTI relapse in women are of microbial origin, i.e., the capacity to form biofilm *in vitro* and the presence of a gene for yersiniabactin. Recurrence has been associated previously with several virulence determinants present in UPEC, and with behavioural or other factors that facilitate vaginal colonisation or entry of colonising uropathogens into the bladder. However, Mulvey *et al*. [9] demonstrated that uropathogens can persist within the bladder tissue in underlying epithelial cells and may be a source of recurrent UTI. Anderson *et al*. [10] observed that intracellular bacteria mature into biofilms, creating pod-like bulges on the bladder surface. This bacterial structural organisation may explain the persist-

ence of bladder infections despite robust host defences. Yersiniabactin and aerobactin, two virulence factors related to iron-uptake systems, have also been associated with relapse [11]; the present data confirmed these observations, which could be related to the need of the bacteria to capture iron for growth in a stressful environment. However, biofilm production may be the key determinant for the persistence of UPEC in either the vaginal reservoir, the bladder epithelial cells or both. An in-vitro biofilm assay could therefore be useful in clinical practice to select patients who may require a therapeutic approach directed at erradicating persistent biofilm-forming *E. coli* strains in order to prevent subsequent relapses.

ACKNOWLEDGEMENTS

This work was supported by grants FIS 02/0327 and FIS 02/0453 from the Ministry of Health, Spain, and 2005 SGR00444 from the Departament d'Universitats, Recerca I Societat de la Informació de la Generalitat de Catalunya, Spain (to J.V.). S. M. Soto is a fellow of Fondo de Investigación Sanitaria of the Spanish Health and Consumption Ministry (BEFI BF03/00037).

REFERENCES

1. Svanborg C, Goldaly G. Bacterial virulence in urinary tract infection. *Infect Dis Clin North Am* 1997; **11**: 513–529.
2. Madersbacher S, Thalhammer F, Marberger M *et al.* Pathogenesis and management of recurrent urinary tract infection in women. *Curr Opin Urol* 2000; **10**: 29–33.
3. Vila J, Marcos MA, Jiménez de Anta MT. A comparative study of different PCR-based DNA fingerprinting techniques for typing of the *Acinetobacter calcoaceticus–A. baumannii* complex. *J Med Microbiol* 1996; 44: 482–489.
4. Gautom RK. Rapid pulsed-field gel electrophoresis protocol for typing of *Escherichia coli* O157:H7 and other gram-negative organisms in 1 day. *J Clin Microbiol* 1997; **35**: 2977–2980.
5. Johnson JR, Stell A. Extended virulence genotypes of *Escherichia coli* strains from patients with urosepsis in relation to phylogeny and host compromise. *J Infect Dis* 2000; **181**: 261–272.
6. Danese PN, Pratt LA, Dove SL, Kolter R. The outer membrane protein, antigen-43, mediates cell-to-cell interactions within *Escherichia coli* biofilms. *Mol Microbiol* 2000; **37**: 424–432.
7. Silhauy TJ, Berman ML, Enquist LW. *Experiments with gene fusions*. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press, 1984; 217–219.
8. Finer G, Landau D. Pathogenesis of urinary tract infections with normal female anatomy. *Lancet Infect Dis* 2004; 4: 631–635.
9. Mulvey MA, Schilling JD, Martínez JJ, Hultgren SJ. Bad bugs and beleagured bladders: interplay between uropathogenic *Escherichia coli* and innate host defenses. *Proc Natl Acad Sci USA* 2000; **97**: 8829–8835.
10. Anderson GG, Palermo JJ, Schilling JD, Roth R, Heuser J, Hultgren SJ. Intracellular bacterial biofilm-like pods in urinary tract infection. *Science* 2003; **301**: 105–107.
11. Johnson JR, O'Bryan TT, Delavari P *et al.* Clonal relationships and extended virulence genotypes among *Escherichia coli* isolates from women with a first or recurrent episode of cystitis. *J Infect Dis* 2001; **183**: 1508–1517.

ARTICULO 3

EXPRESSION OF INTERLEUKIN-8 RECEPTORS (CXCR1 AND CXCR2) IN PREMENOPAUSAL WOMEN WITH RECURRENT URINARY TRACT INFECTIONS

EXPRESION DE RECEPTORES DE INTERLEUCINA 8 (CXCR1 Y CXCR2) EN MUJERES PREMENOPÁUSICAS CON INFECCIÓN URINARIA RECURRENTE

A. Smithson, M.R. Sarrias, J. Barceló, B. Suárez, J.P. Horcajada, S.M. Soto, A. Soriano, J. Vila, J.A. Martínez, J. Vives, J. Mensa, F. Lozano.

Clinical and Diagnostic Laboratory Immunology 2005; 12: 1358-1363.

RESUMEN

La migración de los polimorfonucleares (PMN) a través de los tejidos infectados está mediada por las quimiocinas CXC y de sus receptores (CXCR1 y CXCR2). Se ha propuesto que los niños con déficit de expresión de CXCR1 tienen un mayor riesgo de desarrollar pielonefritis aguda. Los objetivos del estudio han sido evaluar la expresión en la superficie de los PMN de CXCR1 y de CXCR2 y la presencia de polimorfismos en el gen *CXCR1* en mujeres premenopáusicas con infecciones urinarias recurrentes. Se han incluido 20 mujeres premenopáusicas con infecciónes urinarias recurrentes, sin anomalías del tracto urinario ni enfermedades potencialmente asociadas con infecciones urinarias recurrentes, y 30 controles sanos sin antecedentes de infecciones urinarias previas. Los niveles de expresión de CXCR1 y de CXCR2 se han cuantificado mediante citometría de flujo midiendo el canal de intensidad de fluorescencia media (IFM). Se ha utilizado la metodología SBT (tipaje basado en la secuencia) para evaluar la presencia de polimorfismos en el promotor y en las regiones codificantes del gen *CXCR1*. Las pacientes con infecciones urinarias recurrentes presentaban niveles medios de IFM del CXCR1 similares a la de los controles. Sin embargo los niveles de expresión medios de IFM del CXCR2 hallados en estas pacientes han sido inferiores a los encontrados en los controles sanos sin infecciones urinarias previas (P = 0.002, test de U de Mann-Whitney). No hemos encontrado polimorfismos a nivel del promotor ni del exón 1 del gen *CXCR1* ni en los pacientes ni en los controles. Hemos observado polimorfismos a nivel del exón 2 del gen *CXCR1*, con igual frecuencia entre pacientes y controles. Como conclusión, hemos encontrado unos niveles de expresión de CXCR2 bajos en las pacientes con infecciones urinarias recurrentes. Estos resultados sugieren que las mujeres premenopáusicas con niveles de expresión bajos de CXCR2 podrían tener una mayor susceptibilidad a infecciones del tracto urinario.

CLINICAL AND DIAGNOSTIC LABORATORY IMMUNOLOGY, Dec. 2005, p. 1358–1363 Vol. 12, No. 12
1071-412X/05/$08.00+0 doi:10.1128/CDLI.12.12.1358–1363.2005

Expression of Interleukin-8 Receptors (CXCR1 and CXCR2) in Premenopausal Women with Recurrent Urinary Tract Infections

Alex Smithson,[1]* Maria Rosa Sarrias,[2] Juanjo Barcelo,[2] Belen Suarez,[2] Juan Pablo Horcajada,[1] Sara Maria Soto,[3] Alex Soriano,[1] Jordi Vila,[3] Jose Antonio Martinez,[1] Jordi Vives,[2] Jose Mensa,[1] and Francisco Lozano[2]

Infectious Diseases Unit,[1] Department of Immunology,[2] and Department of Microbiology,[3] Hospital Clínic, IDIBAPS, University of Barcelona, Barcelona, Spain

Received 3 August 2005/Returned for modification 25 August 2005/Accepted 29 September 2005

The migration of neutrophils through infected tissues is mediated by the CXC chemokines and its receptors (CXCR1 and CXCR2). It has been proposed that a CXCR1 deficiency could confer susceptibility to acute pyelonephritis in children. The objective of the study is to assess the surface expression of CXCR1 and CXCR2 and the existence of polymorphisms in the *CXCR1* gene in premenopausal women with recurrent urinary tract infections. The study included 20 premenopausal women with recurrent urinary infections, with normal urinary tracts, and without diseases potentially associated with relapsing urinary infections and 30 controls without previous urinary infections. The levels of CXCR1 and CXCR2 expression on neutrophils were measured and analyzed by flow cytometry by measuring the mean fluorescence intensity (MFI) channel. The promoter and coding regions of the *CXCR1* gene were analyzed for the presence of polymorphisms by a sequence-based typing method. Patients with recurrent urinary tract infections exhibited median levels of CXCR1 expression, determined from MFI values, similar to those of the controls. The analysis of CXCR2 showed that patients with recurrent urinary infections had lower median levels of expression, determined from the MFI values, than the controls ($P = 0.002$, Mann-Whitney U test). No polymorphisms were detected at the promoter or at the exon 1 region of the *CXCR1* gene either in the patients or in the controls. Polymorphisms were detected at the exon 2 of *CXCR1*, but their frequencies did not differ between patients and controls. We have found a low level of CXCR2 expression in patients with recurrent urinary tract infections. These results suggest that a low level of CXCR2 expression may increase the susceptibilities of premenopausal women to urinary tract infections.

Recurrent urinary tract infections (RUTIs) are frequent among healthy young women who generally have anatomically and physiologically normal urinary tracts (23). It has been estimated that each year in the United States about 6 million to 8 million young women have acute cystitis (20). Moreover, about 25% of these women who have had an initial infection will experience RUTIs, resulting in considerable morbidity and the associated health care costs (25). There is no satisfactory theory to explain the predisposition of some young healthy women with normal urinary tracts to RUTIs, but it seems to be the result of the combination of various factors inherited by the host and several behavioral conditions (5).

In response to infection with uropathogenic *Escherichia coli*, the most common pathogen involved in community-acquired urinary tract infections (UTIs) (28), human uroepithelial cells secrete interleukin-8 (IL-8) as well as other members of the CXC cytokine family, which are potent neutrophil chemotactic and activating peptides (15, 10, 16). Neutrophils respond to the CXC cytokine gradient by leaving the general circulation and crossing the epithelial barrier into the lumen of the urinary tract, where they contact and phagocytose uropathogenic bacteria. In humans, the CXC chemokines mediate their biologic activities by binding to two specific seven-span transmembrane receptors coupled to G proteins, CXCR1 and CXCR2. These receptors are expressed on the surfaces of neutrophils and are encoded by two single-copy genes located at chromosome 2q35 (12). They share 78% of their amino acid sequences and bind to the IL-8 chemokine with different affinities. CXCR1 is highly specific for IL-8, while CXCR2 is more promiscuous and binds to IL-8 as well as to other CXC chemokines containing a common amino-terminal Glu-Leu-Arg (ELR) sequence, including the epithelial cell-derived neutrophil-activating protein 78 (ENA-78), growth-related oncogene alpha (GRO-α), GRO-β, GRO-γ, and lipopolysaccharide-induced CXC chemokine (13, 1). The importance of the IL-8 receptors in the process of transepithelial migration of neutrophils has been demonstrated in an experimental UTI model in which IL-8 receptor homologue-knockout mice were unable to clear an *E. coli* infection. Mice lack CXCR1, and CXCR2 exclusively mediates the granulocyte response to ELR-positive chemokines, e.g., macrophage inflammatory protein 2, cytokine-induced neutrophil chemoattractant, and ENA-78 (3). In these IL-8 receptor homologue-knockout mice, neutrophils fail to cross the epithelium, accumulate in the subepithelial tissue, and cause renal scarring and, eventually, end-stage renal disease (9, 29). Additional evidence of the importance of the IL-8 receptors in the neutrophil migration process comes from the observation of the absence of neutrophil recruitment to the urinary tract after the administration of a blocking IL-8 receptor homologue antibody in mice (14).

* Corresponding author. Mailing address: Infectious Diseases Unit, Hospital Clinic, IDIBAPS, Edificio Helios, 1Ş planta. C/ Villarroel 170, 08036 Barcelona, Spain. Phone: 34-93 227 54 00. Fax: 34-93 227 93 22. E-mail: asa30412@hotmail.com.

TABLE 1. Summary of clinical findings for patients with RUTIs included in the study[a]

Patient no.	Age (yr) at time of study	Age (yr) at time of first UTI	Age (yr) of first intercourse	Total no. of UTIs in lifetime	No. of cases of AP	MFI value		CXCR1 exon 2 SNP
						CXCR1	CXCR2	
1	26	≤15	16	≥10	7	58.91	4.57	ND
2	25	≤15	17	≥10	2	49.43	6.05	R335C
3	26	≤15	19	5–9	2	68.25	14.56	S276T
4	21	≤15	N	≥10	1	203.12	30.17	ND
5	36	≤15	20	≥10	7	108.48	13.34	ND
6	24	≤15	18	≥10	2	126.41	15.53	ND
7	24	≤15	16	≥10	7	164.01	16.51	ND
8	21	≤15	N	≥10	1	174.02	17.35	ND
9	23	≤15	18	4	3	112.14	12.65	ND
10	27	24	20	≥10	0	80.86	14.74	ND
11	26	25	24	≥10	1	115.5	15.22	ND
12	21	17	17	≥10	0	143.56	14.77	ND
13	32	31	20	4	1	125.27	18.74	ND
14	39	22	17	≥10	0	172.94	33.33	ND
15	36	26	18	≥10	1	171.03	17.41	ND
16	26	16	20	≥10	0	106.77	15.16	ND
17	23	22	21	5–9	1	103.29	9.78	ND
18	35	23	23	≥10	1	122.31	12.57	ND
19	34	21	20	4	2	127.32	16.87	ND
20	22	19	13	5–9	4	102.45	13.77	S276R

[a] AP, acute pyelonephritis; SNP, single-nucleotide polymorphism; N, not started; ND, not detected.

It has been proposed that the increased susceptibility to UTIs observed in IL-8 receptor-knockout mice could have a human counterpart. Frendeus et al. have demonstrated that neutrophils from children prone to acute pyelonephritis show decreased levels of expression of CXCR1 but not CXCR2 compared with those of age-matched controls, which may explain their susceptibilities to recurrent acute pyelonephritis (6). In addition, preliminary results suggest that the low level of expression of CXCR1 could be related to polymorphisms in the promoter region of the *CXCR1* gene (7). Therefore, the confirmation of a deficient expression or function of the CXC receptors in humans could provide new genetic clues to explain individual susceptibilities to UTIs.

To our knowledge the expression of CXC receptors in adults with RUTIs has not been previously assessed. The objective of the present study was to investigate the surface expression of CXCR1 and CXCR2 on human neutrophils as well as the presence of single-nucleotide polymorphisms in the *CXCR1* gene from premenopausal women with RUTIs and from a healthy control group.

MATERIALS AND METHODS

Study population. The study group included 20 premenopausal women with a history of RUTIs and normal urinary tracts identified from the Infectious Diseases Outpatient Unit between January 2002 and January 2003. For further comparison, 30 volunteer premenopausal females who were not matched with the patients and who did not have a history of UTIs were also studied. The present study was conducted with the approval of the hospital Ethics Committee and the informed consent of all participants. The human experimentation guidelines of the U.S. Department of Health and Human Services and those of the authors' institution were followed in the conduct of the clinical research.

Women were considered to meet the case definition for RUTIs if they had experienced either three or more symptomatic UTIs in the past year or two such episodes in the past 6 months. A UTI was defined by bacterial growth $\geq 10^5$ CFU/ml in a culture of midstream urine from a women experiencing two or more symptoms of cystitis (dysuria, urgency, frequency, suprapubic pain, or hematuria) or, in the absence of culture, the demonstration of pyuria on urine analysis and two or more urinary tract symptoms, as well as the complete and rapid resolution of symptoms after the start of antibiotic therapy. Acute pyelonephritis was clinically defined as an armpit temperature of >38°C, pyuria, and lumbar tenderness.

Premenopausal women with a history of urological abnormalities or urological manipulation or in which the imaging evaluation (echography and pyelography) had demonstrated urological abnormalities potentially related to RUTIs were excluded. Patients with diseases associated with RUTIs (diabetes mellitus, cirrhosis, immunosuppressive treatments, transplant recipients) were also excluded.

Patient samples. Samples were obtained during an infection-free interval. Blood samples were collected by venipuncture into Vacutainers (Becton Dickinson) with EDTA anticoagulant. The blood samples were immediately transported on ice to the laboratory and analyzed by flow cytometry within 1 to 6 h of collection.

Reagents. Phycoerythrin (PE)-labeled mouse immunoglobulin G2a (IgG2a) monoclonal antibodies to human CXCR1 (clone 42705.111) and CXCR2 (clone 48311.211) were from R&D Systems Inc. (Minneapolis, MN). Fluorescein isothiocyanate (FITC)-labeled mouse IgG1 monoclonal antibody to human CD45 (clone 2D1) was from BD Biosciences (San Jose, CA). FITC-labeled mouse IgG2a (clone X39) and PE-labeled mouse IgG1 were from BD Biosciences.

Immunofluorescence and flow cytometry analysis. Direct immunofluorescence staining of whole blood was performed by using a lysis, no-wash procedure, according to the manufacturer's instructions (BD Biosciencies). Briefly, 50 μl of EDTA-anticoagulated whole blood was mixed with 5 to 20 μl fluorochrome-conjugated monoclonal antibodies (PE-labeled anti-CXCR1 and anti-CXRC2 or FITC-labeled anti-CD45) and incubated for 15 min in the dark at room temperature. As negative controls, additional samples were incubated with PE-labeled mouse IgG2a or FITC-labeled mouse IgG1. The staining with FITC-labeled anti-CD45 monoclonal antibody, which recognizes the common leukocyte antigen present on neutrophils, monocytes, and lymphocytes, was used as an internal control to verify the integrity of the target cell population among different individuals and to exclude fragments of red blood cells. Then, 450 μl of fluorescence-activated cell sorter lysing solution was added by gentle vortexing, and the mixture was left for an additional 15 to 30 min in the dark at room temperature before analysis on a FACScan flow cytometer (Becton Dickinson, Mountain View, CA) equipped with an argon laser (excitation wavelength, 488 nm). The data were analyzed with 1.0 CellQuest software (Becton Dickinson). Forward and side light-scatter parameters were used for the gating of the neutrophil population. A histogram of fluorescence distribution was constructed, and the relative mean fluorescence intensity (MFI) was obtained from the histogram and expressed as an index of membrane surface expression.

Sequence-based typing. Genomic DNA was extracted from peripheral blood by using a commercially available kit (QIAamp blood DNA isolation kit; QIAGEN, Hilden, Germany), following the manufacturer's instructions. Specific primers

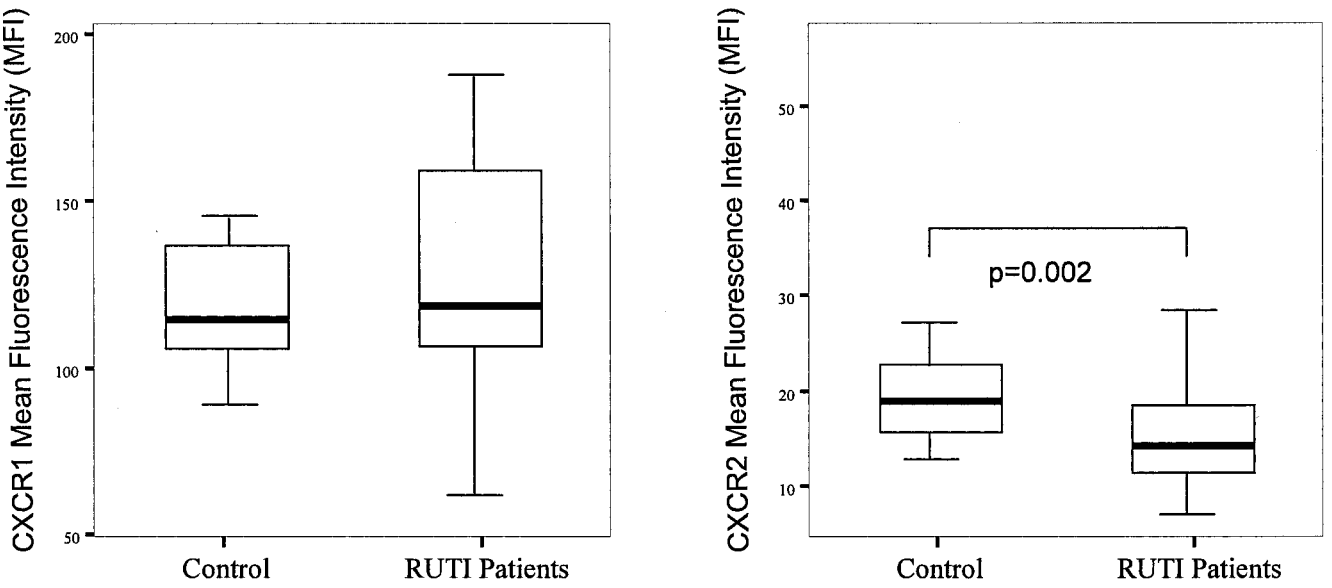


FIG. 1. CXCR1 and CXCR2 staining of neutrophils from healthy controls and RUTI patients. The corresponding MFI values are shown. Data are represented as medians (horizontal bar), 25th and 75th percentiles (boxes), and 10th and 90th percentiles (bars). Significant differences between groups are indicated (Mann-Whitney U test).

were designed for PCR amplification of the promoter and the coding regions of the *CXCR1* gene, according to the published genomic DNA sequences (GenBank accession number L19592) (24). A 519-bp fragment encompassing the promoter region and exon 1 was generated by using primers IL8RAPro.Fw (5′-GAAGTCTCTGCTGTAAGTCA-3′) and IL8RAPro.Rv (5′-CTCAGCTCC CACAGAAATG-3′). The whole coding region of exon 2 (a 1,203-bp fragment), from the end of intron 1 to beyond the natural stop codon, was generated by using primers IL8RAInt1.Fw (5′-GCCTTGAATCCGAGCTACTAAAT-3′) and IL8RAEx2.Rv (5′-CCTCAGGGTGTTGGTTATTCTT-3′). The PCR was performed in a final volume of 50 μl containing 100 to 500 ng of genomic DNA, 2 pmol/ml of specific primers, 0.5 to 2 U *Taq* Expand DNA polymerase (Boehringer Mannheim), 2 mM $MgCl_2$, 5 mM deoxynucleoside triphosphates, and 10 mM Tris-HCl (pH 9.5). The cycling conditions were 8 min at 98°C; 35 cycles of 30 s at 94°C, 30 s at 58°C, and 90 s at 72°C; and a final extension step of 10 min at 72°C. Samples (1 to 2 μl) of the resulting PCR products were subjected to direct sequencing by the dye-terminator method (ABI PRISM dRhodamine Terminator cycle sequencing ready reaction kit) and analyzed with an automated ABI 3100 DNA sequencer (Applied Biosystems). The sequencing primers for the promoter-exon 1 and exon 2 fragments were IL8RAPro.Fw (see above) and IL8RA6072.Fw (5′-GAGGTCCTGGGAAATGACAC-3′), respectively.

Statistical analysis. The levels of expression of CXCR1 and CXCR2 on neutrophils from women with RUTIs and from healthy controls were compared by the Mann-Whitney U test. Values are expressed as medians (25th and 75th percentiles). Statistical significance was defined as a two-tailed P value of <0.05. Statistical analysis was carried out by the program SPSS (version 11.0; SPSS, Inc., Chicago, IL).

RESULTS

Clinical data. The study group was composed of 20 premenopausal women with RUTIs (median age, 26 years; age range, 21 to 39 years) and 30 premenopausal female volunteers who had never experienced an UTI (median age, 27 years; age range, 18 to 46 years). The main clinical findings for the premenopausal patients with RUTIs are shown in Table 1. Nine of the 20 patients with RUTIs had experienced their first UTI before the age of 15 years. The lifetime number of UTIs was ≥10 in 65% of the women with RUTIs. By consideration of the total number of acute pyelonephritis episodes of each patient during their lives, 11 patients (55%) had experienced cystitis with or without one episode of acute pyelonephritis and 9 patients (45%) had experienced cystitis with more than one episode of acute pyelonephritis. The nine patients with an early onset of UTI had experienced a total of 32 episodes of acute pyelonephritis (74% of the total episodes of acute pyelonephritis of the study group), while the 11 patients with a late onset of UTI had experienced a total of 11 episodes of acute pyelonephritis.

Chemokine receptor expression. The surface expression of CXCR1 and CXCR2 was measured on neutrophils and expressed as arbitrary units of MFI. The values obtained for each premenopausal patient with RUTIs included in the study are represented in Table 1. The median MFI values (25th and 75th percentiles) of CXCR1 and CXCR2 in the healthy control group were 116.22 (103.67 and 139.46) and 18.94 (16.2 and 23.62), respectively. The median MFI values of CD45 were comparable between the patients and the healthy controls (18.38 [15.89 and 23.71]) for patients with RUTIs and 18.36 [16.62 and 23.41] for healthy controls for samples simultaneously stained for CXCR1; (19.22 [16.78 and 30.1] for patients with RUTIs and 18.84 [17.21 and 22.7] for the healthy controls for samples simultaneously stained for CXCR2). The median MFI value of CXCR1 observed in the premenopausal patients with RUTIs was 118.9 (102.66 and 158.89), similar to the median MFI values found for the healthy controls (Fig. 1). However, three patients with the onset of UTIs during childhood had CXCR1 MFI values below the 5th percentile of the CXCR1 MFI values for the healthy control group. The analysis of CXCR2 revealed that premenopausal women with RUTIs showed significantly lower median CXCR2 MFI values (14.96 [12.82 and 17.23]; $P = 0.002$) than the controls (Fig. 1). A deeper analysis in which the patients were classified by the age of onset of UTIs showed that although the median MFI values of CXCR1 (112.14 [63.58 and 169.01] for those with the onset of UTIs before the age of 15 years and 122.31 [103.29 and

143.56] for those with the onset of UTIs after the age of 15 years) and CXCR2 (14.56 [9.35 and 16.93] for those with the onset of UTIs before the age of 15 years and 15.16 [13.77 and 17.41] for those with the onset of UTIs after the age of 15 years) were lower in those premenopausal women with the early onset of UTIs, the differences were not statistically significant.

Analysis of CXCR1 polymorphisms. We have performed a sequence-based typing analysis of the promoter region and the coding region (exon 1 and exon 2) of the *CXCR1* gene in our series of patients and controls. We could not detect any polymorphisms in the promoter or the exon 1 region of either the patients or the controls. Sequence analysis of exon 2 (from the initiation methionine to the natural stop codon) revealed the presence of individuals heterozygous for two previously reported nonsynonymous polymorphisms (11, 27): G827C, which leads to the change of serine to threonine at position 276 (S276T), and C1003T, which leads to the change of arginine to cysteine at position 335 (R335C). Among the patients, one individual carried the S276T polymorphism and another one carried the R335C polymorphism. Interestingly, a third patient presented a new variant of the nonsynonymous polymorphism at position 276 (S276R), in which S276 (AGC) was replaced by R276 (AGG). The correlation between the MFI values of CXCR1 and the polymorphisms at exon 2 of the *CXCR1* gene of the RUTI patients included is shown in Table 1. Among the healthy controls, two individuals carried the S276T polymorphism and one individual carried the R335C polymorphism. The presence of two other previously reported synonymous polymorphisms (V247V and Y305Y) could not be detected in any of the individuals analyzed, either the patients or the controls.

DISCUSSION

Most recurring UTIs in women occur in healthy premenopausal individuals who do not have anatomical or functional abnormalities in the urinary tract. Numerous studies have suggested the presence of underlying host genetic factors to explain the increased susceptibilities of certain premenopausal women to RUTIs. Evidence of a genetic predisposition was initially proposed after the demonstration that women with RUTIs have increased adhesion of uropathogenic *E. coli* to vaginal and buccal epithelial cells compared with the level of adhesion for women without a history of recurrences (18). This observation suggested that differences in the density or expression of receptors to uropathogens in the mucosal epithelium of the urinary tract could be genetically determined, in part. Nowadays, it is well known that women with RUTIs are three to four times more likely to be nonsecretors of ABH blood group antigens than women without RUTIs and that vaginal epithelial cells from nonsecreting women express globoseries glysphingolipid receptors that bind to uropathogenic *E. coli* (22, 26). Additional evidence of the existence of a genetic predisposition to UTIs is the demonstration that specific HLA phenotypes are more prevalent in women with RUTIs than in women without such a history (19).

Defective expression of the IL-8 receptors CXCR1 and CXCR2 is another factor that may contribute to a genetic predisposition to RUTIs. Children prone to recurrent acute pyelonephritis show low levels of CXCR1 neutrophil surface expression compared to those for age-matched controls. This deficient CXCR1 expression could be the result of genetic polymorphisms in the promoter region of the *CXCR1* gene (6, 7). In our study, patients with RUTIs had a median CXCR1 MFI value comparable to that for the healthy controls, although the small number of patients included could have influenced these results. However, a closer analysis of the data revealed that three patients (patients 1, 2, and 3) with RUTIs had a median CXCR1 MFI value below the 5th percentile of the median MFI value for the healthy controls. These three patients began having UTIs before the age of 15 years, experienced their first sexual intercourse after that age, and have had more than one episode of acute pyelonephritis. The onset of UTIs before the age of 15 years, particularly before the first sexual intercourse, has been reported to be a risk factor for RUTIs, supporting the idea that inherited factors may be important in some women with relapsing UTIs (21). Therefore, our observation suggests that a deficient expression of CXCR1 could be implicated in the increased susceptibility to RUTIs in the subset of premenopausal women with an early onset of urinary tract infections through deficient neutrophil chemotaxis to the urinary tract.

An unexpected observation of our study is the low median level of MFI CXCR2 surface expression on neutrophils from premenopausal women with RUTIs compared to that on neutrophils from healthy controls. This lower level of surface expression was even more marked in the subgroup of premenopausal women in whom the onset of UTIs occurred before the age of 15 years, which again strengthens the idea that individual factors could be particularly important in the pathogenesis of RUTIs in women with the onset of urinary tract infections during childhood. It is interesting that the two patients (patients 1 and 2) with the lowest levels of CXCR2 surface expression also had very low levels of CXCR1 surface expression. Different studies have suggested that CXCR1 and CXCR2 are regulated by agonist-dependent mechanisms. Binding of IL-8 and ENA-78 rapidly downmodulates CXCR1 and CXCR2 due to internalization of the ligand-receptor complex (4). It is probable that IL-8, ENA-78, as well as other chemotactic cytokines regulate the expression of CXCR1 and CXCR2 through similar or identical pathways. Therefore, the expression of both receptors may be regulated in a similar way.

Neutrophil recruitment is driven not only by IL-8 but also by other chemotactic factors, such as ENA-78 and GRO; and thus, a low level of surface expression of a multiligand-specific receptor such as CXCR2 could cause a deficient neutrophil chemotaxis. Our data demonstrating the low level of expression of CXCR2 in premenopausal women with RUTIs suggests a handicapped capacity of GRO-α and ENA-78, and maybe of other CXCR2 binding chemokines, to exert their chemotactic activities. These data differ from those from previous in vitro studies that used kidney epithelial cell layers infected with *E. coli* and in which the addition of an anti-CXCR1 antibody but not an anti-CXCR2 antibody reduced *E. coli* transepithelial neutrophil migration (8). However, other studies have demonstrated a partial inhibition of neutrophil chemotaxis by using specific neutralizing antibodies directed against ENA-78 and GRO, which are CXCR2-dependent chemokines (14). Further studies are needed to address whether the CXCR2-deficient surface expression in the patients with RUTIs is caused by

specific gene defects or, alternatively, by upstream regulatory mechanisms.

In the second part of the study we screened the promoter and the entire coding region of the *CXCR1* gene in 20 premenopausal patients with RUTIs and in 30 healthy controls and detected three missense exchanges. Two of these have already been reported (11, 27). Although previous studies have suggested the existence of polymorphisms in the promoter region and exon 1 of the *CXCR1* gene in children prone to acute pyelonephritis, we were not able to detect such polymorphisms in either patients or healthy controls (7). The analysis of exon 2 has demonstrated that two patients were heterozygous for the two previously reported nonsynonymous polymorphisms, S276T and R335C of the mature protein. These two patients (patients 2 and 3) also had low levels of MFI CXCR1 surface expression (Table 1). Another patient (patient 20), who presented with the novel nonsynonymous S276R polymorphism, showed an average level of MFI CXCR1 surface expression in the flow cytometry analysis. In conclusion, no significant differences in the presence of single-nucleotide polymorphisms in the promoter and the coding regions (exons 1 and 2) of the *CXCR1* gene were detected between patients and controls. It is worth mentioning that the R335C polymorphism was observed in the only healthy control individual showing reduced surface expression of CXCR1. The same polymorphism was also observed in one of the three patients showing reduced surface expression of CXCR1.

The CXCR1 receptor is a member of the superfamily of G-protein-coupled receptors that consists of seven transmembrane domains, three intracellular loops, and three extracellular loops. The S276T and the S276R polymorphisms are located in the third extracellular loop, while the R335C polymorphism is located in the C-terminal tail of the *CXCR1* gene. The C-terminal tail of CXCR1 is necessary for receptor phosphorylation and desensitization (17), and the replacement of arginine by cysteine at position 335 may confer the potential of homo- or heterodimerization through disulfide bonds (2). Therefore, the R335C polymorphism has the potential to influence receptor functions by changing the secondary or tertiary structure of the receptor. The S276T and S276R variations are not expected to lead to major differences in receptor structure or function. Further studies are needed to evaluate the relevance of the R335C polymorphism on CXCR1 functions.

In conclusion, we have found no differences regarding the average level of neutrophil CXCR1 expression between premenopausal patients with RUTIs and healthy controls. However, three patients that began having UTIs during childhood presented low, below-average CXCR1 levels on the neutrophil surface. These low levels of CXCR1 on the neutrophil surface were not related to specific polymorphisms in the *CXCR1* gene. We have also found an average lower level of expression of CXCR2 in premenopausal women with RUTIs, particularly in those with an early onset of urinary tract infections, compared with that for the healthy controls. These results suggest that a low level of expression of CXCR2 could be associated with increased susceptibilities to RUTIs in certain patients, particularly in those premenopausal women with the onset of UTIs during childhood. The identification of these women at high risk of developing serious and repetitive urinary tract infections could justify the implementation of preventive antibiotic therapeutic strategies.

ACKNOWLEDGMENT

This work was supported by grants from the Fundacion Maximo Soriano.

REFERENCES

1. **Ahuja, S. K., and P. M. Murphy.** 1996. The CXC chemokines growth-regulated oncogene (GRO) alpha, GRObeta, GROgamma, neutrophil-activating peptide-2, and epithelial cell-derived neutrophil activating peptide-78 are potent agonists for the type B, but not the type A, human interleukin-8 receptor. J. Biol. Chem. **271:**20545–20550.
2. **Bockaert, J., and J. P. Pin.** 1999. Molecular tinkering of G protein-coupled receptors: an evolutionary success. EMBO J. **18:**1723–1729.
3. **Cacalano, G., J. Lee, K. Kikly, A. M. Ryan, S. PittsMeek, B. Hultgren, W. I. Wood, and M. W. Moore.** 1994. Neutrophil and B cell expansion in mice that lack the murine IL-8 receptor homolog. Science **265:**682–684.
4. **Chuntharapai, A., and K. J. Kim.** 1995. Regulation of the expression of IL-8 receptor A/B by IL-8: possible functions of each receptor. J. Immunol. **155:**2587–2594.
5. **Finer, G., and D. Landau.** 2004. Pathogenesis of urinary tract infections with normal female anatomy. Lancet Infect. Dis. **4:**631–635.
6. **Frendeus, B., G. Godaly, L. Hang, D. Karpman, A. C. Lundstedt, and C. Svanborg.** 2000. Interleukin 8 receptor deficiency confers susceptibility to acute experimental pyelonephritis and may have a human counterpart. J. Exp. Med. **192:**881–890.
7. **Godaly, G., G. Bergsten, L. Hang, H. Fischer, B. Frendeus, A. C. Lundstedt, M. Samuelsson, P. Samuelsson, and C. Svanborg.** 2001. Neutrophil recruitment, chemokine receptors and resistance to mucosal infection. J. Leukoc. Biol. **69:**899–906.
8. **Godaly, G., L. Hang, B. Frendeus, and C. Svanborg.** 2000. Transepithelial neutrophil migration is CXCR1 dependent in vitro and is defective in IL-8 receptor knock-out mice. J. Immunol. **165:**5287–5294.
9. **Hang, L., B. Frendeus, G. Godaly, and C. Svanborg.** 2000. Interleukin-8 receptor knockout mice have subepithelial neutrophil entrapment and renal scarring following acute pyelonephritis. J. Infect. Dis. **182:**1738–1748.
10. **Horcajada, J. P., M. Velasco, X. Filella, L. Alvarez, E. De Lazzari, J. L. Marin, B. Collvinent, A. Smithson, J. A. Martínez, M. Noguero, J. Vila, and J. Mensa.** 2004. Evaluation of inflammatory and renal-injury markers in women treated with antibiotics for acute pyelonephritis caused by *Escherichia coli*. Clin. Diagn. Lab. Immunol. **11:**142–146.
11. **Kato, H., N. Tsuchiya, and K. Tokunaga.** 2000. Single nucleotide polymorphisms in the coding regions of human CXC-chemokine receptors CXCR1, CXCR2 and CXCR3. Genes Immun. **1:**330–337.
12. **Morris, S. W., N. Nelson, M. B. Valentine, D. N. Shapiro, A. T. Look, C. J. Kozlosky, M. P. Beckmann, and D. P. Cerreti.** 1992. Assignment of the genes encoding human interleukin-8 receptor types 1 and 2 and an interleukin-8 receptor pseudogene to chromosome 2q35. Genomics **14:**685–691.
13. **Murphy, P. M.** 1997. Neutrophil receptors for interleukin-8 and related CXC chemokines. Semin. Hematol. **34:**311–318.
14. **Olszyna, D. P., S. Florquin, M. Sewnath, J. Branger, P. Speelman, S. J. van Deventer, R. M. Strieter, and T. van der Poll.** 2001. CXC chemokine receptor 2 contributes to host defense in murine urinary tract infection. J. Infect. Dis. **184:**301–317.
15. **Olszyna, D. P., S. M. Opal, J. M. Prins, D. L. Horn, P. Speelman, S. J. van Deventer, and T. van der Poll.** 2000. Chemotactic activity of CXC chemokines interleukin-8, growth related oncogen-alpha and epithelial cell-derived neutrophil-activating protein-78 in urine in patients with urosepsis. J. Infect. Dis. **182:**1731–1737.
16. **Otto, G., M. Burdick, R. Strieter, and G. Godaly.** 2005. Chemokine response to febrile urinary tract infection. Kidney Int. **68:**62–70.
17. **Richardson, R. M., R. J. Marjoram, L. S. Barak, and R. Snyderman.** 2003. Role of the cytoplasmatic tails of CXCR1 and CXCR2 in mediating leukocyte migration, activation, and regulation. J. Immunol. **170:**2904–2911.
18. **Schaeffer, A. J., J. M. Jones, and J. K. Dunn.** 1981. Association of in vitro Escherichia coli adherence to vaginal and buccal epithelial cells with susceptibility of women to recurrent urinary-tract infections. N. Engl. J. Med. **304:**1062–1066.
19. **Schaeffer, A. J., R. M. Radvany, and J. S. Chmiel.** 1983. Human leukocyte antigens in women with recurrent urinary tract infections. J. Infect. Dis. **148:**604.
20. **Schappert, S. M.** 1999. Ambulatory care visits to physician offices, hospital outpatient departments, and emergency departments: United States, 1997. Vital Health Stat. Ser. 13 **143:**i–iv, 1–39.
21. **Scholes, D., T. M. Hooton, P. L. Roberts, A. E. Stapleton, K. Gupta, and W. Stamm.** 2000. Risk factors for recurrent urinary tract infection in young women. J. Infect. Dis. **182:**1177–1182.

22. **Sheinfeld, J., A. J. Schaeffer, C. Cordon-Cardo, A. Rogatko, and W. R. Fair.** 1989. Association of Lewis blood-groups phenotype with recurrent urinary tract infections in women. N. Engl. J. Med. **320:**773–777.
23. **Sobel, J. D.** 1997. Pathogenesis of urinary tract infection: role of host defenses. Infect. Dis. Clin. N. Am. **11:**531–549.
24. **Sprenger, H., A. R. Lloyd, R. G. Meyer, J. A. Johnston, and D. J. Kelvin.** 1994. Genomic structure, characterization, and identification of the promoter of the human IL-8 receptor A gene. J. Immunol. **153:**2524–2532.
25. **Stamm, W. E., and T. M. Hotoon.** 1993. Management of urinary tract infections in adults. N. Engl. J. Med. **329:**1328–1334.
26. **Stapleton, A., E. Nudelman, H. Clausen, S. Hakomori, and W. E. Stamm.** 1992. Binding of uropathogenic Escherichia coli R45 to glycolipids extracted from vaginal epithelial cells is dependent on histo-blood group secretor status. J. Clin. Investig. **90:**965–972.
27. **Stemmler, S., U. Arinir, W. Klein, G. Rohde, S. Hoffjan, N. Wirkus, K. Reinitz-Rademacher, A. Bufe, G. Schultze-Werninghaus, and J. T. Epplen.** 2005. Association of interleukin-8 receptor alpha polymorphisms with chronic obstructive pulmonary diseases and asthma. Genes Immun. **6:**225–230.
28. **Svanborg, C., and G. Godaly.** 1997. Bacterial virulence in urinary tract infection. Infect. Dis. Clin. N. Am. **11:**513–529.
29. **Svensson, M., H. Irjala, P. Alm, B. Holmqvist, A. C. Lundstedt, and C. Svanborg.** 2005. Natural history of renal scarring in susceptible mIL-8Rh−/− mice. Kidney Int. **67:**103–110.

ARTICULO 4

ASSOCIATION BETWEEN MANNOSE-BINDING LECTIN DEFICIENCY AND SEPTIC SHOCK FOLLOWING ACUTE PYELONEPHRITIS DUE TO *ESCHERICHIA COLI*

ASOCIACIÓN ENTRE EL DÉFICIT DE LECTINA FIJADORA DE MANOSA Y SHOCK SÉPTICO TRAS PIELONEFRITIS AGUDA CAUSADA POR *ESCHERICHIA COLI*

A. Smithson, A. Muñoz, B. Suárez, S.M. Soto, R. Perelló, A. Soriano. J.A. Martínez, J. Vila, J.P. Horcajada, J. Mensa, F. Lozano.

Clinical and Vaccine Immunology 2007; 14: 256-261.

RESUMEN

La presencia de polimorfismos a nivel del promotor y de la región estructural del gen *MBL2*, causantes de niveles circulantes bajos de MBL, se ha asociado con un riesgo incrementado de infecciones. El objetivo de este estudio ha sido evaluar la posible asociación entre la presencia de polimorfismos en el gen *MBL2* y la incidencia de shock séptico y bacteriemia en pacientes con pielonefritis aguda causadas por *E. coli*. Se han incluido 62 mujeres con pielonefritis aguda causadas por *E. coli* que requerían ingreso hospitalario, así como 133 controles sanos. Se han analizado seis polimorfismos de nucleótido único (SNPs) a nivel del promotor y del exón 1 (-550 G/C, -221 C/G, +4 C/T, codón 52 CGT/TGT, codón 54 GGC/GAC and codón 57 GGA/GAA) del gen *MBL2* utilizando la metodología SBT (tiraje basado en la secuencia). No se han encontrado diferencias estadísticamente significativas en la frecuencia de genotipos de *MBL2* de baja expresión (O/O y LXA/O) entre los pacientes con pielonefritis aguda y los controles sanos. Las pacientes con pielonefritis aguda y shock séptico han presentado una mayor incidencia de genotipos de *MBL2* de baja expresión comparado con los pacientes con pielonefritis aguda sin shock séptico asociado (odds ratio = 9.019, intervalo de confianza 95%= 1.23 a 65.93; *P* = 0.03). No hemos encontrado asociación entre la presencia de pielonefritis aguda bacteriémica y la presencia de genotipos de baja expresión de *MBL*. Los resultados de este estudio sugieren que los genotipos *MBL2* de baja expresión predisponen al shock séptico pero no al desarrollo de bacteriemia en pacientes con pielonefritis aguda causadas por *E. coli*. La determinación de los polimorfismos del gen *MBL2* podría ser de utilidad para evaluar el riesgo de shock séptico en mujeres con pielonefritis aguda.

CLINICAL AND VACCINE IMMUNOLOGY, Mar. 2007, p. 256–261 Vol. 14, No. 3
1556-6811/07/$08.00+0 doi:10.1128/CVI.00400-06

Association between Mannose-Binding Lectin Deficiency and Septic Shock following Acute Pyelonephritis Due to *Escherichia coli*[▽]

Alex Smithson,[1]* Ana Muñoz,[1] Belen Suarez,[2] Sara Maria Soto,[3] Rafael Perello,[1] Alex Soriano,[1] Jose Antonio Martinez,[1] Jordi Vila,[3] Juan Pablo Horcajada,[1] Jose Mensa,[1] and Francisco Lozano[2]

Infectious Diseases Unit,[1] Department of Immunology,[2] and Department of Microbiology,[3] Hospital Clínic, IDIBAPS, University of Barcelona, Barcelona, Spain

Received 27 October 2006/Returned for modification 5 December 2006/Accepted 18 December 2006

Structural and promoter *MBL2* gene polymorphisms responsible for low MBL levels are associated with increased risk of infection. The objective of this study was to assess the possible association between polymorphisms of the *MBL2* gene and the incidence of septic shock and bacteremia in patients with acute pyelonephritis due to *Escherichia coli*. The study included 62 female patients with acute pyelonephritis due to *E. coli* who required hospital admission, as well as 133 healthy control subjects. Six single-nucleotide polymorphisms (−550 G/C, −221 C/G, +4 C/T, codon 52 CGT/TGT, codon 54 GGC/GAC, and codon 57 GGA/GAA) in the *MBL2* gene were genotyped by using a sequence-based typing technique. No significant differences were observed in the frequencies for low-expression *MBL2* genotypes (O/O and LXA/O) between patients with acute pyelonephritis and healthy controls. Patients with acute pyelonephritis and septic shock had a higher incidence of low-expression *MBL2* genotypes than patients with acute pyelonephritis without septic shock (odds ratio = 9.019, 95% confidence interval = 1.23 to 65.93; $P = 0.03$). No association was found between bacteremic acute pyelonephritis and low-expression *MBL2* genotypes. We found that low-expression *MBL2* genotypes predispose to septic shock but not to bacteremia in patients with *E. coli*-induced acute pyelonephritis. Determination of *MBL2* polymorphisms could be useful for assessing the risk of septic shock in women undergoing acute pyelonephritis.

Annually in the United States, at least 250,000 episodes of acute pyelonephritis (AP) in adults occur, with nearly 200,000 hospitalizations and an estimated mortality rate among female patients of 7.3 cases per 1,000 hospital admissions (7, 19). Despite the fact that most cases of AP have a good prognosis, they often require prolonged therapy and, when accompanied by bacteremia, AP has a mortality rate of 10 to 20% (31). Several host conditions, such as immunosuppression (44), age (27), diabetes (29, 32), pregnancy (14), and bedridden status (6), have been associated with more serious forms of AP.

Besides the existence of clinical conditions that predispose to severe AP, the biological substrate of such predisposition is not yet clearly understood. In recent years increasing evidence suggests that different host factors could be involved in the pathogenesis of urinary tract infections (UTIs), particularly in recurrent UTIs (38). Evidence of the importance of these host factors includes the facts that women with recurrent UTIs or with uncomplicated AP are more likely to be nonsecretors of blood group antigens (20, 36) and that specific HLA phenotypes are more prevalent in women with recurrent UTIs (34). Additional evidence includes the demonstration that children prone to recurrent AP have a decreased expression of the interleukin-8 receptor CXCR1 (8), although these data differ from those observed by our study group in which premenopausal women with recurrent UTIs, including women with recurrent AP, showed a decreased expression of CXCR2 (37).

The mannose-binding lectin (MBL) is a circulating C-type plasma lectin, mainly produced by the liver, that plays an important role in innate immunity. MBL is a pattern recognition molecule that binds with high affinity to the terminal mannose, fucose, glucose, and *N*-acetyl-D-glucosamine moieties present on the surface of various pathogens (26, 35), including *Escherichia coli*, the pathogen most commonly involved in UTIs (42). In addition to acting as an opsonin for phagocytosis for numerous pathogens, MBL activates the complement cascade through the lectin pathway, using MBL-associated serine proteases, namely, MASP-2 (25).

The *MBL2* gene (MBL-1 is a pseudogene) is located on chromosome 10q11.2-q21 (33). Three missense single-nucleotide polymorphisms (SNPs) have been reported at codons 52 (allele D), 54 (allele B), and 57 (allele C) of the exon 1 of the *MBL2* gene, which result in amino acid substitutions interfering with oligomerization of MBL monomers into multimers and reducing serum MBL levels (17, 45). In addition to these structural variant alleles, three SNPs in the promoter region of the *MBL2* gene, at positions −550 (H/L), +4 (P/Q) and, particularly, −221 (Y/X), influence the rate of transcription and are also associated with low concentrations of serum MBL (40). The SNPs at exon 1 are in strong linkage disequilibrium with those at the promoter and give rise to seven common haplotypes (HYPA, LYQA, LYPA, LXPA, LYPB, LYQC, and HYPD), which show considerable variation in their frequencies between ethnic groups (23, 24). The HY haplotype induces high MBL concentrations, whereas exon 1 mutations (O variants) and the LX haplotypes cause reduced MBL con-

* Corresponding author. Mailing address: Infectious Diseases Unit, Hospital Clínic, IDIBAPS, Edificio Helios, 1 Planta C/Villarroel 170, 08036 Barcelona, Spain. Phone: 34-93 227 54 00. Fax: 34-93 227 93 22. E-mail: asa30412@hotmail.com.

▽ Published ahead of print on 3 January 2007.

centrations (24). Thus, based on previous reports, patients can be classified into high (HYA/HYA, HYA/LYA, HYA/LXA, LYA/LYA, and LYA/LXA), intermediate (LXA/LXA, HYA/O, and LYA/O), and low (LXA/O and O/O) MBL expression groups (40). Although MBL deficiency appears to predispose to serious infections (43), particularly during early childhood (22) and in patients undergoing chemotherapy (30), as well as in adults with concomitant diseases (10, 11), the association between MBL deficiency and severe forms of AP has not been established.

The aim of the present study was to investigate whether the existence of low-expression *MBL2* genotypes confers an increased risk for septic shock and bacteremia in women with AP due to *E. coli*.

MATERIALS AND METHODS

Study population. We prospectively collected blood samples from 62 female Caucasian patients with community-acquired AP caused by *E. coli* who required admission to our tertiary hospital between January 2003 and January 2004. Inclusion criteria for patients were >18 years of age, the presence of clinical symptoms of AP (armpit temperature of >38°C, pyuria, and lumbar tenderness), and a positive uroculture for *E. coli*. For further comparison, 133 healthy control subjects (104 Spanish blood donors from the geographic area of Barcelona and 29 female members of the hospital staff without previous urinary tract infections) were included in the study. The present study was conducted with the approval of the hospital Ethics Committee and the informed consent of all participants. The human experimentation guidelines of the U.S. Department of Health and Human Services and those of the authors' institutions were followed in conducting the clinical research.

Urine samples were obtained by use of a clean midstream catch method. Urine samples were spread on MacConkey and cysteine lactose electrolyte-deficient agar plates for quantification, followed by incubation for 48 h. Positive urine culture results were defined as pathogen growth of $\geq 10^5$ CFU/ml. Identification of *E. coli* was performed by standard methods. Blood cultures were processed by means of an automatic infrared culture system (Bactec 9240; Becton Dickinson) for 5 days.

Septic shock was defined according to the 1992 American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference guidelines (3). In summary, septic shock was diagnosed in cases of AP with an arterial systolic pressure of <90 mm Hg for at least 1 h after fluid resuscitation or required vasopressor therapy (dopamine at 5 μg/kg of body weight or more per min or any dose of epinephrine, norepinephrine, or vasopressin) to maintain a systolic blood pressure of >90 mm Hg.

Age and the following comorbid host conditions were included in the analysis: cirrhosis, heart failure, chronic kidney failure (defined as a creatinine level of >1.5 mg/dl), diabetes mellitus, underlying neoplastic disease, and immunosuppressive or corticosteroid therapy.

DNA samples. Genomic DNA was extracted from EDTA-treated whole blood samples by using the QIAamp DNA blood minikit according to the manufacturer's instructions (QIAGEN GmbH, Hilden, Germany) and then stored at −80°C until used.

Sequence analysis of MBL gene variants. Genotyping of the *MBL2* gene was performed by using a sequencing-based typing technique. Specific primers were designed according to the published genomic DNA sequences (GenBank accession number AF360991). A 969-bp fragment encompassing from the promoter to the end of exon 1 of the *MBL2* gene was obtained by PCR amplification using sense (5′-GGG GAA TTC CTGCCA GAAAGT-3′) and antisense (5′-CAT ATC CCCAGG CAG TTT CCT C-3′) primers and the Expand 20Kb Plus PCR system (Roche Diagnostics GmbH, Mannheim, Germany). The cycling conditions used for amplification of the *MBL2* gene were 94°C for 8 min, followed by 35 cycles at 94°C for 45 s, 58°C for 30 s, 72°C for 90 s, and 72°C for 10 min. Portions (5 μl) of the resulting PCR product were treated with ExoSAP-IT (USB Corp., Cleveland, OH) and then subjected to direct sequencing with the BigDye Terminator v1.1 cycle sequencing kit (Applied Biosystems, Warrington, United Kingdom) according to the manufacturer's instructions with the sense and antisense gene-specific primers described above.

Statistical analysis. Continuous variables were compared by the Student *t* test or Mann-Whitney U test when the distribution departed from normality and are given as means (±standard deviations) or medians (range of values), respectively. Categorical data were compared by using the chi-square or Fisher exact test as appropriate. Variables associated to septic shock or bacteremia in the univariate analysis ($P < 0.1$) were included in a binary logistic regression analysis with septic shock and bacteremia as separate dependent variables. In the logistic models, age was analyzed with the median as a dichotomizing value (the two groups were values less than the median and values greater than or equal to the median). Statistical significance was defined as a two-tailed *P* value of <0.05. Statistical analysis was carried out by the program SPSS (version 11.0; SPSS, Inc., Chicago, IL).

RESULTS

Clinical data. The study included 62 women with AP due to *E. coli.* The mean age of the patients included in the study was 43 years (±20.6). Twenty-six (42%) patients had bacteremic AP. A total of 18 (29%) patients had associated comorbidity; 3 (4.8%) had cirrhosis, 5 (8%) had past history of heart failure, 7 (11.3%) had chronic kidney failure, 8 (13%) had diabetes mellitus, 4 (6.4%) had underlying neoplastic diseases, and 5 (8%) were receiving immunosuppressive or corticosteroid therapy. The patients with underlying neoplastic diseases included one patient with a cervix neoplasia, another with a hypernephroma (neither of whom had obstruction of the urinary tract), and two with hematological disorders (one patient with a polycythemia vera and another with a multiple myeloma). Patients under immunosuppressive or corticosteroid therapy included two with systemic lupus erythematosus that were on corticosteroid treatment and three kidney transplant recipients that were on corticosteroid therapy plus immunosuppressive therapy. Among the patients with chronic kidney failure, one received dialysis therapy. Seven patients (11.3%) met the criteria for septic shock; one had nonbacteremic AP and six had bacteremic AP. One patient with septic shock (haplotype LYPB/LYPB) died of a cause unrelated to UTI (stroke).

Analysis of the MBL polymorphisms. The sequence-based typing analysis of the exon 1 and the promoter region of the *MBL2* gene allowed the categorization of individuals according to haplotypes and genotypes responsible for high (HYA/HYA, HYA/LYA, HYA/LXA, LYA/LYA, and LYA/LXA), intermediate (HYA/O, LYA/O, LXA/LXA), and low (O/O, LXA/O) MBL serum levels as described previously (40). Table 1 shows the frequency for the *MBL2* haplotypes and genotypes found among the healthy controls and the patients with AP. No significant differences in the frequencies for the different haplotypes were found among the healthy control group and the patients with AP. LYPB was the predominant variant type haplotype both in the healthy control group and in the patients with AP. No overall statistical significant differences were observed for the frequencies of high (57.1% versus 51.6% [$P = 0.46$]), intermediate (30.1% versus 30.6% [$P = 0.93$]), and low (12.8% versus 17.7% [$P = 0.35$]) *MBL2* genotypes among the healthy controls and the patients with AP, respectively (Table 1). The differences found in the frequencies for high (65.5%), intermediate (31.8%), and low (3.4%) *MBL2* genotypes among the 29 members of the personal staff without previous UTI and the patients with AP were not statistically significant ($P = 0.21$, 0.97, and 0.09, respectively).

In the univariate analysis, low-*MBL2*-expression genotypes and chronic kidney failure were the only variables associated with septic shock (Table 2). To further assess the independent value of the previous associations, we performed a logistic

TABLE 1. Frequency of the *MBL2* haplotypes and genotypes found among healthy controls and patients with acute pyelonephritis

Expression type[a]	No. (%) of subjects		*P*
	Healthy controls	Patients	
MBL2 haplotypes			
LYQA	59 (22.2)	19 (15.3)	0.11
HYPA	72 (27)	38 (30.6)	0.46
LYPA	23 (8.6)	6 (4.8)	0.18
LYPB	42 (15.8)	20 (16.1)	0.93
LXPA	50 (18.8)	29 (23.3)	0.29
LYQC	5 (1.8)	4 (3.2)	0.47
HYPD	15 (5.6)	8 (6.4)	0.75
MBL2 genotypes			
High expression	76 (57.1)	32 (51.6)	0.46
HYA/HYA	6 (4.5)	6 (9.6)	
HYA/LYA	24 (18)	8 (12.9)	
HYA/LXA	18 (13.5)	8 (12.9)	
LYA/LYA	12 (9)	1 (1.6)	
LYA/LXA	16 (12)	9 (14.5)	
Intermediate expression	40 (30)	19 (30.6)	0.93
LXA/LXA	4 (3)	3 (4.8)	
HYA/O	18 (13.5)	9 (14.5)	
LYA/O	18 (13.5)	7 (11.3)	
Low expression	17 (12.8)	11 (17.7)	0.35
LXA/O	8 (6)	6 (9.6)	
O/O	9 (6.7)	5 (8)	

[a] Y and X indicate base exchanges at codon −221. A, normal structural allele; O, variant alleles (B, codon 54; C, codon 57; and D, codon 52).

regression with septic shock as a dependent variable. In the logistic regression model, heart failure and age were also included (P [in the univariate analysis] < 0.1). Only low-*MBL2*-expression genotypes (odds ratio [OR] = 9.019, 95% confidence interval [CI] = 1.23 to 65.93; $P = 0.03$) were independently associated with the development of septic shock. In contrast, chronic kidney failure did not reach statistical significance (Table 2).

Bacteremia was associated in the univariate analysis with old age, neoplasia, chronic kidney failure, and the existence of any comorbidity but not with the presence of low-expression *MBL2* genotypes. By logistic regression analysis, old age was the only variable significantly associated with bacteremia (Table 3).

DISCUSSION

Despite the fact that AP is one of the most common infectious diseases and a potentially life-threatening disorder, little is known about the predictor variables associated with an unfavorable outcome. The presence of septic shock has been described as one of the clinical variables related to death in patients with AP (6). In recent years many studies have stressed the implication of the innate immune response on the pathogenesis of septic shock and particularly of one of its components, the MBL (12, 15). In light of the accumulated evidence, we have evaluated the implication of the *MBL2* genotypes associated with MBL deficiency on the incidence of septic shock and secondarily of bacteremia in women with AP due to *E. coli*.

The MBL is a circulating human collectin (a family of proteins that possess both collagenous regions and lectin domains) with the ability to activate the complement and mediate phagocytosis after binding to specific carbohydrates on the surface of several bacteria, fungi, and viruses. The serum concentration and functional activity of the MBL are mainly determined by SNPs at the promoter and the exon 1 of the *MBL2* gene. Genotypes associated with low serum MBL levels have been correlated with an increased risk, severity, and frequency of infections, particularly those mediated by capsulated bacteria (46). Genetically defined MBL deficiency is remarkably common in the general population, with an estimated prevalence of more than 10 to 15% in several Caucasian populations (17, 18). In our study, the *MBL2* genotype frequencies observed in the healthy control group closely resembled those previously reported in a Canary Islands (Spain) population (9), which in turn was similar to that observed in other healthy control groups from several European studies performed with Caucasian populations (1, 12, 13). LYPB, as previously reported, was the predominant variant type haplotype both in the healthy control group and in the patients with AP. An interesting

TABLE 2. Univariate and multivariate analyses of the association between different clinical characteristics and the presence of septic shock

Characteristic	No. (%) of subjects[a]		P^b	Multivariate analysis[d]	
	Presence of septic shock ($n = 7$)	Absence of septic shock ($n = 55$)		OR (95% CI)	*P*
Cirrhosis	1 (14.3)	2 (3.6)	0.306		
Heart failure	2 (28.6)	3 (5.4)	0.093	NS	
Neoplasia	1 (14.3)	3 (5.4)	0.389		
Immunosuppressive or corticosteroid treatment	1 (14.3)	4 (7.3)	0.462		
Chronic kidney failure	3 (42.8)	4 (7.3)	0.026	NS	
Diabetes	1 (14.3)	7 (12.7)	1		
Presence of comorbidity	4 (57.1)	14 (25.4)	0.179		
Low-*MBL2*-expression genotypes	4 (57.1)	7 (12.7)	0.015	9.019 (1.23–65.93)	0.03
Median age in yr (range)[c]	52 (34–75)	35 (18–94)	0.087	NS	

[a] Except where otherwise noted, data are the numbers (%) of subjects with the indicated characteristics, and the results are expressed as proportions of patients with septic shock in the presence or absence of the clinical condition.
[b] Determined by univariate analysis of the correlations between the presence of septic shock and each characteristic.
[c] In the logistic model, age was dichotomized by means of the median age (35.5 years) of the patients with AP.
[d] Clinical variables associated with septic shock ($P < 0.1$) were included in the multivariate analysis. NS, nonsignificant differences.

TABLE 3. Univariate and multivariate analyses of the association between different clinical characteristics and the presence of bacteremia

Characteristic	No. (%) of patients[a]		P^b	Multivariate analysis[d]	
	Presence of bacteremia (n = 26)	Absence of bacteremia (n = 36)		OR (95% CI)	P
Cirrhosis	3 (11.5)	0	0.069	NS	
Heart failure	4 (15.4)	1 (2.8)	0.152		
Neoplasia	4 (15.4)	0	0.027	NS	
Immunosuppressive or corticosteroid treatment	4 (15.4)	1 (2.8)	0.152		
Chronic kidney failure	7 (26.9)	0	0.001	NS	
Diabetes	5 (19.2)	3 (8.3)	0.262		
Presence of any comorbidity	13 (50)	5 (13.9)	0.002	NS	
Low-*MBL2*-expression genotypes	7 (26.9)	4 (11.1)	0.177		
Median age in yr (range)[c]	58 (26–94)	26 (18–84)	<0.0001	8.32 (1.91–36.23)	0.005

[a] Except where otherwise noted, data are the numbers (%) of subjects with the indicated characteristics, and results are expressed as proportions of patients with bacteremia in the presence or absence of the clinical condition.
[b] Determined by means of univariate analysis of the correlations between the presence of bacteremia and each characteristic.
[c] In the logistic model, age was dichotomized by means of the median age (35.5 years) of the patients with AP.
[d] Clinical variables associated with bacteremia ($P < 0.1$) were included in the multivariate analysis. NS, nonsignificant differences.

observation of our study was the relatively low frequency of *MBL2* genotypes associated with MBL deficiency detected in the staff members without previous UTI. Although these low genotype frequencies were probably related to the small number of individuals with this condition included in the study, the potential implication of the MBL lectin pathway in the pathogenesis of UTI may justify further studies.

To our knowledge, this is the first study on *MBL2* genotyping that has focused on patients with AP. Our results suggest that patients with *MBL2* genotypes associated with serum MBL deficiency undergoing AP due to *E. coli* have a higher risk for developing septic shock but not bacteremia. The multivariate analysis has revealed that the presence of *MBL2* genotypes associated with low MBL production was the only variable associated with septic shock even though other clinical variables, such as age, immunosuppression, and diabetes mellitus, previously related to AP with an unfavorable outcome (27, 29, 32, 44) were also included in the study. Although one possible limitation of our study is the fact that serum MBL levels were not measured, the relationship between *MBL2* genotypes and serum MBL levels has been clearly established in numerous studies (12, 13, 40). Therefore, *MBL2* genotyping could be of clinical interest as a molecular marker defining patients with AP at risk for septic shock. The correlation between *MBL2* genotypes associated with low MBL production and septic shock is not novel and has been reported in prior studies. Garred et al. demonstrated that MBL gene polymorphisms causing low serum levels of MBL were associated with the development of septic shock in intensive care unit patients with systemic inflammatory response syndrome (12). More recently, Gordon et al. found a higher incidence of exon 1 polymorphisms among patients with septic shock than among normal healthy adults (15).

The method by which low serum MBL levels seem to favor the progression of localized infections and the development of septic shock remains unclear. Although activation of the immune system during microbial invasion is generally protective, septic shock may develop as a consequence of an exacerbated inflammatory response (2). MBL is known to activate the complement cascade and, as a result, to induce the release of proinflammatory cytokines, particularly of tumor necrosis factor alpha, from monocytes (4, 39). An excess of complement activation could lead to enhanced inflammation, which could be deleterious for the host. One might hypothesize that low MBL levels could in fact be beneficial since they may reduce inflammation and therefore the development or the severity of septic shock. A novel mechanism by which MBL could influence the development of septic shock is through a direct effect as a modulator of proinflammatory cytokine production. Jack et al. addressed this issue by incubating *Neisseria meningitidis* with increasing concentrations of MBL before adding MBL-deficient whole blood. Release of tumor necrosis factor alpha, interleukin-6, and interleukin-1β from monocytes was enhanced at low MBL concentrations and suppressed at higher concentrations, which suggests that MBL is not only involved in complement activation but is also a potent regulator of inflammatory pathways (21). Another mechanism by which MBL could interfere in the development of shock is through an increased clearance of endotoxin, one of the most powerful inductors of septic shock. Ono et al. have demonstrated that MBL is able to enhance the uptake of lipid A, the molecular component responsible for the toxic effects of the endotoxin, by increasing the cell surface expression of scavenger receptor A by Kupffer cells (28).

However, the implication of MBL in the pathogenesis of septic shock, in patients with AP, is complex and could depend not only on host (quantity of MBL) but also on bacterial characteristics (the binding capacity of MBL to different types or strains of bacteria). In our study all of the episodes of AP included were due to *E. coli*, which is responsible for most of the cases of AP. The MBL binding capacity to *E. coli* has been evaluated by means of flow cytometry (26) and enzyme-linked lectin assay (35), with conflicting results depending on the method used. While in the study by Neth et al. (26) only one isolate of *E. coli* bound to MBL, in the study by Shang et al. *E. coli* demonstrated a high binding capacity to MBL (35). In both studies different isolates of *E. coli* showed different intraspecies binding rates. These results could be related to differences in the sugar array compositions of the membranes of *E. coli*. The membrane of *E. coli* is mainly composed of lipopolysaccharide, which is the major acceptor molecule for MBL on gram-negative bacteria (5). It has been demonstrated that several serotypes of *E. coli*, which possess different lipopolysaccharide

compositions, show different binding capacities to MBL (47). Unfortunately, the strains of *E. coli* involved in the episodes of AP were not serotyped in our study. In vitro studies have demonstrated that MBL is able to increase the phagocytosis of *E. coli* by Kupffer cells (28). Therefore, one would expect a higher incidence of bacteremia in patients with MBL deficiency, which was not the case in our study, thus suggesting that bacterial factors are important when analyzing the implication of host MBL genotypes in different infectious disease scenarios. To add another degree of complexity, functionally relevant polymorphisms of *MASP2* have recently been described, and therefore genotyping of the MBL alone may not be sufficient to evaluate the MBL pathway (16, 41).

In conclusion, the present study suggests that patients with AP due to *E. coli* with *MBL2* genotypes associated with MBL deficiency have a higher risk for developing septic shock but not for bacteremia. A rapid determination of the *MBL2* genotype could be an important tool to identify patients with AP who are at risk for developing septic shock.

REFERENCES

1. **Aittoniemi, J., H. Soranummi, A. T. Rovio, M. Hurme, T. Pessi, M. Nieminen, and J. Karjalainen.** 2005. Mannose-binding lectin 2 (MBL2) gene polymorphism in asthma and atopy among adults. Clin. Exp. Immunol. **142:**120–124.
2. **Annane, D., E. Bellisant, and J. M. Cavaillon.** 2005. Septic shock. Lancet **365:**63–78.
3. **Bone, R. C., R. A. Balk, F. B. Cerra, R. P. Dellinger, A. M. Fein, W. A. Knaus, R. M. Schein, W. J. Sibbald, et al.** 1992. Definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. Chest **101:**1644–1655.
4. **Chaka, W., A. F. Verheul, V. V. Vaishnav, R. Cherniak, J. Scharring, J. Verhoef, H. Snippe, and A. I. Hoepelman.** 1997. Induction of TNF-alpha in human peripheral blood mononuclear cells by the mannoprotein of *Cryptococcus neoformans* involves human mannose binding protein. J. Immunol. **159:**2979–2985.
5. **Dumestre-Perard, C., E. Doerr, M. G. Colomb, and M. Loos.** 2007. Involvement of complement pathways in patients with bacterial septicemia. Mol. Immunol. **44:**1642–1649.
6. **Efstathious, S. P., A. V. Pefanis, D. I. Tsioulos, I. D. Zacharos, A. G. Tsiakou, A. G. Mitromaras, S. E. Mastorantonakis, S. N. Kanavaki, and T. D. Mountokalakis.** 2003. Acute pyelonephritis in adults: prediction of mortality and failure of treatment. Arch. Intern. Med. **163:**1206–1212.
7. **Foxman, B., K. L. Klemstine, and P. D. Brown.** 2003. Acute pyelonephritis in US hospitals in 1997: hospitalization and in-hospital mortality. Ann. Epidemiol. **13:**144–150.
8. **Frendeus, B., G. Godaly, L. Hang, D. Karpman, A. C. Lundstedt, and C. Svanborg.** 2000. Interleukin 8 receptor deficiency confers susceptibility to acute experimental pyelonephritis and may have a human counterpart. J. Exp. Med. **192:**881–890.
9. **Garcia-Laorden, M. I., A. Manzanedo, A. Figuerola, F. Sanchez-Garcia, and C. Rodriguez-Galleo.** 2001. Mannose-binding lectin polymorphisms in a Canary Islands (Spain) population. Genes Immun. **2:**292–294.
10. **Garred, P., H. O. Madsen, U. Baslev, B. Hofman, C. Pedersen, J. Gerstoft, and A. Svejgaard.** 1997. Susceptibility to HIV infection and progression of AIDS in relation to variant alleles of mannose-binding lectin. Lancet **349:** 236–240.
11. **Garred, P., H. O. Madsen, P. Halberg, J. Petersen, G. Kronborg, S. Svejgaard, V. Andersen, and S. Jacobsen.** 1999. Mannose binding polymorphisms and susceptibility to infection in systemic lupus erythematosus. Arthritis Rheum. **42:**2145–2152.
12. **Garred, P., J. Strom, L. Quist, E. Taaning, and H. O. Madsen.** 2003. Association of mannose-binding lectin polymorphisms with sepsis and fatal outcome in patients with systemic inflammatory response syndrome. J. Infect. Dis. **188:**1394–1403.
13. **Geleijns, K., A. Roos, J. Houwing-Duistermaat, W. van Rijs, A. P. Tio-Guillen, J. D. Laman, P. A. van Doorn, and B. C. Jacobs.** 2006. Mannose binding lectin contributes to the severity of Guillain-Barre syndrome. J. Immunol. **177:**4211–4217.
14. **Gilstrap, L. C., III, and S. M. Ramin.** 2001. Urinary tract infections during pregnancy. Obstet. Gynecol. Clin. N. Am. **28:**581–591.
15. **Gordon, A. C., U. Waheed, T. K. Hansen, G. A. Hitman, C. S. Garrad, M. W. Turner, N. J. Klei, S. J. Brett, and C. J. Hinds.** 2006. Mannose-binding lectin polymorphisms in severe sepsis: relationship to levels, incidence, and outcome. Shock **25:**88–93.
16. **Granell, M., A. Urbano-Ispizua, B. Suarez, M. Rovira, F. Fernandez-Aviles, C. Martinez, M. Ortega, C. Uriburu, A. Gaya, J. M. Roncero, A. Navarro, E. Carreras, J. Mensa, J. Vives, C. Rozman, E. Montserrat, and F. Lozano.** 2006. Mannan-binding lectin pathway deficiencies and invasive fungal infections following allogenic stem cell transplantation. Exp. Hematol. **34:**1435–1441.
17. **Guardia, A., and F. Lozano.** 2003. Mannose binding lectin deficiencies in infectious and inflammatory disorders. Rev. Med. Microbiol. **14:**41–52.
18. **Holmskov, U., S. Thiel, and J. C. Jensenius.** 2003. Collectins and ficolins: humoral lectins of the innate immune defense. Annu. Rev. Immunol. **21:** 547–578.
19. **Hooton, T. M., and W. E. Stamm.** 1997. Diagnosis and treatment of uncomplicated urinary tract infection. Infect. Dis. Clin. N. Am. **11:**551–581.
20. **Ishitoya, S., S. Yamamoto, K. Mitsumori, O. Ogawa, and A. Terai.** 2002. Non-secretor status is associated with female acute uncomplicated pyelonephritis. BJU Int. **89:**851–854.
21. **Jack, D. L., R. C. Read, A. J. Tenner, M. Frosch, M. W. Turner, and N. J. Klein.** 2001. Mannose-binding lectin regulates the inflammatory response of human professional phagocytes to *Neisseria meningitidis* serogroup B. J. Infect. Dis. **184:**1152–1162.
22. **Koch, A., M. Melbye, P. Sorensen, P. Homoe, H. O. Madsen, K. Molbak, C. H. Hansen, L. H. Andersen, G. W. Hahn, and P. Garred.** 2001. Acute respiratory tract infections and mannose-binding lectin insufficiency during early childhood. JAMA **285:**1316–1321.
23. **Lozano, F., B. Suarez, A. Muñoz, J. C. Jensenius, J. Mensa, J. Vives, and J. P. Horcajada.** 2005. Novel MASP2 variants detected among North African and Sub-Saharan individuals. Tissue Antigens **66:**131–135.
24. **Madsen, H. O., P. Garred, S. Thiel, J. A. Kurtzhals, L. U. Lamm, L. P. Ryder, and A. Svejgaard.** 1995. Interplay between promoter and structural gene variants control basal serum level of mannan-binding protein. J. Immunol. **155:**3013–3020.
25. **Matsushita, M.** 1996. The lectin pathways of the complement system. Microbiol. Immunol. **40:**887–893.
26. **Neth, O., D. L. Jack, A. W. Dodds, H. Holzel, N. J. Klein, and M. W. Turner.** 2000. Mannose-binding lectin binds to a range of clinically relevant microorganisms and promotes complement deposition. Infect. Immun. **68:**688–693.
27. **Nicolle, L. E.** 2001. Urinary tract pathogens in complicated infection and in elderly individuals. J. Infect. Dis. **183**(Suppl. 1)**:**S5–S8.
28. **Ono, K., C. Nishitani, H. Mitsuzawa, T. Shimizu, H. Sano, H. Suzuki, T. Kodama, N. Fjii, K. Fukase, K. Hirata, and Y. Kuroki.** 2006. Mannose-binding lectin augments the uptake of lipid A, *Staphylococcus aureus*, and *Escherichia coli* by Kupffer cells through increased cell surface expression of scavenger receptor A. J. Immunol. **177:**5517–5523.
29. **Patterson, J. E., and V. T. Andriole.** 1997. Bacterial urinary tract infections in diabetes. Infect. Dis. Clin. N. Am. **11:**735–750.
30. **Peterslund, N. A., C. Koch, J. C. Jensenius, and S. Thiel.** 2001. Association between deficiency of mannose-binding lectin and severe infection after chemotherapy. Lancet **358:**637–638.
31. **Roberts, F. J., I. W. Geere, and A. Coldman.** 1991. A three-year study of positive blood cultures, with emphasis on prognosis. Rev. Infect. Dis. **13:**34–46.
32. **Ronald, A., and E. Ludwig.** 2001. Urinary tract infections in adults with diabetes. Int. J. Antimicrob. Agents **17:**287–292.
33. **Sastry, K., G. A. Herman, L. Day, E. Deignan, G. Bruns, C. C. Morton, and R. A. Ezekowitz.** 1989. The human mannose-binding protein gene. Exon structure reveals its evolutionary relationship to a human pulmonary surfactant gene and localization to chromosome 10. J. Exp. Med. **170:**1175–1189.
34. **Schaeffer, A. J., R. M. Radvany, and J. S. Chmiel.** 1983. Human leukocyte antigens in women with recurrent urinary tract infections. J. Infect. Dis. **148:**604.
35. **Shang, S. Q., G. X. Chen, J. Shen, X. H. Yu, and K. Y. Wang.** 2005. The binding to common bacteria in infectious diseases of children. J. Zhejiang Univ. Sci. **6:**53–56.
36. **Sheinfeld, J., A. J. Schaeffer, C. Cordon–Cardo, A. Rogatko, and W. R. Fair.** 1989. Association of Lewis blood-group phenotype with recurrent urinary tract infections in women. N. Engl. J. Med. **320:**773–777.
37. **Smithson, A., M. R. Sarrias, J. Barcelo, B. Suarez, J. P. Horcajada, S. M. Soto, A. Soriano, J. Vila, J. A. Martinez, J. Vives, J. Mensa, and F. Lozano.** 2005. Expression of interleukin-8 receptors (CXCR1 and CXCR2) in premenopausal women with recurrent urinary tract infections. Clin. Diagn. Lab. Immunol. **12:**1358–1363.
38. **Sobel, J. D.** 1997. Pathogenesis of urinary tract infection: role of host defenses. Infect. Dis. Clin. N. Am. **11:**531–549.
39. **Soell, M., E. Lett, F. Holveck, M. Scholler, D. Wachsmann, and J. P. Klein.** 1995. Activation of human monocytes by streptococcal rhamnose glucose polymers is mediated by CD 14 antigen, and mannan binding protein inhibits TNF-alpha release. J. Immunol. **154:**851–860.
40. **Steffensen, R., S. Thiel, K. Varming, C. Jersild, and J. C. Jensenius.** 2000. Detection of structural gene mutations and promoter polymorphisms in the mannan-binding lectin (MBL) gene by polymerase chain reaction with sequence-specific primers. J. Immunol. Methods **241:**33–42.
41. **Stengaard-Pedersen, K., S. Thiel, M. Gadjeva, M. Moller-Kristensen, R. Sorensen, L. T. Jensen, A. G. Sjoholm, L. Fugger, and J. C. Jensenius.** 2003.

Inherited deficiency of mannan-binding-associated serine proteases 2. N. Engl. J. Med. **349:**554–560.
42. **Svanborg, C., and G. Godaly.** 1997. Bacterial virulence in urinary tract infection. Infect. Dis. Clin. N. Am. **11:**513–529.
43. **Takahashi, K., and A. Ezekowitz.** 2005. The role of the mannose binding lectin in innate immunity. Clin. Infect. Dis. **41**(Suppl. 7)**:**S440–S444.
44. **Tolkoff-Rubin, N. E., and R. H. Rubin.** 1997. Urinary tract infection in the immunocompromised host. Infect. Dis. Clin. N. Am. **11:**707–717.
45. **Tuner, M. W., and R. M. Hamvas.** 2000. Mannose-binding lectin: structure, function, genetics, and disease associations. Rev. Immunogenet. **2:**305–322.
46. **Worthley, D. L., P. G. Bardy, and C. G. Mullighan.** 2005. Mannose-binding lectin: biology and clinical implications. Intern. Med. J. **35:**548–555.
47. **Zhao, L., Y. Ohtaki, K. Yamaguchi, M. Matsushita, T. Fujita, T. Yokochi, H. Takada, and Y. Endo.** 2002. LPS-induced platelet response and rapid shock in mice: contribution of O-antigen region of LPS and involvement of the lectin pathway of the complement system. Blood **100:**3233–3239.

8. DISCUSIÓN.

8.1. Estudio 1: Formación de biopelícula por cepas uropatógenas de *E. coli*: relación con prostatitis, factores de virulencia y resistencia a antibióticos.

Este estudio demuestra que la producción de biopelícula es uno más de los FV que poseen las cepas de UPEC causantes de PA y que su producción se asocia con la presencia de hemolisina y con la expresión de fimbrias tipo 1. Aunque la producción de biopelícula no parece estar directamente implicada en la invasividad, que es una propiedad aparentemente asociada con la hemolisina, su formación parece estar relacionada con la mayor capacidad de las cepas de *E. coli* causantes de PA de permanecer en el sistema secretor prostático y conducir a ITURs, que es una de las características propias de la prostatitis crónica bacteriana.

Tras un episodio de PA, aproximadamente 1/3 de los pacientes presentan un cultivo positivo de las secreciones prostáticas a los 3 meses de haber completado 6 semanas de tratamiento antibiótico[163]. Este hecho podría estar en relación a la capacidad de la bacteria de formar biopelícula. La formación de biopelícula permite al microorganismo persistir en el tracto urinario o sobre superficies de biomaterial, protegiéndola del efecto de arrastre de las fuerzas hidrodinámicas de la orina, de la acción de los mecanismos defensivos del huésped y de los antibióticos[164]. La formación de biopelícula confiere a la bacteria ventajas como la adquisición de tolerancia a los antibióticos, la expresión de diversos FV, y una mayor resistencia a la fagocitosis y a otros mecanismos defensivos del huésped. El estudio de los factores que contribuyen a la formación de las biopelículas podría ser importante para diseñar nuevas

estrategias terapéuticas para tratar estas infecciones. En este sentido, Wu *et al*[25] sugirieron que la inhibición de la adhesión a las superficies uroepiteliales, que es un elemento crucial en la patogénesis de las ITUs, podría inhibir la formación de las biopelículas.

En este estudio hemos observado un mayor porcentaje de cepas de *E. coli* productoras de biopelícula entre aquellas cepas aisladas de PA que entre las aisladas de CA o PNA. Sin embargo, la presencia de hemolisina ha constituido el principal confusor de la asociación dado que las cepas de *E. coli* productoras de hemolisina, FV que se ha asociado con las cepas causantes de PA, también producían con mayor frecuencia biopelícula *in vitro*. En este estudio también hemos evidenciado que las cepas de *E. coli* productoras de biopelícula expresan con mayor frecuencia fimbrias tipo 1 que las cepas no productoras de biopelícula. Las fimbrias tipo 1 son consideradas como un elemento importante en las primeras fases, adhesión a las células epiteliales del huésped, de la formación de la biopelícula. La mayor producción de hemolisina por parte de las cepas de *E. coli* causantes de PA que hemos encontrado en nuestro estudio coincide con los resultados obtenidos por otros grupos[19,43,68,165,166]. Mitsumori *et al*[167] detectaron la presencia de hemolisina en un 65% de las 107 cepas de prostatitis estudiadas, que representa un porcentaje similar al encontrado en nuestro trabajo (63%). Nuestros datos confirman que el tropismo e invasividad de las cepas de *E. coli* por la próstata recae principalmente en la hemolisina, pero también ofrecen una posible explicación sobre la persistencia de dichas cepas en el sistema secretor prostático debido a su mayor capacidad para formar biopelículas.

Los datos de nuestro estudio también están en consonancia con los resultados obtenidos en estudios previos en cuanto a la mayor presencia de FV y la mayor capacidad de producir biopelículas entre los aislados procedentes de la orina en relación a los de procedencia fecal[165]. La menor expresión de fimbrias tipo 1 ($P < 0.00001$) entre las cepas de *E. coli* de procedencia fecal podría explicar su menor capacidad para la formación de biopelículas respecto a las cepas de procedencia urinaria. De hecho, datos actuales indican que la expresión de las fimbrias tipo 1 por parte de las cepas de UPEC se asocia de forma independiente con la formación de biopelícula. Está bien establecido que la mayor parte de las cepas de *E. coli* aisladas de las heces pertenecen a los grupos filogenéticos A y B1 mientras que los aislados de procedencia urinaria suelen pertenecer a los grupos B2 y D. Las diferencias en el grupo filogenético entre los aislados urinarios y los fecales indican que la próstata no está colonizada, en la mayor parte de los casos, por bacterias comensales del tracto intestinal.

Actualmente se recomienda prolongar el tratamiento de la PA bacteriana durante 6 semanas para evitar la aparición de recurrencias. Esta estrategia terapéutica parece justificada dado que un elevado porcentaje de las cepas de *E. coli* causantes de PA producen biopelícula. Son precisos nuevos estudios a fin de establecer si la detección de la producción *in vitro* de biopelícula puede seleccionar qué pacientes con PA precisan realizar tratamientos antibióticos más prolongados y cuales se pueden beneficiar de pautas terapéuticas más cortas.

8.2. Estudio 2: Implicación de la formación de biopelículas en la persistencia de infección del tracto urinario causada por *E. coli*.

Las ITURs son frecuentes entre las mujeres jóvenes sanas a pesar de que habitualmente tienen tractos urinarios anatómicamente y fisiológicamente normales[135]. En este estudio, los factores microbiológicos han sido los únicos que se han asociado con la aparición de recidivas tras un episodio de ITU, especialmente la formación *in vitro* de biopelícula y la presencia del gen de la yersiniobactina. Estudio previos han permitido identificar diversos FV presentes en las cepas de UPEC así como factores del huésped, particularmente los de tipo conductual, que facilitan la colonización vaginal y/o la entrada de microorganismos uropatógenos a la vejiga urinaria. En relación a los factores dependientes del microorganismo, Mulvey *et al*[31] demostraron como los microorganismos uropatógenos pueden persistir a nivel de la vejiga urinaria, por debajo del uroepitelio, y ser fuente potencial de ITURs. Por otra parte Anderson *et al*[34] observaron como las bacterias intracelulares maduran hacia la formación de biopelículas, creando "pods" en la superficie de la vejiga urinaria. La formación de estas estructuras bacterianas puede explicar la persistencia de las infecciones vesicales a pesar de los mecanismos defensivos del huésped. La yersiniobactina y la aerobactina, dos FV asociados con los sistemas de captación de hierro, también se han asociado con la aparición de recurrencias[138]. Los datos de nuestro estudio confirman estas observaciones, poniendo de manifiesto las necesidades de la bacteria por captar hierro para su crecimiento en condiciones de estrés ambiental. Sin embargo, la producción de biopelícula podría ser el elemento clave que permitiría a las cepas de UPEC

persistir en el reservorio vaginal, a nivel del epitelio vesical o en ambos. La realización de estudios *in vitro* para determinación la formación de biopelícula podría resultar de utilidad en la práctica clínica diaria para seleccionar aquellas pacientes que requieran un abordaje terapéutico dirigido a la erradicación de las cepas de *E. coli* productoras de biopelícula a fin de prevenir futuras recidivas.

8.3. Estudio 3: Expresión de receptores de interleucina 8 (CXCR1 y CXCR2) en mujeres premenopáusicas con infección urinaria recurrente.

De acuerdo con los datos de nuestro estudio, las pacientes con ITURs presentan unos valores de intensidad de fluorescencia media (IFM) de CXCR1, medida a nivel de la superficie de los PMN, comparables con las encontradas en el grupo de controles sanos, aunque el escaso número de pacientes incluidas puede haber influenciado los resultados del estudio. Sin embargo un análisis más detallado de los resultados obtenidos ha revelado que tres pacientes con ITURs presentaban valores de IFM de CXCR1 por debajo del percentil 5 respecto a los valores de IFM de CXCR1 encontrados en el grupo de controles sanos. Estas tres pacientes empezaron a presentar ITUs antes de los 15 años, iniciaron las relaciones sexuales después de esta edad y tenían antecedentes de haber presentado más de un episodio de PNA. El haber iniciado las ITUs antes de los 15 años, particularmente antes del inicio de las relaciones sexuales, ha sido identificado como un factor de riesgo de ITURs, lo que refuerza la idea de la implicación de los factores hereditarios en algunas mujeres con ITURs[139]. Por tanto, nuestras observaciones sugieren que una expresión deficiente de CXCR1, con el consiguiente defecto en la quimiotaxis de los PMN hacia el tracto urinario, podría estar implicada en la mayor susceptibilidad a ITURs en el subgrupo de mujeres premenopáusicas con un inicio de las ITUs durante la infancia.

Un hallazgo inesperado de nuestro estudio ha sido la observación de unos niveles de expresión bajos de IFM de CXCR2 en la superficie de los PMN de las mujeres premenopáusicas con ITURs respecto a los valores encontrados

en el grupo de controles sin ITUs previas. Estos niveles bajos de expresión de CXCR2 han resultado ser aún más marcados en el subgrupo de pacientes con un inicio de las ITUs antes de los 15 años, lo que de nuevo refuerza la idea sobre la importancia de los factores individuales en la patogénesis de las ITURs, especialmente en aquellas mujeres que presentan ITUs desde la infancia. También resulta de interés la observación, en las dos pacientes con los niveles de expresión de superficie de CXCR2 más bajos, de unos niveles de expresión de CXCR1 muy bajos. Diferentes estudios han sugerido que la expresión de CXCR1 y de CXCR2 está regulada por mecanismos dependientes de sus agonistas. La unión de la IL-8 y de la ENA-78 a sus receptores induce una rápida disminución de la expresión de CXCR1 y de CXCR2 a través de la internalización del complejo ligando-receptor[168]. Es posible que otras citocinas quimiotácticas regulen la expresión de CXCR1 y de CXCR2 mediante mecanismos idénticos o similares.

El reclutamiento de los PMN está guiada, no sólo por la IL-8, sino también por otros factores quimiotácticos como el ENA-78 y el GRO-α. Por tanto es posible que una baja expresión de un receptor con múltiples ligandos, como es el CXCR2, pudiera causar una quimiotaxis deficiente de los PMN. La observación en nuestro estudio de bajos niveles de expresión de superficie de CXCR2 en mujeres premenopáusicas con ITURs sugiere la presencia de una capacidad deficiente de GRO-α, ENA-78, y quizás de otras quimiocinas ligandos del CXCR2, de ejercer su actividad quimiotáctica. Estos datos difieren de los obtenidos en estudios *in vitro* previos en los que, usando células epiteliales de riñón infectadas con cepas de *E. coli*, se ha demostrado una disminución de la migración transepitelial de PMN cuando se añadían anticuerpos anti-CXCR1

pero no cuando se utilizaban anticuerpos anti-CXCR2[169]. Sin embargo, otros estudios han observado una inhibición parcial de la quimiotaxis de los PMN mediante la utilización de anticuerpos neutralizantes dirigidos frente a ENA-78 y al GRO-α, que son quimiocinas dependientes del CXCR2[108]. Son precisos más estudios para clarificar si los defectos en la expresión de CXCR2 encontrados en las pacientes con ITURs se deben a defectos genéticos específicos o a la existencia de alteraciones en los mecanismos de regulación.

En la segunda parte de este estudio se ha utilizado la metodología SBT (tipaje basado en la secuencia) para evaluar la presencia de polimorfismos en el promotor y en las regiones codificantes del gen *CXCR1*. Tras estudiar a las 20 pacientes premenopáusicas con ITURs y a los 30 controles sanos sin ITUs previas, hemos detectado la presencia de 3 SNPs responsables de cambios aminoacídicos, dos de los cuales ya habían sido descritos en estudios previos[170,171]. Aunque se había sugerido la existencia de polimorfismos en la región del promotor y en el exón 1 del gen *CXCR1* en niños con PNA de repetición[52], no hemos podido detectar su presencia ni en las pacientes ni en los controles sanos. El análisis del exón 2 del gen *CXCR1* demostró que dos pacientes eran heterozigotas para dos polimorfismos previamente ya descritos, el S276T y el R335C, de la proteína madura. Estas dos pacientes presentaban bajos niveles de IFM de CXCR1. Además, hemos encontrado un polimorfismo previamente no descrito, el S276R, en una tercera paciente con niveles de IFM de CXCR1 normales.

En resumen, no encontramos diferencias significativas en la presencia de SNPs ni en el promotor ni en las regiones codificantes (exón 1 y 2) del gen *CXCR1* entre las pacientes y los controles sanos. Es de interés reseñar que el

polimorfismo R335C se observó en el único control sano con bajos niveles de expresión de CXCR1. El mismo polimorfismo se observó en uno de las tres pacientes con unos niveles de expresión bajos de CXCR1.

El CXCR1 pertenece a la superfamilia de receptores acoplados a proteínas G que están constituidos por siete dominios transmembrana, y tres asas intracelulares y extracelulares. Los polimorfismos S276T y S276R se localizan en el tercer asa extracelular, mientras que el polimorfismo R335C se localiza en el extremo C terminal del CXCR1, de localización intracelular. El extremo C terminal del CXCR1 resulta necesario para los procesos de fosforilación y de desensibilización del receptor[172], y la substitución de arginina por cisteína en la posición 335 puede conferir el potencial de homo o heterodimerización a través de la formación de puentes disulfuro[173]. Por tanto, el polimorfismo R335C tiene el potencial de influir en la funcionalidad del receptor mediante cambios en la estructura secundaria o terciaria del receptor. No sería esperable que los polimorfismos S276T y S276R inducieran cambios mayores en la estructura o función del receptor. De nuevo, se precisan más estudios para evaluar la relevancia del polimorfismo R335C en la funcionalidad del CXCR1.

En conclusión, no hemos encontrado diferencias significativas en los niveles de expresión de CXCR1 entre las mujeres con ITURs y los controles sanos sin ITUs previas. Sin embargo, tres pacientes con historia de ITUs desde la infancia presentaban niveles bajos de expresión de CXCR1 en la superficie de los PMN, que no se relacionaron con la presencia de polimorfismos en el gen *CXCR1*. En relación a los niveles de expresión de CXCR2 se han evidenciado bajos niveles de expresión en las mujeres premenopáusicas con ITURs, especialmente en aquellas con historia de ITUs desde la infancia. Estos

resultados sugieren que unos niveles de expresión bajos de CXCR2 podrían estar asociados a un incremento en la susceptibilidad a ITURs de ciertas mujeres, especialmente en las premenopáusicas con un inicio precoz de las ITUs. La identificación de este grupo de mujeres podría justificar la implementación de estrategias terapéuticas preventivas.

8.4. Estudio 4: Asociación entre el déficit de lectina fijadora de manosa y shock séptico tras pielonefritis aguda causada por *E. coli*.

Aunque las PNA representan uno de los procesos infecciosos más frecuentes, y que ocasionalmente pueden amenazar la vida del paciente, no son bien conocidas las variables predictivas que se asocian a una mala evolución clínica. La presencia de shock séptico se ha descrito como una de las variables clínicas asociadas a un mal pronóstico en pacientes con PNA[156]. En los últimos años numerosos estudios han implicado a la respuesta inmune innata, en particular la dependiente de la MBL, en la patogénesis del shock séptico[174,175]. En base a dichas evidencias científicas, en este estudio se ha evaluado la posible asociación entre la existencia de genotipos de *MBL2* asociados con déficit sérico de MBL y la evolución a shock séptico o la presencia de bacteriemia en mujeres con PNA causadas por *E. coli*.

La MBL es una colectina humana circulante, familia de proteínas que poseen tanto una región colágena como dominios de lectina, que tiene la capacidad de activar el complemento y mediar la fagocitosis tras unirse con una serie de carbohidratos específicos presentes en la superficie de diversas bacterias, virus y hongos. La concentración sérica y el funcionalismo de la MBL están determinados por la presencia de SNPs a nivel del promotor y el exón 1 del gen *MBL2*. Aquellos genotipos responsables de bajos niveles séricos de MBL se han asociado con una mayor frecuencia y gravedad de ciertos procesos infecciosos, particularmente aquellos causados por gérmenes capsulados[114,115]. El déficit de MBL es bastante común con una prevalencia estimada en torno al 10-15% en población caucásica[114,176].

En nuestro estudio, las frecuencias de los genotipos *MBL2* observadas en nuestro grupo control han sido ser muy parecidas a las descritas previamente en una población de las Islas Canarias[177] que a su vez son similares a las observadas en otras poblaciones sanas caucásicas utilizadas como controles en varios estudios europeos[174,178,179]. El haplotipo LYPB, al igual que en estudios previos, ha sido la variante de haplotipo predominante tanto en el grupo control como en las pacientes con PNA.

Este es el primer estudio en el que se ha genotipado el gen *MBL2* en pacientes con PNA. Los resultados de nuestro estudio sugieren que las pacientes con PNA por *E. coli* tienen un mayor riesgo de desarrollar shock séptico pero no bacteriemia. En el análisis multivariado, la presencia de genotipos de *MBL2* deficientes ha sido la única variable que se ha visto asociada a shock séptico a pesar de que otras variables clínicas como la edad, los estados de inmunosupresión y la diabetes, vinculadas todas ellas a PNA de peor pronóstico[6,180,181], también se han incluido en el estudio. En este estudio no se han determinado las concentraciones séricas de MBL, lo que podría ser interpretado como una posible limitación. Sin embargo la relación entre los genotipos *MBL2* y los niveles de MBL ha sido claramente establecida en numerosos estudios previos[116,174,179]. Por tanto el genotipado de la MBL2 podría ser de interés clínico como marcador molecular de riesgo de shock séptico en pacientes con PNA. La asociación entre genotipos de *MBL2*, responsables de bajos niveles de MBL, y el shock séptico no es nueva. Garred *et al*[174] demostraron una asociación entre la presencia de polimorfismos en el gen de la *MBL*, responsables de bajos niveles circulantes de MBL, y el desarrollo de shock séptico en pacientes de cuidados intensivos con síndrome de respuesta

inflamatoria sistémica. Más recientemente, Gordon *et al*[175] encontraron una mayor incidencia de polimorfismos en el exón 1 del gen de la MBL en pacientes con shock séptico en relación a un grupo de controles sanos.

Los mecanismos por los que el déficit de MBL facilita la progresión de las infecciones localizadas y el desarrollo de shock séptico no están del todo claros. Aunque la activación del sistema inmune tras la invasión microbiana se considera un elemento protector, cuando la respuesta inflamatoria es excesiva esta puede conducir al desarrollo de shock séptico[182]. Como ya se ha dicho, la MBL es capaz de activar la cascada del complemento y, como consecuencia, es capaz de inducir la liberación de citocinas proinflamatorias, especialmente factor de necrosis tumoral α (TNF- α), por parte de los monocitos[183,184]. Una excesiva activación del complemento conduciría a la aparición de una gran respuesta inflamatoria que sería deletérea para el huésped. Uno puede hipotetizar que la presencia de niveles bajos de MBL podrían, de hecho, ser beneficioso dado que reduciría la inflamación y por tanto el desarrollo o la severidad del shock séptico. Un nuevo mecanismo por el que la MBL podría influir en el desarrollo del shock séptico sería a través de un efecto directo como modulador de la producción de citocinas proinflamatorias. En este sentido es de especial relevancia el estudio de Jack *et al*[185] en el que incubaron cepas de *Neisseria meningitidis* con concentraciones crecientes de MBL antes de añadir sangre total sin MBL. La liberación por parte de los monocitos del TNF- α, IL-6, y IL-1β se incrementaba ante concentraciones bajas de MBL y se suprimía con concentraciones elevadas de MBL, lo que sugería que la MBL no sólo participa en la activación del complemento sino que además era un potente regulador de las vías de la inflamación. Otro mecanismo por el que la MBL podría interferir en

el desarrollo de shock séptico sería a través de un incremento del aclaramiento de la endotoxina, uno de los inductores más potentes de shock séptico. Ono *et al*[186] han demostrado como la MBL es capaz de incrementar la recaptación del lípido A, el componente molecular responsable de los efectos tóxicos de la endotoxina, por medio de un incremento en la expresión de superficie de los receptores "*scavenger"* de tipo A a nivel de las células de Kupffer.

Sin embargo, la implicación de la MBL en la patogénesis del shock séptico en pacientes con PNA es compleja y podría depender no sólo del huésped (cantidad de MBL) sino también de las características propias del microorganismo (capacidad de unión de la MBL a los diferentes tipos o cepas de la bacteria). En este estudio, todos los episodios de PNA incluidos estaban causados por *E. coli*. La capacidad de unión de la MBL a *E. coli* ha sido previamente evaluada mediante citometría de flujo[110] y por "*enzyme-linked lectin assay"*[111], con resultados contradictorios según la técnica utilizada. Mientras que en el estudio de Neth *et al*[110] únicamente un aislado de *E. coli* se unía a la MBL, en el estudio de Shang *et al*[111], *E. coli* demostró tener una gran capacidad de unión a la MBL. En ambos estudios distintos aislados de *E. coli* mostraron diferencias (intraespecie) en su capacidad de unión a la MBL, lo que podría estar en relación a la existencia de diferencias en la composición hidrocarbonada de las membranas de *E. coli*. La membrana de *E. coli* está compuesta principalmente por el LPS, que es la principal molécula aceptora de la MBL en los BGN[187]. Se ha demostrado como distintos serotipos de *E. coli*, que poseen diferencias en la composición del LPS, exhiben diferencias en su capacidad de unión a la MBL[188]. Desafortunadamente en este estudio no se serotiparon las cepas de *E. coli* implicadas en los episodios de PNA.

Como se ha expuesto previamente, estudios *in vitro* han demostrado que la MBL es capaz de incrementar la fagocitosis de *E. coli* por parte de las células de Kupffer[186]. Por tanto uno podría esperar una mayor incidencia de bacteriemia en los pacientes con déficit de MBL, lo que no se ha observado en este estudio, lo que sugiere que los factores dependientes del microorganismo deben tenerse en consideración cuando se analiza la implicación de los genotipos de la MBL del huésped en diferentes procesos infecciosos. Para añadir un grado superior de dificultad, recientemente se han descrito la presencia de polimorfismos relevantes en el gen de la *MASP2* por lo que el análisis aislado del gen *MBL2* podría no ser suficiente para evaluar la vía de la MBL[189,190].

9. CONCLUSIONES.

1. La producción de biopelícula es más fecuente entre las cepas de *E. coli* causantes de PA. Su producción se asocia con la presencia de hemolisina y con la expresión de fimbrias tipo 1. La producción de biopelícula por parte de las cepas de *E. coli* causantes de PA permite explicar su persistencia en la próstata y la aparición de recurrencias. Las cepas de UPEC producen biopelícula con mayor frecuencia que las cepas fecales de *E. coli.*
2. La detección de la producción *in vitro* de biopelícula por cepas de *E. coli* podría ser de utilidad para ajustar la duración del tratamiento antibiótico en los pacientes con PA.
3. La producción de biopelícula parece jugar un papel en las ITURs, particularmente en las recidivas. La yersiniobactina es otro FV que se asocia a ITURs.
4. La detección de la producción *in vitro* de biopelícula podría resultar útil para seleccionar aquellas pacientes que se podrían beneficiar de un abordaje terapéutico dirigido a la erradicación de las cepas de *E. coli* productoras de biopelícula con el objetivo de prevenir recidivas.
5. Algunas mujeres premenopáusicas con ITURs, sin enfermedades subyacentes y vías urinarias normales presentan niveles de expresión de CXCR1 disminuidos, en particular las mujeres premenopáusicas con ITUs desde la infancia, aunque estos niveles no se asocian con la presencia de polimorfismos ni en la región del promotor ni en el exón 1 del gen *CXCR1*.

6. Los niveles de expresión de CXCR2 podrían aumentar la susceptibilidad a ITURs en las mujeres premenopáusicas.
7. Las pacientes con PNA por *E. coli* que evolucionan a shock séptico tienen una mayor frecuencia de genotipos de *MBL2* asociados a déficit de MBL.
8. En las PNA causadas por *E. coli* la presencia de genotipos deficientes de MBL no se asocia con la presencia de bacteriemia.
9. La determinación del genotipo de la *MBL2* podría ser una herramienta útil para identificar aquellas pacientes con PNA con un mayor riesgo de desarrollar shock séptico.

10. REFERENCIAS BIBLIOGRÁFICAS.

1. Nicolle LE. Epidemiology of urinary tract infections. Infect Med 2001; 18:153-162.

2. Schappert, S.M. Ambulatory care visits to physician offices, hospital outpatient departments, and emergency departments. United States, 1997. Vital Health Stat 13. 1999; 143: i-iv, 1-39.

3. Stamm WE, Hotoon TM. Management of urinary tract infections in adults. N Engl J Med 1993; 329: 1328-1334.

4. Saint S, Chenoweth CE. Biofilms and catheter-associated urinary tract infections. Infect Dis Clin North Am 2003; 17: 411-432.

5. Foxman B. Epidemiology of urinary tract infections: incidence, morbidity, and economic costs. Dis Mon 2003; 49: 53-70.

6. Nicolle LE. Urinary tract pathogens in complicated infection and in elderly individuals. J Infect Dis 2001; 183 (Suppl 1): 5-8.

7. Velasco M, Horcajada JP, Mensa J, Moreno-Martinez A, Vila J, Martinez JA, Ruiz J, Barranco M, Roig G, Soriano E. Decreased invasive capacity of quinolone resistant Escherichia coli in patients with urinary tract infections. Clin Infect Dis 2001; 33:1682-1686.

8. Ronald A. The etiology of urinary tract infection: traditional and emerging pathogens. Am J Med 2002; 113 Suppl 1A: 14-19.

9. Stamey TA. Urinary tract infection in women. En Stamey TA ed. Pathogenesis and treatment of urinary tract infections. Williams & Wilkins, Baltimore, 1980: 122-209.

10. Johnson JR. Evolution of pathogenic Escherichia coli. En: Escherichia coli: virulence mechanisms of a versatile pathogen. Elsevier Science (USA); 2002: 55-77.

11. Herzer PJ, Inouye S, Inouye M, Whittam TS. Phylogenetic distribution of branched RNA-linked multicopy single-stranded DNA among natural isolates of Escherichia coli. J Bacteriol 1990; 172: 6175-6161.

12. Clermont O, Bonacorsi S, Bingen E. Rapid and simple determination of the Escherichia coli phylogenetic group. Appl Environ Microbiol 2000; 66: 4555-4558.

13. Andreu A, Stapleton AE, Fennell C, Lockman HA, Xercavins M, Fernandez F, Stamm WE. Urovirulence determinants of Escherichia coli strains causing prostatitis. J Infect Dis 1997; 176: 464-469.

14. Johnson JR. Stell AL. Extended virulence genotypes of Escherichia coli strains from patients with urosepsis in relation to phylogeny and host compromise. J Infect Dis 2000; 181: 261-272.

15. Moreno E, Planells I, Prats G, Planes AM, Moreno G, Andreu A. Comparative study of Escherichia coli virulence determinants in strains causing urinary tract bacteremia versus strains causing pyelonephritis and other sources of bacteremia. Diagn Microbiol Infect Dis 2005; 53: 93-99.

16. Picard B, Garcia JS, Gouriou S, Duriez P, Brahimi N, Bingen E, Elion J, Denamur J. The link between phylogeny and virulence in Escherichia coli extraintestinal infection. Infect Immun 1999; 67: 546-553.

17. Moreno E, Prats G, Planells I, Planes AM, Perez T, Andreu A. Characterization of Escherichia coli isolates derived from phylogenetic groups A y B1 causing extraintestinal infection. Enferm Infecc Microbiol Clin 2006; 24:483-489.

18. Soto SM. Expression of virulence factors by extraintestinal strains of Escherichia coli. Enfer Infecc Microbio Clin 2006; 24: 479-480.

19. Johnson JR, Kuskowsky MA, Gajewski A, Soto S, Horcajada JP, Jimenez de Anta MT, Vila J. Extended virulence genotypes and phylogenetic background of Escherichia coli isolates from patients with cystitis, pyelonephritis, or prostatitis. J Infect Dis 2005; 191: 46-50.

20. Russo TA. Johnson JR. Proposal for a new inclusive designation for extraintestinal pathogenic isolates of Escherichia coli: ExPEC. J Infect Dis 2000; 181; 1753-1754.

21. Johnson JR, Moseley SL, Roberts PL, Stamm WE. Aerobactin and other virulence factors genes among strains of Escherichia coli causing urosepsis: association with patients characteristics. Infect Immun 1988; 56: 405-412.

22. Linder H, Engberg I, Baltzer IM, Jann K, Svanborg-Eden C. Induction of inflammation by Escherichia coli on the mucosal level: requirements for adherence and endotoxin. Infect Immnu 1988; 56: 1309-1313.

23. Connell I, Agace W, Klemm P, Schembri M, Marild S, Svanborg C. Type 1 fimbrial expression enhances Escherichia coli virulence for the urinary tract. Proc Natl Acad Sci USA 1996; 93: 9827-9832.

24. Schilling JD, Mulvey MA, Hultgren SJ. Structure and function of Escherichia coli type 1 pili: new insight into the pathogenesis of urinary tract infections. J Infect Dis 2001; 183 Suppl 1: 36-40.

25. Wu XR, Sun TT, Medina JJ. In vitro binding of type 1-fimbriated Escherichia coli to uroplakins Ia and Ib: relation to urinary tract infections. Proc Natl Acad Sci USA 1996; 93: 9630-9635.

26. Pak J, Pu Y, Zhang ZT, Hasty DL, Wu XR. Tamm-Horsfall protein binds to type 1 fimbriated Escherichia coli and prevents E. coli from binding to uroplakin Ia and Ib receptors. J Biol Chem 2001; 276: 9924-9930.

27. Balish MJ, Jensen J, Uehling DT. Bladder mucin: a scanning electron microscopy study in experimental cystitis. J Urol 1982; 128: 1060-1063.

28. Hultgren SJ, Porter TN, Schaeffer AJ, Duncan JL. Role of type 1 and effects of phase variation on lower urinary tract infections produced by Escherichia coli. Infect Immun 1985; 50: 370-377.

29. Bergthorsson U, Ochman H. Heterogenicity of genome sizes among natural isolates of Escherichia coli. J Bacteriol 1995; 177: 5784-5789.

30. Schembri MA, Klemm P. Biofilm formation in a hydrodynamic environment by novel FimH variants and ramifications for virulence. Infect Immun 2001; 69:1322-1328.

31. Mulvey MA, Lopez-Boado YS, Wilson CL, Roth R, Parks WC, Heuser J, Hultgren SJ. Induction and evasion of host defenses by type 1-piliated uropathogenic Escherichia coli. Science 1998; 282:1494-1497.

32. Martinez JJ Mulvey MA, Schilling JD, Pinker JS, Hultgren SJ. Type 1 pilus-mediated bacterial invasion of bladder epithelial cells. EMBO J 2000; 19: 2803-2812.

33. Mulvey MA, Schilling JD, Hultgren SJ. Establishment of a persistent Escherichia coli reservoir during the acute phase of a bladder infection. Infect Immun 2001; 69: 4572-4579.

34. Anderson GG, Palermo JJ, Schilling JD, Roth R, Heuser J, Hultgren SJ. Intracellular bacterial biofilm-like pods in urinary tract infections. Science 2003; 301: 105-107.

35. Langermann S, Palaszynski S, Barnhart M, Auguste G, Pinker JS, Burlein J Barren P, Koenig S, Leath S, Jones CH, Hultgren SJ. Prevention of mucosal Escherichia coli infection by FimH-adhesin based systemic vaccination. Science 1997; 276: 607-611.

36. Goluszko P, Moseley SL, Truong DL, Kaul A, Williford JR, Selvarangan R, Nowicki S, Nowicki B. Devlopment of experimental model of chronic pyelonephritis with Eschrichia coli O75:K5:H-bearing Dr fimbriae: mutation in the *dra* region prevented tubulointerstitial nephritis. J. Clin Invest 1997; 99: 1662-1672.

37. Roberts JA, Marklund BI, Ilver D, Haslam D, Kaack MB, Baskin G, Louis M, Möllby R, Winberg J, Normark S. The Gal(alpha l-4)Gal-specific tip adhesion of Escherichia coli P-fimbriae is needed for pyelonephritis to occur in the normal urinary tract. Proc Natl Acad Sci USA 1994: 11889-11893.

38. Leffler H, Svanborg-Eden C. Chemical identification of a glycosphingolipid receptor for Escherichia coli attaching to human urinary tract epithelial cells and agglutinating human erythrocytes. FEMS Microbiol Lett 1980; 8:127-134.

39. Svanborg C, Eriksson B, Hanson LA, Jodal U, Kaijser B, Lidin-Janson G, Lindberg U, Olling S. Adhesion to normal human uro-epithelial cells of Escherichia coli of children with various forms of urinary tract infection. J Pediatr 1978; 93: 398-403.

40. Johnson JR. Virulence factors in Escherichia coli urinary tract infection. Clin Microbiol Rev 1991; 4: 80-128.

41. Johanson IM, Plos K, Marklund BI, Svanborg C. Pap, papG and prs DNA sequences in Escherichia coli from the fecal flora and the urinary tract. Microb Pathol 1993; 15: 121-129.

42. Otto G, Sandberg T, Marklund BI, Ulleryd P, Svanborg C. Virulence factors and *pap* genotype in Escherichia coli isolates from women with acute pyelonephritis, with and without bacteriemia. Clin Infect Dis 1993; 17: 448-456.

43. Ruiz J, Simon K, Horcajada JP, Velasco M, Barranco M, Roig G, Moreno-Martinez A, Martinez JA, Jimenez de Anta T, Mensa J, Vila J. Differences in virulence factors among clinical isolates of Escherichia coli causing cystitis and pyelonephritis in women and prostatitis in men. J Clin Microbiol 2002; 40: 4445-4449.

44. Otto G, Magnusson M, Svensson M, Braconier J, Svanborg C. Pap genotype and P fimbrial expression in Escherichia coli causing bacteremic and nonbacteremic febrile urinary tract infection. CID 2001; 32: 1523-1531.

45. Godaly G, Otto G, Burdick MD, Strieter RM, Svanborg C. Fimbrial lectins influence the chemokine repertoire in the urinary tract mucosa. Kidney Int 2007; 71: 721-723.

46. Wult B, Bergsten G, Samuelsson M, Gebretsadik N, Hull R, Svanborg C. The role of P fimbriae for colonization and host response induction in the human urinary tract. J Infect Dis 2001; 183 Suppl 1: 43-46.

47. Smithson A, Ruiz J, Soto S, Horcajada JP, Martinez JA, Moreno-Martinez A, Vila J, Mensa J. “Influence of Escherichia coli virulence factors in the levels of C reactive protein in febrile urinary tract infections”. En: Programa y abstracts del 14th European Congress of Clinical Microbiology and Infectious Diseases (Praga). CMI, 2004, 262.

48. Frendeus B, Wachtler C, Hedlund M, Fisher H, Samuelsson P, Swensson M, Svanborg C. Escherichia coli P fimbriae utilize the Toll-like receptor 4 pathway for cell activation. Mol Microbiol 2001; 40: 37-50.

49. Hedlund M, Svensson M, Nilsson A, Duan RD, Svanborg C. Role of ceramide-signaling pathway in cytokine response to P-fimbriated Escherichia coli. J Exp Med 1996; 183: 1037-1044.

50. Hedlund M, Wachtler C, Johansson E, Hang L, Somerville E, Darveau RP, Svanborg C. P fimbriae-dependent, lypopolysaccharide-independent activation of epithelial cytokines responses. Mol Microbiol 1999; 33: 693-703.

51. Samuelsson P, Hang L, Wullt B, Irjala H, Svanborg C. Toll-like receptor 4 expression in the human urinary tract mucosa. Infect Immun 2004; 72: 3179-3186.

52. Godaly G, Bergsten G, Hang L, Fischer H, Frendeus B, Lundsted AC, Samuelsson M, Samuelsson P, Svanborg C. Neutrophil recruitment, chemokine receptors, and resistance to mucosal infection. J Leuk Biol 2001; 69: 899-906.

53. Fischer H, Yamamoto M, Akira S, Beutler B, Svanborg C. Mechanisms of pathogen-specific TLR4 activation in the mucosa: fimbriae, recognition receptors an adaptador protein selection. Eur J Immunol 2006; 36: 267-277.

54. Nowicki B, Labigne A, Moseley S, Hull R, Hull S, Moulds J. The Dr hemagglutinin, afimbrial adhesins AFA-I and AFA-III, and F1845 fimbriae of uropathogenic and diarrhea-associated Escherichia coli belong to a family of hemagglutinins with Dr receptor recognition. Infect Immun 1990; 58: 279-281.

55. Marre R, Kreft B, Hacker J. Genetically engineered S and F1C fimbriae differ in their contribution to adherence of Escherichia coli to cultured renal tubular cells. Infect Immun 1990; 58: 3434-3437.

56. Pham TQ, Goluszko P, Popov V, Nowicki S, Nowicki BJ. Molecular cloning and characterization of Dr-II, a nonfimbrial adhesin-I-like adhesin isolated from gestational pyelonephritis-associated Escherichia coli that binds to decay-accelerating factor. Infect Immun 1997; 65: 4309-4318.

57. Marre R, Hacker J. Role of S- and common-type 1 fimbriae of Escherichia coli in experimental upper and lower urinary tract infection. Microb Pathogen 1987; 2: 223-226.

58. Guyer DM, Gunther NW 4th, Mobley HL. Secreted proteins and other features specific to uropathegenic Escherichia coli. J Infect Dis 2001; 183 Suppl 1: 32-35.

59. Oelschlaeger TA, Dobrindt U, Hacker J. Virulence factors of uropathogens. Curr Opin Urol 2002; 12: 33-38.

60. Linggood MA, Ingram PL. The role of alpha hemolysin in the virulence of Escherichia coli for mice. J Med Microbiol 1982; 15: 23-30.

61. Cavalieri SJ, Zinder IS. Effect of Escherichia coli alpha-hemolysin on human peripheral leukocyte viability in vitro. Infect Immun 1982; 36:455-461.

62. Svanborg C, Godaly G. Bacterial virulence in urinary tract infection. Infect Dis Clin North Am 1997; 513-529.

63. Guyer DM, Radulovic S, Jones FE, Mobley HL. Sat, the secreted autotransporter toxin of uropathogenic Escherichia coli, is a vacuolating cytotoxin for bladder and kidney epithelial cells. Infect Immun 2000; 70:4539-4546.

64. Guignot J, Chaplais C, Coconnier-Polter MH, Servin AL. The secreted autotransporter toxin, Sat, functions as a virulence factor in Afa/Dr diffusely adhering Escherichia coli by promoting lesions in tight junctions of polarized cells. Cell Microbiol 2007; 9: 204-221.

65. Russo TA, Carlino UB, Johnson JR. Identification of a new iron-regulated virulence gene, ire A, in an extraintestinal pathogenic isolate of Escherichia coli. Infect Immun 2001; 69: 6209-6216.

66. Russo TA, Brown JJ, Jodush ST, Johnson JR. The O4 specific antigen moiety of lipopolysaccharide but not the K54 group 2 capsule is important for urovirulence in an extraintestinal isolate of Escherichia coli. Infect Immun 1996; 64; 2343-2348.

67. Linder H, Engberg I, Baltzer IM, Jann K, Svanborg-Eden C. Induction of inflammation by Escherichia coli on the mucosal level: requirements for adherence and endotoxin. Infect Immun 1988; 56:1309-1313.

68. Vila J, Simon K, Ruiz J, Horcajada JP, Velasco M, Barranco M, Moreno A, Mensa J. Are quinolone resistant uropathogenic Escherichia coli less virulent? J Infect Dis 2002; 186:1039-1042.

69. Horcajada JP, Soto S, Gajewski A, Smithson A, Jimenez de Anta MT, Mensa J, Vila J, Johnson JR. Quinolone-resistant uropathogenic Escherichia coli strains from phylogenetic group B2 have fewer virulence factors than their susceptible counterparts. J Clin Microbiol 2005; 43: 2962-2964.

70. Hacker J, Blum-Oehler G, Müldorfer I, Tschäpe H. Pathogenicity islands of virulent bacteria: structure, function and impact on microbial evolution. Mol Microbiol 1997; 23:1089-1097.

71. Soto SM Jimenez de Anta MT, Vila J. Quinolones induce partial or total loss of pathogenicity islands in uropathogenic Escherichia coli by SOS-dependent or independent pathways, respectively. Antimicrob Agents

Chemother 2006; 50:649-653.

72. Einstein BI. Phase variation of type 1 fimbriae in Escherichia coli is under transcriptional control. Science 1981; 337-339.

73. Guyer DM, Gunther NW 4th, Mobley HL. Secreted proteins and other features specific to uropathogenic Escherichia coli. J Infect Dis 2001; 183 Suppl 1: 32-35.

74. Dobrindt U, Blum-Oehelr G, Nagy G, Schneider G, Johann A, Gottschalk G, Hacker J. Genetic structure and distribution of four pathogenicity islands (PAI (536) to PAI IV(536)) of uropathogenic Escherichia coli strain 536. Infect Immun 2002; 70: 6365-6372.

75. Hacker J, Kaper JB. Pathogenicity islands and the evolution of microbes. Annu Rev Microbiol 2000; 54;641-679.

76. Bergthorsson U, Ochman H. Heterogenicity of genome sizes among natural isolates of Escherichia coli. J Bacteriol 1995; 177:5784-5789.

77. Sabate M, Moreno E, Perez T, Andreu A, Prats G. Pathogenicity island markers in commensal and uropathogenic E. coli isolates. Clin Microbiol Infect 2006; 12: 880-886.

78. Costerton JW, Stewart PS, Greenberg EP. Bacterial biofilms: a common cause of persistent infections. Science 1999; 284: 1318-1322.

79. Nickel JC. Catheter-associated urinary tract infection: new perspectives on old problems. Can J Infect Control 1991; 6:38-42.

80. Farrieres L, Hancock V, Klemm P. Specific selection for virulent urinary tract infectious Escherichia coli strains during catheter-associated biofilm formation. FEMS Immunol Med Microbiol 2007; 51:212-219.

81. Sheikh J, Hicks S, Dall´Agnol M, Phillips AD, Nataro JP. Roles for FIS and YafK in biofilm formation by enteroaggregative Escherichia coli. Mol Microbiol 2001; 41:983-997.

82. Nickel JC, Costerton JW. Coagulase–negative staphylococcus in chronic prostatitis. J Urol 1992; 147: 398-401.

83. Nickel JC, Costerton JW, McLean RJ, Olson M. Bacterial biofilms: influence on the pathogenesis, diagnosis and treatment of urinary tract infections. J Antimicrob Chemother 1994; 33 Suppl A: 31-41.

84. Nickel JC, Olson M, McLean RJ, Grant SK, Costerton JW. An ecological study of infected urinary stone genesis in an animal model. Br J Urol 1987; 59: 21-30.

85. Hancock V, Ferrieres L, Klemm P. Biofilm formation by asymptomatic and

virulent urinary tract infectious Escherichia coli strains. FEMS Microbiol Lett 2007; 267: 30-37.

86. Stapleton A, Stamm WE. Prevention of urinary tract infection. Infect Clin North Am 1997; 11: 719-733.

87. Soto S.M, Smithson A, Horcajada JP, Martinez JA, Vila J, Mensa J. "Caracterización de cepas de Escherichia coli causantes de infección urinaria recurrente". En: Programa y abstracts del XI Congreso de la Sociedad Española de Enfermedades Infecciosas y Microbiología Clínica (SEIMC). Enfermedades Infecciosas y Microbiología Clínica, 2004, 202.

88. Davey ME, O´Toole GA. Microbial biofilms: from ecology to molecular genetics. Microbiology Mol Biol Rev 2000; 64:847-865.

89. Pratt LA, Kolter R. Genetic analysis of Escherichia coli biofilm formation: roles of flagella, motility, chemotaxis and type I pili. Mol Microbiol 1998; 30: 285-293.

90. Mah TF, O´Toole GA. Mechanisms of biofilm resistance to antimicrobial agents. Trends Microbiol 2001; 9:34-39.

91. Reid G, Hsiehl J, Potter P, Mighton J, Lam D, Warren D, Stephenson J. Cranberry juice consumption may reduce biofilms on uroepithelial cells: pilot study in spinal cord injured patients. Spinal Cord 2001; 39: 26-30.

92. Yassien M, Khardori N. Interaction between biofilms formed by Staphylococcus epidermidis and quinolones. Diagn Microbiol Infect Dis 2001; 40: 79-89.

93. Kumon H. Management of biofilm infections in the urinary tract. World J Surg 2000; 24:1193-1196.

94. Ghigo JM. Natural conjugative plasmids induce bacterial biofilm development. Nature 2001; 412:412:442-445.

95. Reisner A, Holler BM. Molin S, Zechner E. Synergistic effects in mixed Escherichia coli biofilms: conjugative plasmid transfer drives biofilm formation. J Bacteriol 2006; 188: 3582-3588.

96. Sobel JD. Pathogenesis of urinary tract infection. Role of host defenses. Infect Dis North Am 1997; 11: 531-549.

97. Svanborg C, Bergsten G, Fischer H, Frendeus B, Godaly G, Gustafsson E, Hang L, Hedlund M, Karpman D, Lundstedt AC, Samuelsson M, Samuelsson P, Svensson M, Wullt B. The innate host response protects and damages the infected urinary tract. Ann Med 2001; 33: 563-570.

98. Olszyna DP, Opal SM, Prins JM, Horn DL, Speelman P, van Deventer SJ, van der Poll T. Chemotactic activity of CXC chemokines interleukin-8,

growth related oncogen-alpha and epithelial cell-derived neutrophil-activating protein-78 in urine in patients with urosepsis. J Infect Dis 2000; 182:1731-1737.

99. Horcajada JP, Velasco M, Filella X, Alvarez L, De Lazzari E, Marin JL, Collvinent B, Smithson A, Martinez JA, Noguero M, Vila J, Mensa J. Evaluation of inflammatory and renal-injury markers in women treated with antibiotics for acute pyelonephritis caused by Escherichia coli. Clin Diagn Lab Immunol 2004; 11: 142-146.

100. Frendeus B, Godaly G, Hang L, Karpman D, Svanborg C. Interleukin-8 receptor deficiency confers susceptibility to acute pyelonephritis. J Infect Dis 1999; 183 Suppl 1: 56-60.

101. Agace W, Patarroyo M, Svensson M, Calerman, Svanborg C. Escherichia coli induce trans-uroepithelial neutrophil migration by an intercelular adhesion molecule-1-dependent mechanisms. Infect Immun 1995; 63: 4045-4062.

102. Morris SW, Nelson N, Valentine MB, Shapiro DN, Look AT, Kozlosky CJ, Beckmann MP, Cerreti DP. Assignment of the genes encoding human interleukin-8 receptor types 1 and 2 and an interleukin-8 receptor pseudogene to chromosome 2q35. Genomics 1992; 14: 685-691.

103. Ahuja SK, Murphy PM. The CXC chemokines growth-regulated oncogene (GRO) alpha, GRObeta, GROgamma, neutrophil-activating peptide-2, and epithelial cell-derived neutrophil activating peptide-78 are potent agonists for the type B, but not the type A, human interleukin-8 receptor. J Biol Chem 1996; 271: 20545-20550.

104. Murphy PM. Neutrophil receptors for interleukin-8 and related CXC chemokines. Semin Hematol 1997; 34: 311-318.

105. Cacalano G, Lee J, Kikly K, Ryan AM, Pitts-Meek S, Hultgren B, Wood WI, Moore MW. Neutrophil and B cell expansion in mice that lack the murine IL-8 receptor homolog. Science 1994; 265:682-684.

106. Hang L, Frendeus B, Godaly G, Svanborg C. Interleukin-8 receptor knockout mice have subepithelial neutrophil entrapment and renal scrarring following acute pyelonephritis. J. Infect. Dis 2000 ; 182: 1738-1748.

107. Svensson M, Irjala H, Alm P, Holmqvist B, Lundstedt AC, Svanborg C. Natural history of renal scarring in susceptible mIL-8Rh-/- mice. Kidney Int 2005; 67: 103-110.

108. Olszyna DP, Florquin S, Sewnath M, Branger J, Speelman P, van Deventer SJ, Strieter RM, van der Poll T. CXC chemokine receptor 2 contributes to host defense in murine urinary tract infection. J Infect Dis 2001; 184: 301-317.

109. Frendeus B, Godaly G, Hang L, Karpman D, Lunstedt A, Svanborg C. Interleukin 8 receptor deficiency confers susceptibility to acute experimental pyelonephritis and may have a human counterpart. J Exp Med 2000; 192: 881-890.

110. Neth O, Jack DL, Dodds AW, Holzel H, Klein NJ, Turner MW. Mannose-binding lectin binds to a range of clinically relevant microorganisms and promotes complement deposition. Infect Immun 2000; 68:688-693.

111. Shang SQ, Chen GX, Shen J, Yu XH, Wang KY. The binding of MBL to common bacteria in infectious diseases of children. J Zhejiang Univ Sci 2005; 6:53-56.

112. Matsushita M. The lectin pathway of the complement system. Microbiol Immunol 1996; 40:887-893.

113. Sastry K, Herman GA, Day L, Deignan E, Bruns G, Morton CC, Ezekowitz RA. The human mannose-binding protein gene. Exon structure reveals its evolutionary relationship to a human pulmonary surfactant gene and localization to chromosome 10. J Exp Med 1989; 170:1175-1189.

114. Guardia A, Lozano F. Mannose binding lectin deficiencies in infectious and inflammatory disorders. Rev Med Microbiol 2003; 14:41-52.

115. Tuner MW, Hamvas RM. Mannose-binding lectin: structure, function, genetics and disease associations. Rev Immunogenet 2000; 2:305-322.

116. Steffensen R, Thiel S, Varming K, Jersild C, Jensenius JC. Detection of structural gene mutations and promoter polymorphisms in the mannan-binding lectin (MBL) gene by polymerase chain reaction with sequence-specific primers. J Immunol Methods 2000; 241:33-42.

117. Lozano F, Suarez B, Muñoz A, Jensenius JC, Mensa J, Vives J, Horcajada JP. Novel MASP2 variants detected among North African and Sub-Saharan individuals. Tissue Antigens 2005; 66:131-135.

118. Madsen HO, Garred P, Thiel S, Kurtzhals JA, Lamm LU, Ryder LP, Svejgaard A. Interplay between promoter and structural gene variants control basal serum level of mannan-binding protein. J. Immunol 1995; 155: 3013-3020.

119. Madsen HO, Garred P, Thiel S, Kurtzhals JA, Lamm LU, Ryder LP, Svejgaard A. Interplay between promoter and structural gene variants control basal serum level of mannan-binding protein. J Immunol 1995; 155:3013-3020.

120. Takahashi K, Ezekowitz RA. The role of the mannose binding lectin in innate immunity. Clin Infec Dis 2005; 41 Suppl 7: 440-444.

121. Koch A, Melbye M, Sorensen P, Homoe P, Madsen HO, Molbak K, Hansen

CH, Andersen LH, Hahn GW, Garred P. Acute respiratory tract infections and mannosse-binding lectin insufficiency during early childhood. JAMA 2001; 285:1316-1321.

122. Peterslund NA, Koch C, Jensenius JC, Thiel S. Association between deficiency of mannose-binding lection and severe infection after chemotherapy. Lancet 2001; 358: 637-638.

123. Garred P, Madsen HO, Baslev U, Hofman B, Pedersen C, Gerstoft J, Svejgaard A. Susceptibility to HIV infection and progression of AIDS in relation to variant alleles of mannose-binding lectin. Lancet 1997; 349:236-240.

124. Garred P, Madsen HO, Halberg P, Petersen J, Kronborg G, Svejgaard S, Andersen V, Jacobsen S. Mannose binding polymorphisms and susceptibility to infection in systemic lupus erythematosus. Arthritis Rheum 1999 ; 42:2145-2152.

125. Nicolle LE. Asymptomatic bacteriuria. When to screen and when to treat. Infect Disc Clin N Am 2003; 17:367-394.

126. Patterson, Andriole T. Detection, significance and therapy of bacteriuria in pregnancy. Infect Dis Clin North Am 1997; 11:593-608.

127. Nicolle LE. Asymptomatic bacteriuria in the elderly. Infect Dis Clin North Am 1997; 11: 647-662.

128. Tambyah PA, Maki DG. Catheter-associated urinary tract infection is rarely symptomatic:a prospective study of 1.497 catheterized patients. Arch Intern Med 2000; 160:678-682.

129. Cardenas DD, Hooton TM. Urinary tract infection in persons with spinal cord injury. Arch Phys Med Rehabil 1995; 76: 272-280.

130. Harding GK, Zhanel GG, Nicolle LE, Cheang M. Antimicrobial treatment in diabetic women with asymptomatic bacteriuria. N Engl J Med 2002; 347: 1576-1583.

131. Zdziarski J, Svanborg C, Wullt B, Hacker J, Dobrint U. Molecular basis of commensalism in the urinary tract: low virulence or virulence attenuation? Infect Immun 2008; 76: 695-703.

132. Ragnarsdottir B, Samuelsson M, Gustafsson M, Leijonhufvud I, Karpman D, Svanborg C. Reduced Toll-like receptor 4 expression in children with asymptomatic bacteriuria. J Infect Dis 2007; 196; 475-484.

133. Stamm WE, Norrby SR. Urinary tract infections: disease panorama and challenges. J Infect Dis 2001;183 Suppl 1: 1-4.

134. Kunin CM. Urinary tract infections in females. Clin Infect Dis 1994; 18:1-12.

135. Finer G, Landau D. Pathogenesis of urinary tract infections with normal female anatomy. Lancet Infect Dis 2004; 4: 631-635.

136. Foxman B, Zhang L, Tallman P, Palin K, Rode C, Bloch C, Gilllespie B, Marrs CF. Virulence characteristics of Escherichia coli causing first urinary tract infection predict risk of second infection. J Infect Dis. 1995; 172: 1536-1541.

137. Stapleton A, Moseley S, Stamm WE. Urovirulence determinants in Escherichia coli isolates causing first episode and recurrent cystitis in women. J Infect Dis 1991: 163: 773-779.

138. Johnson JR, O´Bryan TT, Delavari P, Kuskowsky M, Stapleton A, Carlino U, Russo TA. Clonal relationships and extended virulence genotypes among Escherichia coli isolates from women with a first or recurrent episodes of cystitis. J Infect Dis 2001; 183: 1508-1517.

139. Scholes D, Hooton T, Roberts PL, Stapleton AE, Gupta K, Stamm WE. Risk factors for recurrent urinary tract infection in young women. J Infect Dis 2000; 182:1177-1182.

140. Schaeffer AJ, Stamey TA. Studies of introital colonization in women with recurrent urinary infections. J Urol 1975; 118: 221-224.

141. Brumfitt W, Gargan RA, Hamilton-Miller JM. Periurethral enterobacterial carriage preceding urinary infection. Lancet 1987; 1: 824-826.

142. Schaeffer AJ, Jones JM, Dunn JK. Association of in vitro Escherichia coli adherence to vaginal and buccal epithelial cells with susceptibility of women with recurrent urinary tract infections. N Engl J Med 1981; 304:1062-1066.

143. Sheinfeld J, Schaeffer AJ, Cordon-Cardo C, Rogatko A, Fair WR. Association of Lewis blood group phenotype with recurrent urinary tract infections in women. N Engl J Med 1989; 320:773-777.

144. Stapleton AE, Stround MR, Hakamori SI, Stamm WE. The globoseries glycosphingolipid sialosyl galactosyl globoside is found in urinary tract tissues and is a preferred binding receptor in vitro for uropathogenic Escherichia coli expressing pap-encoded adhesions. Infect Immun 1998; 66: 3856-3861.

145. Lomberg H, Hanson LA, Jacobsson B, Jodal U, Leffler H, Svanborg EC. Correlation of P blood group, vesicoureteral reflux and bacterial attachment in patients with recurrent pyelonephritis. N Eng J Med 1983; 308:1189-1192.

146. Tomisawa S, Kogure T, Kuruome T, Leffler H, Lomberg H, Shimabukoro N, Terao K, Svanborg C. P blood group and pronensses to urinary tract

infection in Japanese children. Scand J Infect Dis 1989; 21: 403-408.

147. Schaeffer AJ, Radvany RM, Chmiel JS. Human leukocyte antigens in women with recurrent urinary tract infections. J Infect Dis 1983: 148:604.

148. Condron C, Toomey D, Casey RG, Shaffii M, Creagh T, Bouchier-Hayes D. Neutrophil bactericidal function is defective in patients with recurrent urinary tract infections. Urol Res 2003; 31: 329-334.

149. Raz R, Gennesin Y, Wasser J, Stoler Z, Rosenfeld S, Rottensterich E. Recurrent urinary tract infection in postmenopausal women. Clin Infect Dis 2000; 30: 152-156.

150. Raz R, Stamm WE. A controlled trial of intravaginal estriol in postmenopausal women with recurrent urinary tract infections. N Engl J Med 1993; 329: 753-756.

151. Geerlings SE, Meiland R, Hoepelman AI. Pathogenesis of bacteriuria in women with diabetes mellitus. Int J Antimicrob Agents 2002; 19: 539-545.

152. Hooton TM, Stapleton AE, Roberts PL, Winter C, Scholes D, Bavendam T. Perineal anatomy and urine voiding characteristics of young women with and without recurrent urinary tract infections. Clin Infect Dis 1999; 29: 1600-1601.

153. Gupta K, Hillier SL, Hooton TM, Roberts PL, Stamm WE. Effects of contraceptive method on the vaginal microbial flora: a prospective evaluation. J Infect Dis 2000; 181: 595-601.

154. Smith HS, Hughes JP, Hooton TM, Roberts P, Scholes D, Stergachis A, Stapleton A, Stamm WE. Antecedent antimicrobial use increases the risk of uncomplicated cystitis in young women. Clin Infect Dis 1997; 25:63-68.

155. Foxman B, Klemstine KL, Brown PD. Acute pyelonephritis in US hospitals in 1997: hospitalization and in-hospital mortality. Ann Epidemiol 2003; 13:144-150.

156. Efstathiou SP, Pefanis AV, Tsioulos DI, Zacharos ID, Tsiakou AG, Mitromaras AG, Mastorantonakis SE, Kanavaki SN, Mountokalakis TD. Acute pyelonephritis in adults: prediction of mortality and failure of treatment. Arch Intern Med 2003; 163: 1206-1212.

157. Scholes D, Hooton TM, Roberts PL, Gupta K, Stapleton AE, Stamm WE. Risk factors associated with acute pyelonephritis in healthy women. Ann Intern Med 2005; 142: 20-27.

158. Ishitoya S, Yamamoto S, Mutsumori K, Ogawa O, Terai A. Non-secretor status is assocated with female acute uncomplicated pyelonephritis. BJU Int 2002; 89: 851-854.

159. Krieger JN, Ross SO, Deutsch L, Riley DE. Seminal fluid analysis in chronic prostatitis/chronic pelvic pain síndrome. Andrologia 2003; 35:266-270.

160. Smithson A, Mateos JJ, Horcajada JP, Velasco M, Lomeña F, Moreno A, Martinez JA, Vila J, Mensa J. "Predictive value of physical examination in the topographic diagnosis of febrile urinary tract infections in males". En: Programa y abstracts del 43rd Interscience Conference on Antimicrobial Agents and Chemotherapy (Chicago). ICAC 2003, 407.

161. Warren JW. The catheter and urinary tract infection. Infect Dis Clin North Am 1997; 11: 609-622.

162. Guyer DM, Kao JS, Mobley HL. Genomic analysis of a pathogenicity island in uropathogenic Escherichia coli CFT073: distribution of homologous sequences among isolates from patients with pyelonephritis, cistitis and catheter-associated bacteriuria and from fecal samples. Infect Immun 1998; 66: 4411-4417.

163. Kravchick S, Cytron S, Agulansky L, Ben-Dor D. Acute prostatitis in middle-aged men: a prospective study. BJU Int 2004; 93: 93-96.

164. Hanna A, Berg M, Stout V, Razatos A. Role of capsular colanic acid in adhesion of uropathogenic Escherichia coli. App Environ Microbiol 2003; 69: 4474-4481.

165. Andreu A, Stapleton AE, Fennell C, Lockman HA, Xercavins M, Fernandez E, Stamm WE. Urovirulence determinants in Escherichia coli strains causing prostatitis. J Infect Dis 1997; 176: 464-469.

166. Terai A, Yamamoto S, Mitsumori K, Okada Y, Kurazono H, Takeda Y, Yoshida O. Escherichia coli virulence factors and serotypes in acute bacterial prostatitis. Int J Urol 1997; 4: 289-294.

167. Mitsumori K, Terai A, Yamamoto S, Ishitoya S, Yoshida O. Virulence characteristics of Escherichia coli in acute bacterial prostatitis. J Infect Dis 1999; 180: 1378-1381.

168. Chuntharapai A, Kim KJ. Regulation of the expression of IL-8 receptor a/B by IL-8: possible functions of each receptor. J Immunol 1995 ; 155 : 2587-2594.

169. Godaly G, Hang L, Frendeus B, Svanborg C. Transepithelial neutrophil migration is CXCR1 dependent in vitro and is defective in IL-8 receptor knock-out mice. J Immunol 2000 ; 165 : 5287-5294.

170. Kato H, Tsuchiya N, Tokunaga K. Single nucleotide polymorphisms in the coding regions of human CXC-chemokine receptors CXCR1, CXCR2 and CXCR3. Genes Immun 2000; 1: 330-337.

171. Stemmler S, Arinir U, Klein W, Rohde G, Hoffjan S, Wirkus N, Reinitz-

Rademacher K, Bufe A, Schultze-Werninghaus G, Epplen JT. Association of interleukin-8 receptor alpha plymorphisms with chronic obstructive pulmonary diseases and asthma. Genes Immun 2005; 6: 225-230.

172. Richardson RM, Marjoram RJ, Barak LS, Snydermann R. Role of the cytoplasmatic tails of CXCR1 and CXCR2 in mediating leukocyte migration, activation, and regulation. J Immunol 2003; 170: 2904-2911.

173. Bockaert J, Pin JP. Molecular tinkering of G protein-coupled receptors: an evolutionary success. EMBO J 1999; 18: 1723-1729.

174. Garred P, Strom J, Quist L, Taaning E, Madsen HO. Association of mannose-binding lectin polymorphisms with sepsis and fatal outcome in patients with systemic inflammatory response syndrome. J Infect Dis 2003; 188 : 1394-1403.

175. Gordon AC, Waheed U, Hansen TK, Hitman GA, Garrad CS, Turner MW, Klei NJ, Brett SJ, Hinds CJ. Mannose-binding lectin polymorphisms in severe sepsis: relationship to levels, incidence, and outcomes. Shock 2006; 25: 88-93.

176. Holmskov U, Thiel S, Jensenius JC. Collectins and ficolins: humoral lectins of the innate immune defense. Annu Rev Immunol 2003; 21: 547-578.

177. Garcia-Laorden MI, Manzanedo A, Figuerola A, Sanchez-Garcia F, Rodriguez-Galleo C. Mannose-binding lectin polymorphisms in a Canary Island (Spain) population. Genes Immun 2001; 2 :292-294.

178. Aittoniemi J, Soranummi H, Rovio AT, Hurme M, Pessi T, Nieminen M, Karjalainen J. Mannose-binding lectin 2 (MBL2) gene polymorphism in asthma and atopy among adults. Clin Exp Immunol 2005;142 :120-124.

179. Geleijns K, Roos A, Houwing-Duitermaat J, van Rijs W, Tio Guillen AP, Laman JD, van Door A, Jacobs CB. Mannose binding lectin contributes to the severity of Guillain-Barre syndrome. J Immunol 2006; 177: 4211-4217.

180. Patterson JE, Andriole VT. Bacterial urinary tract infections in diabetes. Infect Dis Clin N Am 1997; 11: 735-750.

181. Tolkoff-Rubin NE, Rubin RH. Urinary tract infection in the immunocompromised host. Infect Dis Clin N Am 1997; 11: 707-717.

182. Annane D, Bellisant E, Cavaillon JM. Septic shock. Lancet 2005; 365: 63-78.

183. Chaka W, Verheul AF, Vaishnav VV, Cherniak R, Scharring J, Verhoef J, Snippe H, Hoepelman AI. Induction of TNF-alpha in human peripheral blood mononuclears cells by the mannoprotein of Cryptococcus neoformans involves human mannose binding protein. J Immunol 1997; 159: 2979-2985.

184. Soell M, Lett E, Holveck F, Scholler M, Wachsmann D, Klein JP. Activation of human monocytes by streptococcal rhamnose glucose polymers is mediated by CD 14 antigens, and mannan binding protein inhibits TNF-alpha release. J Immunol 1995; 154: 851-860.

185. Jack DL, Read RC, Tenner AJ, frosch M, Turner MW, Klein NJ. Mannose-binding lectin regulates the inflammatory response of human professional phagocytes to Neisseria meningitidis serogrup B. J Infect Dis 2001; 184: 1152-1162.

186. Ono K, Nishitani C, Mitsuzawa H, Shimizu T, Sano H, Suzuki H, Kodama T, Fijii N, Kukase K, Hirata K, Kuroki Y. Mannose-binding lectin augments the uptake of lipid A, Staphylococcus aureus and Escherichia coli by Kupffer cells through increased cell surface expression of scavanger receptor A. J Immunol 2006; 177: 5517-5523.

187. Dumestre-Perard C, Doerr E, Colomb MG, Loos M. Involvement of complement pathways in patient with bacterial septicemia. Mol Immunol 2007; 44: 1642-1649.

188. Zhao L, Ohtaki Y, Yamaguchi K, Matsushita M, Fujita T, Yokochi T, Takada H, Endo Y. LPS-induced platelet response and rapid shock in mice: contribution of O-antigen region of LPS and involvement of the lectin pathway of the complement system. Blood 2002; 100: 3233-3239.

189. Stengaard-Pedersen K, Thiel S, Gadjeva M, Moller-Kristensen M, Sorensen R, Jensen LT, Sjoholm AG, Fugger L, Jensenius JC. Inherited deficiency of mannan-binding associated serine proteases 2. N Engl J Med 2003; 349: 554-560.

190. Granell M, Urbano-Ispizua A, Suarez B, Rovira M, Fernandez Aviles F, Martinez C, Ortega M, Uriburu C, Gaya A, Roncero JM, Navarro A, Carreras E, Mensa J, Vives J, Rozman C, Montserrat E, Lozano F. Mannan-binding lectin pathway deficiencies and invasive fungal infections following allogenic stem cell transplantation. Exp Hematol 2006; 34: 1435-1441.

11. OTRAS PUBLICACIONES RELACIONADAS CON EL TEMA DE LA TESIS.

Artículo 1.

Título: "Acute pyelonephritis in adults: an update".
Autores: J.P. Horcajada, A. Smithson.
Publicación: Rev Med Microbiol 2003; 14: 119-127.
Factor de impacto: 1,0.

Artículo 2.

Título: "Evaluation of inflammatory and renal-injury markers in women treated with antibiotics for acute pyelonephritis caused by *E. coli*".
Autores: J.P. Horcajada, M. Velasco, X. Filella, L. Álvarez, E. De Lazzari, J.L. Marín, B. Collvinent, A. Smithson, J.A. Martínez, M. Noguero, J. Vila, J. Mensa.
Publicación: Clin Diagn Lab Immunol 2004; 11: 142-146.
Factor de impacto: 2,511.

Artículo 3.
Título: "Quinolone-resistant uropathogenic *E. coli* strains from phylogenetic group B2 have fewer virulence factors than their susceptible counterparts".
Autores: J.P. Horcajada, S.M. Soto, A. Gajewski, A. Smithson, M.T. Jiménez de Anta, J. Mensa, J. Vila, J.R. Johnson.
Publicación: J Clin Microbiol 2005; 43: 2962-2964.
Factor de impacto: 3,708.

Artículo 4.
Título: "Is mannose-binding lectin (MBL) deficiency associated with gram positive infections?".
Autores: A. Smithson, R. Perelló, J.P. Horcajada, J.M. Nicolás, F. Lozano.
Publicación: Clin Infect Dis 2008. En prensa.

Factor de impacto: 6,75.

Acute pyelonephritis in adults: an update

Juan P. Horcajada and Alex Smithson

Recent advances in pathogenesis, changes in antimicrobial susceptibility of uropathogens and new therapeutic guidelines have resulted in a growing interest in acute pyelonephritis. New virulence factors of uropathogens have been described as well as a possible link between antibiotic resistance and lower virulence in *Escherichia coli*. Resistance to commonly used antimicrobials, such as ampicillin, first-generation cephalosporins, trimethoprim-sulphametoxazole, fluoroquinolones and even second- and third-generation cephalosporins, is increasing among uropathogens not only in nosocomial urinary tract infections, but in community-acquired infections as well. At the same time, some risk factors associated with resistance have been discovered. From the clinical point of view, while acute pyelonephritis typically produces an easy-to-diagnose clinical picture, in certain groups of patients, such as the elderly or diabetics, the clinical picture may be less obvious and outcomes more severe. Recent advances in diagnostic imaging, including scintigraphy, have helped in the diagnosis of urinary tract infections. The local prevalence of antimicrobial resistance, the patient's risk of infection due to antimicrobial-resistant uropathogens, the need for hospital admission and the severity of the clinical picture have to be taken into account in the selection of empirical antimicrobial therapy of acute pyelonephritis.

Reviews in Medical Microbiology 2003, **14**:119–127

Keywords: acute pyelonephritis, pathogenesis, diagnosis, therapy, review

Introduction

Acute pyelonephritis (APN) is defined as a urinary tract infection (UTI) affecting the renal pelvis and parenchyma and is one of the most common infectious diseases. In the last decade significant advances in the understanding of both bacterial and host factors involved in UTI, a growing frequency of multi-drug-resistant uropathogens and clinical trials with new therapeutic strategies have appeared. With this in mind, this review provides both updated basic and clinical information about APN.

Epidemiology

Annually in the USA, at least 250 000 episodes of APN occur in young women resulting in as many as 100 000 hospitalizations per year [1]. APN often requires prolonged therapy and when accompanied by bacteraemia, APN has a mortality rate of 10–20% [2]. Although there are no specific data concerning the costs of APN, the financial implications of community-acquired and nosocomial UTI are of great importance and result in $2 billion in medical expenditure in the USA each year [3,4].

Classification

From a practical point of view APN are divided into complicated and non-complicated. This distinction is important because both have distinct host features, pathogenic mechanisms, causative pathogens, clinical presentation, and therefore antibiotic therapy. APN that occur in a normal genitourinary tract with no prior instrumentation are considered uncomplicated, generally affecting women aged 18–40 years. Complicated infections are diagnosed in genitourinary tracts that have structural or functional abnormalities. In men any UTI is potentially complicated for anatomical reasons. There are multiple diseases and interventions responsible for complicated APN, including obstructive lesions, metabolic diseases, instrumentation, foreign bodies or

From the Service of Infectious Diseases. Hospital Clínic Universitari-IDIBAPS, Barcelona, Spain.

Correspondence to J. P. Horcajada, Service of Infectious Diseases, Hospital Clínic Universitari-IDIBAPS, Villarroel 170. 08036 Barcelona. Spain. Tel: +34 3 2275586; fax: +34 3 4514438; e-mail: jhorcaja@clinic.ub.es

Received: 1 August 2003; revised: 22 August 2003; accepted: 14 October 2003.

ISSN 0954-139X © 2003 Lippincott Williams & Wilkins

dysfunctional voiding, primarily due to neurological diseases.

Although little is known about the specific risk factors for uncomplicated APN in young women, recognized factors for uncomplicated cystitis would be predicted to predispose to APN also. Several inherited and acquired conditions facilitate the appearance of UTI and can be divided into genetic, anatomic–functional and behavioural factors. This is a practical classification as genetic factors cannot be solved, anatomic–functional ones are more or less reversible, and the behavioural factors can be easily modified (Table 1).

Aetiology

Uropathogens cause APN following an ascending route from the urinary bladder to the renal pelvis through the ureters. In most cases, the pathogens arise from the hosts' own intestinal flora [5]. More rarely the infectious route is haematogenous particularly following *Staphylococcus aureus* bacteraemia or *Candida* fungaemia.

Escherichia coli is responsible for most cases (> 80%) of uncomplicated APN. *Klebsiella pneumoniae*, *Proteus mirabilis*, *Enterococcus faecalis* (especially in the elderly) and *Staphylococcus saprophyticus* (particularly in young women) are the next most frequent isolates (Table 2). A characteristic of complicated APN is the variety of infecting organisms isolated that are relatively resistant to antimicrobial agents (Table 2). Although *E. coli* is still the single most common pathogen in complicated APN (50–60% of infections) these strains are often more resistant to antimicrobials and probably less virulent than strains that cause uncomplicated APN [6].

The aetiological spectrum of APN has remained constant over the last 50 years. However a gradual increasing in antimicrobial resistance rates among uropathogens has been observed, not only in complicated APN, but also in uncomplicated-acquired APN. In Spain, recent data demonstrate that 20% of uropathogenic *E. coli* (UPEC) strains are resistant to fluorquinolones, 50% to ampicillin and 35% to trimethoprim–sulphamethoxazole (TMP–SMX) while 70–80% of these strains are susceptible to first-generation cephalosporins and 95–99% are susceptible to

Table 1. Risk factors for urinary tract infection (UTI).

Genetic factors	Anatomic–functional factors	Behavioural factors
Non-secretor status of blood group antigens[a]	Congenital and acquired urologic abnormalities	Sexual intercourse
A_1P_1 blood group phenotype[b]	Vesicoureteral reflux	Use of spermicides and/or diaphragm as contraceptives
	Urinary incontinence	
High density of epithelial cell receptors	Oestrogen deficiency (menopause)[c]	Recent use of certain antibiotics[d]
History of UTI in mother	History of UTI after puberty	
History of UTI during childhood		
	Diabetes mellitus	
	Urolithiasis[e]	
	Urinary in-dwelling catheter or urological instrumentation	

[a]Secretion or expression of certain blood groups on the surface of the uroepithelial cells, which is genetically determined, influences susceptibility to UTI. Women with recurrent UTI have an increased frequency of non-secretor Lewis blood group phenotype. [b]Individuals of blood group A_1P_1 express globo-A receptors in their uroepithelial cells and become infected with bacteria recognizing this receptor. [c]The lack of oestrogens that occurs in the menopause causes changes in the vaginal flora, epithelial atrophy and urinary incontinence and therefore increases the risk of UTI. [d]Recent intake of anaerobicidal antibiotics causes changes in the normal vaginal flora facilitating colonization by enterobacteriaceae. [e]Urolithiasis increases the risk for UTI by: (i) acting as reservoir for uropathogens; (ii) causing obstruction of the urine flow.

Table 2. Aetiology of acute pyelonephritis.

In previously healthy patients	In patients with risk factors for antimicrobial-resistant uropathogens[a]
Escherichia coli	*Escherichia coli*
Klebsiella spp.	*Pseudomonas aeruginosa*
Proteus spp.	*Enterococcus* spp.
Staphylococcus saprophyticus	Extended-spectrum beta-lactamase-producing *E. coli* and *Klebsiella* spp.
	Staphylococcus aureus
	Group B streptococci
	Candida spp.
	Other bacteria[b]
	Polymicrobial[c]

[a]Patients with one or more of the following conditions: urological abnormalities, associated diseases (diabetes, cirrhosis, transplant recipient patients), recent urologic manipulation, in-dwelling urinary catheters, prior antimicrobial treatment and nosocomial urinary tract infection. [b]*Haemophilus influenzae, Gardnerella vaginalis, Mycoplasma hominis, Ureaplasma urealyticum, Corynebacterium urealyticum.* [c]More frequent in patients with neurogenic bladder or with vesico-intestinal or vesico-vaginal fistula.

third-generation cephalosporins [7]. The prevalence of extended-spectrum beta-lactamase-producing *E. coli* and *Klebsiella pneumoniae* has increased in recent years, particularly in patients with prior antibiotic therapy [8]. Several risks factors have been identified to be associated with antibiotic *E. coli* resistance. Recent exposure to TMP–SMX or to other antimicrobials, diabetes mellitus, male sex, previous UTI and recent hospitalization have been demonstrated to be risk factors for TMP–SMX resistance [9,10]. In-dwelling urinary catheters, prior UTI or antibiotic treatment are conditions that predispose to amoxicillin-clavulanic acid resistance, while male sex, age $>$ 65 years, urinary abnormalities and prior UTI, urinary instrumentation or treatment with fluoroquinolones are circumstances that favour fluoroquinolone resistance [10,11]. Finally, the elderly from nursing homes form a group of population in which it is common to find several risk factors for antimicrobial resistance and, thus have higher resistance rates than the general population [12].

Bacterial virulence

UPEC differ from intestinal commensal non-pathogenic *E. coli* by the presence of chromosomal pathogenicity islands that encode multiple virulence factors, mainly adhesins and toxins [13,14]. The initial step in the pathogenesis of APN involves the adherence of the microbial pathogen to the epithelium of the urinary tract. The majority of UPEC express a number of different adhesive organelles including type 1 and P fimbriae. Type 1 fimbriae, encoded by virtually all UPEC isolates, mediate bacterial attachment to the host bladder cells and have been shown to be significantly associated with UTI [15,16]. An adhesin molecule, FimH, located at the tip of type I fimbriae, binds mannose-containing glycoprotein receptors expressed on the luminal surface of the bladder and mediates bacterial attachment to the epithelium allowing bacteria to remain in the urinary tract despite the diluting effect of continuous urine flow [17]. FimH not only mediates bacterial adherence but also stimulates host cell signalling cascades that leads to the induction of cytoskeletal rearrangements and the envelopment and internalization of adherent UPEC [18]. Following invasion of superficial bladder epithelial cells, UPEC can replicate intracellularly and eventually mature into biofilms forming massive foci of *E. coli* creating a chronic quiescent reservoir in the bladder. Thus in addition to the intestine and vagina as reservoir for UPEC, the bladder itself may serve as the source of recurrent cystitis and asymptomatic bacteriuria [19,20]. P fimbriae is another class of fimbriae-associated adhesins with two predominant subtypes depending on the receptor specificity of PapG, the corresponding adhesin of P fimbriae. This class of fimbriae participates at different stages in the pathogenesis of UTI. It has been demonstrated that P fimbriae enhances early bacterial colonization of the urinary tract [21] and that the class II G adhesins are more common among strains that cause APN and bacteraemic UTI, whereas the class III G adhesin predominates in isolates from cystitis [22]. P fimbriae also have the capacity to trigger the local host response with production of chemokines that leads to recruitment of neutrophils and clearance of the infection [21]. This response is mediated through the Toll-like receptors (TLR) signalling pathway, specifically the TLR-4, a group of receptors that form part of the innate immunity [23]. As well as adhesins UPEC secrete specific proteins (toxins) the role of which is still controversial in the pathogenesis of UTI. Examples of such toxins are haemolysin and cytotoxic necrotizing factor type 1 that act by favouring tissue invasion, and aerobactin which is a siderophore [24].

The repertoire of virulence factors present in the bacteria determines the kind (e.g., cystitis versus pyelonephritis), severity and the group of patients affected by the disease. Strains of *E. coli* isolated from patients with cystitis have fewer virulence factors than those isolated from patients with APN [25], and *E. coli* strains isolated from patients with recent urological manipulation have fewer virulence factors than those strains from non-manipulated patients [6]. Recently it has been shown that quinolone-resistant UPEC have fewer virulence factors, and lower bacterial fitness, than quinolone-susceptible strains [26,27]. These quinolone-resistant *E. coli* strains are isolated more frequently from patients with cystitis and from UTI after recent urinary tract manipulation than from uncomplicated APN, suggesting that uncomplicated APN is caused by *E. coli* strains with more virulence properties than those that cause acute cystitis [6].

Clinical features

The clinical spectrum of APN varies from septic shock to a cystitic syndrome with slight flank pain. A typical history of the classic non-complicated APN in otherwise healthy young women includes fever with shaking chills, pain in the costovertebral region or flanks and symptoms of bladder inflammation. The most characteristic finding during the physical examination is the presence of tenderness on one or both costovertebral angles.

Atypical presentations are common particularly in the elderly or in patients with associated diseases in which the classical picture of APN is frequently associated with dehydration, delirium or with complications such as strokes [28]. In diabetic patients APN may occur

with severe complications such as emphysematous pyelonephritis or renal abscesses. Another characteristic of APN in this group of patients is that they are eventually caused by more resistant uropathogens, frequently proceed with absence of local signs of kidney infection and with decreased level of consciousness and require more prolonged hospitalization than APN in non-diabetic patients [29].

Complications of APN

When fever persists for more than 72 h after the onset of antimicrobial empirical treatment or when a worsening of the clinical state occurs, one of the following situations has to be suspected: (i) infection is caused by a uropathogen resistant to the initial empirical treatment. (ii) Acute focal or multifocal nephritis. This is a severe form of APN characterized by infiltration of leukocytes in one (focal) or several (multifocal) renal lobes without frank abscess, which is probably the stage before renal abscess formation. These forms of APN are more frequent among diabetic patients, are often bacteraemic and respond poorly to antimicrobial treatment. Diagnosis is made with ultrasound and computerized tomography [30,31]. (iii) Development of pyogenic collections (abscesses, infected cysts, hydronephrosis or pyonephrosis). Renal cortical abscesses are usually caused from haematogenous spread from a primary focus elsewhere in the body. *Staphylococcus aureus* is the aetiologic agent in 90% of such cases. Renal corticomedullary abscesses are usually associated with an underlying urinary tract abnormality and are caused by enteric Gram-negative bacilli, which reach the kidney following an ascending route. Perinephritic abscesses are usually the result of the rupture of an intra-renal abscess into the perinephritic space. Renal abscesses can be drained by percutaneous puncture. Pyonephrosis, usually caused by ureteral obstruction secondary to nephrolithiasis, has to be urgently drained by retrograde ureteral catheterization or percutaneous nephrostomy [30,32]. (iv) Renal papillary necrosis. It is a disorder of the kidney producing necrosis of part of the renal papillae occurring with haematuria, lumbar pain, renal insufficiency and septic shock. It is more frequent in patients with vascular atheromathosis such as diabetic patients [33]. (v) Emphysematous pyelonephritis. This is an uncommon entity presenting as a severe, necrotizing form of acute multifocal bacterial nephritis with presence of gas in the kidney and perinephric areas. It is more frequent in diabetics and the treatment options include antimicrobials combined with nephrectomy in patients with non-functional kidneys and absence of obstructive uropathy whereas in patients with functional kidneys or obstructive uropathy, nephrectomy should be performed only if percutaneous aspiration or ureteral catheterization are unable to control the infection [30].

Diagnosis

Diagnosis of APN is based on the combination of characteristic symptoms and signs together with laboratory tests.

Urine analysis

The minimal laboratory test to diagnose APN is the microscopic examination of a voided urine specimen to evaluate the presence of pyuria. The presence of pyuria can be determined by counting leukocytes present in unspun urine using a counting chamber such as a haemocytometer, by microscopic evaluation of the presence of leukocytes in a high-powered field ($\times$ 400 magnification) in a resuspended urine sediment or using the dipstick leukocyte esterase test. The most accurate method to assess the presence of pyuria is by examining an unspun voided midstream urine specimen with a haemocytometer; the presence of 10 or more leukocytes per mm^3 is considered abnormal. Microscopic detection of pyuria has a superior margin of error when it is evaluated using a haemocytometer; pyuria is indicated when there are more than 5 leukocytes/high power field of view. The dipstick leukocyte esterase test is a rapid screening test for detecting pyuria. It is both sensitive (90%) and specific ($>$ 95%) for detecting more than 10 leukocytes/μl. It should be emphasized that the finding of pyuria is non-specific, and patients without pyuria may or may not have an UTI, although pyuria is present in the vast majority of patients with symptomatic UTI. For instance pyuria can be absent when UTI is associated with urinary tract obstruction or with neutropenia. Inversely, leukocyturia is not specific of UTI and can be present in the following circumstances: interstitial nephropathies, ureterolithiasis and kidney tuberculosis [30].

Microbiological analysis

Urine Gram stain

Albeit rarely performed it can be used in certain circumstances when it is important to rule out the presence of Gram-positive bacteria; if present antimicrobial therapy should include agents active against *Enteroccocus* sp.

Urine culture

Urine culture is the 'gold standard' by which to establish the diagnosis of UTI as it allows the identification of the aetiologic microorganism and establishes the antimicrobial pattern of susceptibility, as well as the confirmation of bacterial eradication. Urine culture is performed from a first morning fresh urine specimen or

from a urine that has remained for 4 h or more inside the bladder. It has to be collected from a clean midstream urine sample, with previous cleaning of the genital area, avoiding the use of antiseptics. More than 80% of the patients with APN have a positive urine culture with more than 1×10^5 colony-forming units (c.f.u.)/μl urine. It is considered as significant when there are $> 1 \times 10^4$ c.f.u. of a uropathogen/μl in a midstream urine (sensitivity of 90–95%). Urine culture can be negative or with low numbers of c.f.u./μl urine in the following circumstances: (i) previous antibacterial treatment; (ii) recent micturation, usually as a result of the cystitic syndrome; (iii) ureteral obstruction; (iv) very low urinary pH; (v) infection due to 'demanding' or slow-growing microorganism.

Blood cultures

Although bacteraemia occurs in about 20–30% of the patients with APN, clinical outcomes are similar regardless of the presence or absence of bacteraemia [34]. Elderly patients, diabetes mellitus, obstruction to urine flow, renal insufficiency, and more of 5 days of symptoms before the beginning of antibacterial treatment are predictive factors for bacteraemia in men with febrile UTI [35].

Imaging studies

Radiological workup is useful when the diagnosis is in doubt, in severely ill or immunocompromised patients, in those who fail to improve with antibiotic therapy, in recurrent UTI or when complications are suspected. Indications for imaging studies are shown in Table 3.

Plain abdominal X-ray

When radiological studies are indicated, a plain film of the abdomen should be the first test to be performed. It is useful for the detection of urinary tract calculi (80% are radio-opaque), soft tissue masses, and kidney gas collections (emphysematous pyelonephritis).

Table 3. Indications for radiological evaluation studies in patients with acute pyelonephritis (intravenous urography with voiding cystouretethrogram)[a].

Men
Women
Less than 5 years old
With recurrent urinary tract infection[b]
With suspicion of underlying urological abnormalities[c]

[a]In some patients this radiological evaluation can be substituted by an abdominal ultrasonography and a plain abdominal X ray. [b]Evidence is limited. It has not been demonstrated which subgroup of women with recurrent urinary tract infection benefit from radiological evaluation. [c]The following data suggest the presence of urological abnormalities: haematuria, colic pain, stranguria, lithiasis and recurrent urinary tract infection caused by *Proteus*.

Ultrasonography and abdominal computed tomography (CT) scan

The practice of an urgent abdominal ultrasonography is indicated in order to rule out the presence of urinary obstruction or pyogenic collections, when an APN is accompanied by: septic shock, acute renal insufficiency, colic pain, haematuria, presence of a renal mass or persistence of fever after 3 days' treatment with active antimicrobial treatment against the bacterial isolate. A routine ultrasonography should also be considered in patients with recurrent UTI or when the presence of underlying urological abnormalities is suspected. Contrast-enhanced CT is more sensitive than ultrasonography to detect small abscesses (< 2 cm diameter) and areas of acute focal nephritis [30].

Intravenous urography

Together with the practice of a voiding cystourethrogram this allows the detection of: (i) urological abnormalities that predispose to UTI, particularly vesicoureteral reflux; (ii) possible complications such as abscesses, lithiasis, pyonephrosis, scars of chronic pyelonephritis and papillar necrosis. The practice of an intravenous urography has to be delayed 2–4 weeks after the diagnosis of APN (8 weeks postpartum), except when a complication is suspected and it is not possible to perform ultrasonography. In men with UTI, the combination of ultrasonography with a plain abdominal X-ray is as useful as the practice of intravenous urography to diagnose the presence of urological abnormalities [36]. In fact, with an accurate clinical history, routine imaging studies of the upper urinary tract seem dispensable in men with febrile UTI and if abnormalities of the urinary tract are suspected urological evaluation should primarily be focused on the lower urinary tract [37].

Nuclear scintigraphy

The role of radioisotope studies in the management of patients with APN is limited to the detection of renal scars with technetium-99 scanning in children with recurrent pyelonephritis and/or with vesicoureteral reflux. Recently the utility of indium-111 white blood labelled gammagraphy has been demonstrated in the topographic localization (kidney and/or prostate) in men with febrile UTI. This gammagraphic technique could be particularly useful for the diagnosis of APN versus acute prostatitis when focal infectious symptoms are not apparent as in cord-injured patients, diabetics or in patients with indwelling urinary catheters [38].

Antimicrobial therapy

Two main questions must to be answered when deciding the empirical antimicrobial treatment in APN: (i) does the patient need hospitalization? and (ii) what

is the susceptibility profile for *E. coli* and other uropathogens in the local community?

The following groups of patients with APN have to be hospitalized: patients with septic shock or with local pyogenic complications, patients affected with chronic conditions likely to influence the causal uropathogen or the response to antimicrobial therapy (diabetes mellitus, elderly, cirrhotic, oncological and transplant-recipient patients), those who do not improve after 6–12 h of empirical antibacterial treatment at the Emergency Department and finally those not suitable for oral therapy (vomiting or non-compliant patients) [30,39].

The appropriate empirical antimicrobial treatment for APN has to accomplish the following: (i) be active against $>95\%$ of the UPEC strains; (ii) reach high urinary and serum concentrations, considering that 30% of APN will develop bacteraemia; (iii) respect rectal and vaginal flora. Agents that adversely affect the faecal and vaginal anaerobic flora facilitate colonization with enterobacteriaceae (particularly *E. coli*) and subsequently bacterial recurrence. Fluoroquinolones and TMP–SMX achieve high urinary concentrations for long periods of time with little effect on the anaerobic flora and therefore are the optimal antimicrobial choices for the treatment of UTI [5].

Recently the Infectious Disease Society of America (IDSA) has developed evidence-based practice guidelines for antimicrobial treatment of uncomplicated APN in women. As the antimicrobial susceptibility pattern of urinary isolates from Spain and other southern European countries is significantly different from that of isolates from the USA, the IDSA recommended guidelines may be not fully applicable in these countries [7,39,40]. The major differences refer to the TMP–SMX and fluoroquinolone susceptibility profiles. In these countries TMP–SMX is not included in the empirical treatment guidelines for APN as 40% of *E. coli* and *Proteus* spp and 30% of *Klebsiella* urinary isolates are TMP–SMX resistant. Although 20% of the *E. coli* isolated from cystitis are resistant to fluoroquinolones the quinolone resistance rate from APN *E. coli* isolates is quite low (around 10%) [6], and in our experience, even less frequent in *E. coli* isolates from patients with acute prostatitis (unpublished data).

A summary of the recommended guidelines for APN is

Table 4. Therapeutic management of acute pyelonephritis (APN): five possible situations.

Situation	Treatment	Grades of evidence
1. APN in patients without risk factors for antimicrobial-resistant uropathogens[a] and not requiring hospitalization[b]	Parenteral monodosis of a broad spectrum cephalosporin, an aminoglycoside or a fluoroquinolone	B,III
	6–24 h of observation and discharge with an:	B,II
	oral second or third generation cephalosporin, or	B,I
	oral fluoroquinolone	B,I
	complete 14 days of antimicrobial therapy	A,I
	courses of highly active agents for 7 days may be sufficient for mild or moderate cases	B,I
	all antimicrobial regimen orally	A,II
2. APN in patients without risk factors for antimicrobial-resistant uropathogens[a] requiring hospitalization[b]	Hospitalization and parenteral antibiotics:	A,II
	Broad spectrum cephalosporin, or	B,III
	an aminoglycoside	B,III
	with improvement, change to an oral fluoroquinolone,	B,III
	an oral broad spectrum cephalosporin	B,III
	or to TMP–SMX (if the organism is susceptible[c])	B,III
	complete 14 days of treatment	A,I
3. APN in patients with risk factors for antimicrobial-resistant uropathogens	Piperacillin-tazobactam or a carbapenem	B, III
	or ampicillin+cefepime or ceftazidime or aztreonam, followed by:	B, III
	oral fluoroquinolones or TMP–SMX or cephalosporins if the bacteria are susceptible[c]	B, III
	or amoxicillin if a Gram-positive bacteria is isolated	B, III
	complete 14 days of treatment	B, III
4. APN with septic shock	Piperacillin-tazobactam or a carbapenem	B, III
	or ampicillin+cefepime or ceftazidime or aztreonam	B, III
	associated with an aminoglycoside	B, III
5. Obstructive APN	Regimen 2, 3 or 4, depending on the clinical setting, and drainage	–

[a] Patients with: urological abnormalities, associated diseases (diabetes, cirrhosis, transplant recipients), urological manipulation, in-dwelling urinary catheters, prior antimicrobial treatment or nosocomial urinary tract infection. [b] Serious sepsis, suspicion of local complications (intense pain or haematuria, renal mass, acute renal failure), associated diseases (diabetes, cirrhosis, transplant recipients), unstable patients 6–12 h after the onset of empirical antimicrobial treatment and those not suitable for oral therapy (vomiting or non-compliant patients). [c]Antibiotic has to be adjusted to the susceptible antimicrobial pattern of the bacteria. If the initial urine and blood cultures are negative it is recommended to complete the treatment with a third-generation cephalosporin with or without amoxicillin if *Enterococcus* spp is the probably the causative organism. TMP–SMX, trimethoprim–sulphametoxazole.

shown in Table 4. These have been obtained from the IDSA guidelines, and from the review of the published literature since 1999. The grades reflecting the quality of evidence on which recommendations are based are shown in Table 5.

Antibiotic treatment options and administration route

Treatment of APN in patients not requiring hospitalization

Adequate empirical treatment can be initiated with a parenteral broad spectrum cephalosporin, an aminoglycoside or with a fluoroquinolone (ciprofloxacin or levofloxacin). Clinically stable patients after 6–12 h of the onset of parenteral antimicrobial treatment can be switched to oral therapy, be discharged from the Emergency Department and be treated as outpatients (B,III) [41]. In our country the recommended oral treatment includes cefixime 400 mg daily, ceftibuten 400 mg daily, cefuroxime–axetil every 12 h, ciprofloxacin 500–750 mg every 12 h or levofloxacin 500 mg daily. The association of penicillins with beta-lactamase inhibitors (amoxicillin–clavulanate, ampicillin–sulbactam) are also active against most uropathogens but have a negative impact on the vaginal flora with a possible higher incidence of recurrent UTI and are less well recommended [5]. Oral treatment therapy with fluoroquinolones or with cephalosporins has been demonstrated as effective as parenteral regimens in uncomplicated APN (A,II) [42,43]. Thus, a young compliant non-pregnant female with a mild to moderate uncomplicated APN may be treated with an oral antibiotic as an outpatient. Fluoroquinolones and TMP–SMX, are the best therapeutic options to complete oral treatment of APN produced by susceptible uropathogens, due to their lower recurrence rate.

Treatment of APN in patients requiring hospitalization

Patients admitted to hospital for APN are usually treated with intravenous antimicrobials (A,II). The IDSA guidelines recommend parenteral therapy with fluoroquinolones, aminoglycosides with or without ampicillin, or with a broad spectrum cephalosporin associated or not with an aminoglycoside. If *Enterococcus* spp. is suspected, based on the Gram stain, treatment with the combination of ampicillin–sulbactam or amoxicillin–clavulanate, combined or not with an aminoglycoside, are reasonable broad spectrum empirical choices (B,III). Patients admitted to hospital for intravenous therapy, after clinical improvement, measured by resolution of fever (usually at 48–72 h), and based on the susceptibility pattern, can be placed on oral antimicrobial treatment giving preference to fluoroquinolones and to TMP–SMX (B,III).

Treatment of complicated APN

This group of patients should be hospitalized and placed on intravenous antimicrobial therapy. Empirical therapeutic options include active agents against multiresistant enterobacteriaceae, particularly *P. aeruginosa* and *Enterococcus* spp. Treatment may be initiated either with monotherapy with carbapenems or piperacillin–tazobactam or with the combination of ampicillin with an anti-pseudomonas cephalosporin (cefepime or ceftazidime) or aztreonam. It is recommended to add an aminoglycoside to any of the previous therapeutic options in patients with septic shock, at least during the first 3 days of treatment. Once the susceptibility pattern of the infecting organism is known, intravenous therapy can be changed to a less expensive therapy, and finally oral treatment can be initiated after a clinical response has been achieved (B,III).

Duration of therapy

The optimal duration of therapy in APN is still undefined and remains a source of controversy. Most young healthy women with uncomplicated APN will have a satisfactory outcome with 2 weeks of an antimicrobial regimen (A,I). A few randomized trials have demonstrated high cure rates after 7 days of treatment and could be an option in compliant patients with mild APN [44,45]. It is generally recommended

Table 5. Categories reflecting the strength of each recommendation and grades reflecting the quality of evidence on which recommendations are based.

Category or grade	Definition
Strength of recommendation	
A	Good evidence to support a recommendation for use
B	Moderate evidence to support a recommendation for use
C	Poor evidence to support a recommendation against use
D	Moderate evidence to support a recommendation against use
E	Good evidence to support a recommendation against use
Quality of evidence	
I	Evidence from at least one properly randomized controlled trial
II	Evidence from at least one well-designed clinical trial without randomization, from cohort or case-controlled analytic studies (preferably from more than one centre), from multiple time-series studies, or from dramatic results in uncontrolled experiments
III	Evidence from opinions of respected authorities, based on clinical experience, descriptive studies, or reports of expert committees

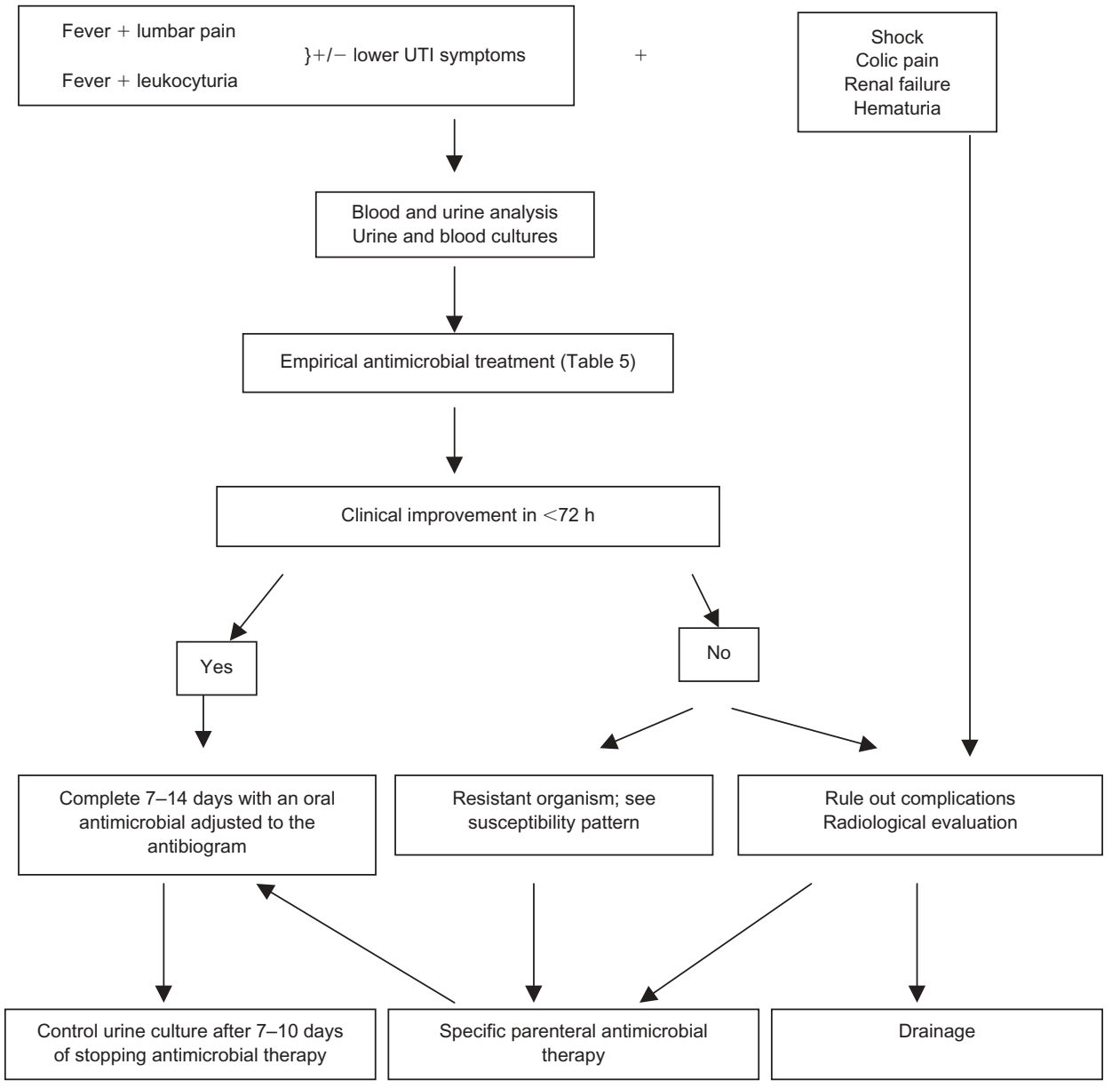


Fig. 1. Diagnostic and therapeutic management of acute pyelonephritis. UTI, urinary tract infection.

that patients with complicated APN should receive a longer course of treatment (14–21 days). The duration of therapy for kidney abscesses must be individualized recommending a course of 2 weeks of antibiotic treatment when percutaneous drainage has been performed or a long course of oral antibiotics (6–8 weeks) if percutaneous drainage has not been practised [32]. Figure 1 shows an algorithm with the therapeutic management of APN.

References

1. Bergeron MG. **Treatment of pyelonephritis in adults.** *Med Clin North Am* 1995, **79**:619–649.
2. Roberts FJ, Geere IW, Coldman A. A three-year study of positive blood cultures, with emphasis on prognosis. *Rev Infect Dis* 1991, **13**:34–46.
3. Foxman B, Barlow R, D'Arcy H, Gillespie B, Sobel JA. **Urinary tract infection: self reported incidence and associated costs.** *Ann Epidemiol* 2000, **10**:509–515.
4. Jarvis WR. **Selected aspects of the socioeconomic impact of nosocomial infections: morbidity, mortality, costs and prevention.** *Infect Control Hosp Epidemiol* 1996, **17**:552–557.
5. Hooton TM, Stamm WE. **The vaginal flora and urinary tract infections.** In: Warren JW, Mololey HLT (editors): *Urinary tract infections: molecular pathogenesis and clinical management.* Washington, DC: American Society of Microbiology Press; 1996:67–94.
6. Velasco M, Horcajada JP, Mensa J, Moreno-Martínez A, Vila J, Martinez JA, *et al.* **Decreased invasive capacity of quinolone-resistant *Escherichia coli* in patients with urinary tract infections.** *Clin Infect Dis* 2001, **33**:1682–1686.
7. Kahlmeter G. **The ECO*SENS Project: a prospective, multina-**

tional, multicentre epidemiological survey of the prevalence and antimicrobial susceptibility of urinary tract pathogens-interim report. *J Antimicrob Chemother* 2000, **46** (Suppl 1):15–22.
8. Lautenbach E, Patel JB, Bilker WB, Edelstein PH, Fishman NO. **Extended-spectrum beta-lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae*: risk factors for infection and impact of resistance on outcomes.** *Clin Infect Dis* 2001, **32**: 1162–1171.
9. Wright SW, Wrenn KD, Haynes ML**. Trimethoprim-sulfamethoxazole resistance among urinary coliform isolates.** *J Gen Intern Med* 1999, **14**:606–609.
10. Sotto A, De Boever CM, Fabbro-Peray P, Gouby A, Sirot D, Jourdan J. **Risk factors for antibiotic-resistant *Escherichia coli* isolated from hospitalized patients with urinary tract infections: a prospective study.** *J Clin Microbiol* 2001, **39**:438–444.
11. Ena J, Amador C, Martínez C, Ortiz de la Tabla V. **Risk factors for acquisition of urinary tract infections caused by ciprofloxacin resistant *Escherichia coli*.** *J Urol* 1995, **153**:117–120.
12. Vromen M, van der Ven AJ, Knols A, Stobberingh EE. **Antimicrobial resistance patterns in urinay isolates from nursing home residents. Fifteen years of data reviewed.** *J Antimicrob Chemother* 1999, **44**:113–116.
13. Oelschlaeger TA, Dobrindt U, Hacker J. **Virulence factors of uropathogens.** *Curr Opin Urol* 2002, **12**:33–36.
14. Johnson JR, Stell AL. **Extended virulence genotypes of *Escherichia coli* strains from patients with urosepsis in relation to phylogeny and host compromise.** *J Infect Dis* 2000, **181**: 261–272.
15. Langermann S, Palaszynski S, Barnhart M, Auguste G, Pinkner JS, Burlein J, *et al.* **Prevention of mucosal *Escherichia coli* infection by FimH-adhesin based systemic vaccination.** *Science* 1997, **276**:607–611.
16. Connell I, Agace W, Klemm P, Schembri M, Marild S, Svanborg C. **Type 1 fimbrial expression enhances *Escherichia coli* virulence for the urinary tract.** *Proc Natl Acad Sci USA* 1996, **93**:9827–9832.
17. Mulvey MA, Schilling JD, Martinez JJ, Hultgren SJ. **Bad bugs and beleaguered bladders: interplay between uropathogenic *Escherichia coli* and innate hosts defenses.** *Proc Natl Acad Sci USA* 2000, **97:**8829–8835.
18. Martinez JJ, Mulvey MA, Schilling JD, Pinkner JS, Hultgren S. **Type 1 pilus-mediated bacterial invasion of bladder epithelial cells.** *EMBO J* 2000, **19**:2803–2812.
19. Mulvey MA, Schilling JD, Hultgren SJ. **Establishment of a persistent *Escherichia coli* reservoir during the acute phase of a bladder infection.** *Infect Immun* 2001, **69**:4572–4579.
20. Anderson GG, Palermo JJ, Schilling JD, Roth R, Heuser J, Hultgren SJ. **Intracellular bacterial biofilm-like pods in urinary tract infections.** *Science* 2003, **301**:105–107.
21. Wullt B, Bergsten G, Samuelsson M, Gebretsadik N, Hull R, Svanborg C. **The role of P fimbriae for colonization and host response induction in the human urinary tract.** *J Infect Dis* 2001, **183** (Suppl 1):43–46.
22. Johanson IM, Plos K, Marklund BI, Svanborg C. **Pap, papG and prs DNA sequences in *Escherichia coli* from the fecal flora and the urinary tract.** *Microbiol Pathol* 1993, **15**:121–129.
23. Frendeus B, Wachtler C, Hedlund M, Fisher H, Samuelsson P, Svensson M, *et al.* ***Escherichia coli* P fimbriae utilize the Toll-like receptor 4 pathway for cell activation.** *Mol Microbiol* 2001, **40**:37–51.
24. Guyer DM, Gunther NW, Mobley HL. **Secreted proteins and other features specific to uropathogenic *Escherichia coli*.** *J Infect Dis* 2001, **183** (Suppl 1):32–35.
25. Ruiz J, Simon K, Horcajada JP, Velasco M, Barranco M, Roig G, *et al.* **Differences in virulence factors among clinical isolates of *Escherichia coli* causing cystitis and pyelonephritis in women and prostatitis in men.** *J Clin Microbiol* 2002, **40**: 4445–4449.
26. Vila J, Simon K, Ruiz J, Horcajada JP, Velasco M, Barranco M, *et al.* **Are quinolone-resistant uropathogenic *Escherichia coli* less virulent?** *J Infect Dis* 2002, **186**:1039–1042.
27. Horcajada JP, Vila J, Moreno-Martínez A, Ruiz J, Martínez JA, Sánchez M, *et al.* **Molecular epidemiology and evolution of resistance to quinolones in *Escherichia coli* after prolonged administration of ciprofloxacin in patients with prostatitis.** *J Antimicrob Chemother* 2002, **49**:55–59.
28. Shortliffe LM McCue JD. **Urinary tract infection at the age extremes: pediatrics and geriatrics.** *Am J Med* 2002, **113** (Suppl 1):55–66.
29. Horcajada JP, Moreno I, Velasco M, Martínez JA, Moreno-Martínez A, Barranco M, *et al.* **Community-acquired febrile urinary tract infection in diabetics could deserve a different management: a case-control study.** *J Intern Med* 2003, **254**: 280–286.
30. Mensa J, Gatell JM, Jiménez de Anta MT, Prats G, Domínguez-Gil A. *Therapeutic Antimicrobial Guide. Acute Pyelonephritis.* Barcelona: Masson; 2002.
31. Nosher JL, Tamminen JL, Amorosa JK, Kallich M. **Acute focal bacterial nephritis.** *Am J Kidney Dis* 1988, **11**:36–42.
32. Dembry LM, Andriole VT. **Renal and perirenal abscesses.** *Infect Clin North Am* 1997, **11**:663–680.
33. Griffin MD, Bergstralhn EJ, Larson TS. **Renal papillary necrosis–a sixteen-year clinical experience.** *J Am Soc Neprhol* 1995, **6**:248–256.
34. Velasco M, Martinez JA, Moreno-Martinez A, Horcajada JP, Ruiz J, Barranco M, *et al.* **Blood cultures in women with uncomplicated acute pyelonephritis: are they necessary?** *Clin Infect Dis* 2003, **37**:1127–1130.
35. Horcajada JP, Martínez JA, Moreno-Martínez A, Mensa J, Almela M, Soriano E. **Predictive factors of the presence bacteremia in males with urinary tract infection.** *Med Clin (Barc)* 1999, **112**:734–735.
36. Andrews SJ, Brooks PT, Hanbury DC, King CM, Prendergast CM, Bostead GB, *et al.* **Ultrasonography and abdominal radiography versus intravenous urography in investigation of urinary tract infection in men: prospective incident cohort study.** *BMJ* 2002, **324**:454–456.
37. Ulleryd P, Zackrisson B, Aus G, Bergdahl S, Hugosson J, Sandberg T. **Selective urological evaluation in men with febrile urinary tract infection.** *BJU Int* 2001, **88**:15–20.
38. Mateos JJ, Velasco M, Lomeña F, Horcajada JP, Setoain FJ, Martin F, *et al.* **111 Indium labelled leukocyte in detection of acute prostatitis.** *Nucl Med Commun* 2002, **23**:1137–1142.
39. Warren JW, Abrutyn E, Hebel JR, Johnson JR, Schaefer AJ, Stamm WE. **Guidelines for antimicrobial treatment of uncomplicated acute bacterial cystitis and acute pyelonephritis in women. Infection Disease Society of America (IDSA).** *Clin Infect Dis* 1999, **29:**745–758**.**
40. Oteo J, Campos J, Baquero F. **Antibiotic resistance in 1962 invasive isolates of *Escherichia coli* in 27 Spanish hospitals participating in the European Antimicrobial Resistance Surveillance System (2001).** *J Antimicrob Chemother* 2002, **50**: 945–952.
41. Sanchez M, Collvinent B, Miro O, Horcajada JP, Moreno A, Marco F, *et al.* **Short-term effectiveness of ceftriaxone single dose in the initial treatment of acute uncomplicated pyelonephritis in women. A randomized controlled trial.** *Emerg Med J* 2002, **19**:19–22.
42. Mombelli G, Pezzoli R, Pinoja-Lutz G, Monotti R, Marone C, Franciolli M. **Oral vs intravenous ciprofloxacin in the initial empirical management of severe pyelonephritis or complicated urinary tract infections: a prospective randomized clinical trial.** *Arch Intern Med* 1999, **159**:53–58.
43. Moreno-Martínez A, Mensa J, Martínez JA, Marco F, Vila J, Almela M, *et al.* **Cefixime versus amoxicillin plus metilmicin in the treatment of community-acquired non-complicated acute pyelonephritis.** *Med Clin (Barc)* 1998, **111**:521–524.
44. Talan DA, Stamm WE, Hooton TM, Moran GJ, Burke T, Iravani A, *et al.* Comparison of ciprofloxacin (7 days) and trimethoprim-sulfamethoxazole (14 days) for acute uncomplicated pyelonephritis in women: a randomized trial. *JAMA* 2000, 283:1583–1590.
45. Mensa J, Moreno-Martínez A, Martínez JA, Marco F, Vila J, Horcajada JP, *et al.* **Treatment of acute uncomplicated pyelonephritis (AUP): a randomized trial comparing 7 vs. 14-day therapy.** *39th Interscience Conference on Antimicrobial Agents and Chemotherapy.* San Francisco, CA, September 25–29, 1999 [abstract 613].

CLINICAL AND DIAGNOSTIC LABORATORY IMMUNOLOGY, Jan. 2004, p. 142–146 Vol. 11, No. 1
1071-412X/04/$08.00+0 DOI: 10.1128/CDLI.11.1.142–146.2004

Evaluation of Inflammatory and Renal-Injury Markers in Women Treated with Antibiotics for Acute Pyelonephritis Caused by *Escherichia coli*

Juan P. Horcajada,[1]* María Velasco,[1] Xavier Filella,[2] Luisa Alvarez,[2] Elisa De Làzzari,[3] Jose Luis Marín,[2] Blanca Collvinent,[4] Alex Smithson,[1] Jose Antonio Martínez,[1] Mariana Noguero,[1] Jordi Vila,[5] and Josep Mensa[1]

Service of Infectious Diseases[1] and Service of Microbiology,[5] Institut Clínic Infeccions i Immunologia, Service of Clinical Biochemistry, Centre de Diagnostic Biomedic,[2] Biostatistics and Epidemiology Unit,[3] and Emergency Room,[4] Hospital Clínic Universitari-IDIBAPS, Barcelona, Spain

Received 13 June 2003/Returned for modification 21 August 2003/Accepted 9 October 2003

The evolution and the relationship between inflammatory and renal-injury markers in women with acute uncomplicated pyelonephritis under antimicrobial therapy were investigated in a prospective study. Markers were measured before and 6 and 24 h after the intravenous administration of 1 g of ceftriaxone. Before treatment, the median levels of all markers except the serum creatinine levels were high. Twenty-four hours after the onset of antibiotic treatment, the C-reactive protein (CRP) level continued to be high, while the serum interleukin-6 (IL-6) levels and the urine IL-6, IL-8, albumin, and immunoglobulin G (IgG) levels decreased significantly. In contrast, serum creatinine and tumor necrosis factor alpha levels and urine *N*-acetyl-β-glucosaminidase, α_1-microglobulin, and β_2-microglobulin levels did not change over time. There was a significant correlation between IL-6 and IL-8 levels and urine albumin and IgG levels (urine albumin and IgG levels are glomerular and urinary tract-injury markers) as well as between serum CRP levels and the levels of the tubular-injury markers. In women with acute pyelonephritis, appropriate antibiotic treatment rapidly decreases serum IL-6 levels and urine IL-6 and IL-8 levels, which correlate well with urine albumin and IgG levels.

Although acute pyelonephritis (APN) causes less morbidity in adults than in the pediatric population, in which APN may lead to renal scars and altered renal function and/or arterial hypertension (3), little is known about the magnitude of renal damage caused by APN in adults or its relationship to the inflammatory process. This is interesting because the antibiotics used to treat infections caused by gram-negative bacteria may exert a proinflammatory effect via endotoxin release, and this effect can be stronger or weaker, depending on the class of antibiotic used (21, 22). Hence, in APN and other more severe infections caused by gram-negative bacteria, it is conceivable that an antibiotic-induced systemic response and/or organ injury mediated by inflammation may occur (16, 22). This could have implications in adult patients with a high risk of renal insufficiency, such as patients with one kidney, renal transplant recipients, or patients with advanced cirrhosis.

Elevations of urine and serum cytokine levels have been observed in patients with different forms of urinary tract infection (UTIs), especially those with upper UTIs (11, 15). Interleukin-6 (IL-6) is an endogenous pyrogen and an activator of acute-phase reactants and lymphocytes (14). In patients with febrile UTIs, IL-6 reflects the degree of inflammatory response in the urinary tract (19). IL-8 is a chemoattractant for neutrophils (1), and in patients with UTIs it has been related to the degree of pyuria and renal scarring (10). A relationship between the degree of inflammation in APN and renal dysfunction and scarring has been reported previously (10, 12, 24). Urinary albumin and immunoglobulin G (IgG) are considered markers of glomerular dysfunction (2, 23). *N*-Acetyl-β-glucosaminidase (NAG) is a renal hydrolytic enzyme located primarily in the lysosomal fraction of the renal tubular cell, and in the event of proximal renal tubular damage, its concentration in urine increases (20). Urinary α_1-microglobulin and β_2-microglobulin have been proposed to be markers of proximal tubular dysfunction (4, 5). Nearly all α_1- and β_2-microglobulins filtered by the glomerulus are reabsorbed by the proximal tubule; increased urinary excretion of these proteins denotes malfunction of the proximal portion of the renal tubule (7). It has been shown that in the absence of nephrotoxic compounds or previous renal diseases, the urine α_1-microglobulin level is a useful marker of tubular damage in patients with APN (5). In fact, patients with APN have elevated urine α_1-microglobulin levels, whereas patients with cystitis do not (6).

In order to establish the evolution of inflammatory- and renal-injury markers (RIMs) in patients with APN receiving antibiotic therapy, as well as the possible correlation between them, we conducted an observational study with women with uncomplicated APN treated with ceftriaxone.

MATERIALS AND METHODS

Women with uncomplicated APN, defined as fever (armpit temperature, >37.9°C), flank pain, and pyuria and a culture of urine positive for a uropathogen (>100,000 CFU/ml) in the absence of urological abnormalities, were ran-

* Corresponding author. Mailing address: Service of Infectious Diseases. Institut Clínic Infeccions i Immunologia, Hospital Clínic Universitari-IDIBAPS, Villaroel 170, 08036 Barcelona, Spain. Phone: 34 932275708. Fax: 34 934514438. E-mail: jhorcaja@clinic.ub.es.

TABLE 1. Levels of inflammation markers at different stages of the study and significance level by Wilcoxon signed-rank test

Inflammation marker and time of evaluation[a]	Median (IQR)	*P* (Wilcoxon test)[b]
Serum CRP (mg/dl)		
Before	11.1 (5.9, 14.9)	0.213
6 h	13.4 (8.5, 15.6)	0.484
24 h	12.4 (6.8, 16.6)	0.064
Serum TNF-α (pg/ml)		
Before	35 (23, 77)	0.995
6 h	45 (29, 56)	0.057
24 h	34 (26, 48)	0.430
Serum IL-6 (pg/ml)		
Before	97 (43, 152)	0.975
6 h	81 (42, 159)	0.005
24 h	44 (23, 90)	0.035
Urine IL-6 (pg/ml)		
Before	81 (36, 207)	0.394
6 h	80 (22, 146)	0.014
24 h	17 (10, 42)	0.014
Urine IL-8 (pg/ml)		
Before	433 (139, 828)	0.975
6 h	353 (136, 660)	0.001
24 h	59 (12, 133)	0.002

[a] Before, 6 h, and 24 h indicate before antibiotic administration and 6 and 24 h after antibiotic administration, respectively. Reference values: CRP, 0.1 to 0.8 mg/dl; TNF-α, <15 pg/ml; serum IL-6, <5 pg/ml; urine IL-6, <5 pg/ml; urine IL-8, <126 pg/ml.

[b] For each marker, the first entry is for the comparison of the values for before and 6 h after antibiotic administration, the second entry is for the comparison of the values for 6 and 24 h after antibiotic administration, and the third entry is for the comparison of the values for 24 h after and before antibiotic administration.

TABLE 2. Levels of RIMs at different stages of the study and significance level by the Wilcoxon signed-rank test

RIM and time of evaluation[a]	Median (IQR)	*P* (Wilcoxon test)[b]
Serum creatinine (mg/dl)		
Before	1 (0.9, 1.1)	0.323
6 h	1 (0.9, 1.1)	0.720
24 h	1 (0.9, 1.1)	0.739
Urine NAG (U/g of creatinine)		
Before	4.5 (2.7, 5.7)	0.576
6 h	4.6 (2.9, 9.3)	0.196
24 h	7.5 (2.7, 13.2)	0.356
Urine β_2-microglobulin (U/g of creatinine)		
Before	387 (160, 1,589)	0.752
6 h	1,077 (148, 3,261)	0.991
24 h	407 (113, 1,926)	0.704
Urine α_1-microglobulin (mg/g of creatinine)		
Before	21.7 (10.5, 37.8)	0.599
6 h	27.5 (10.9, 47.1)	1.000
24 h	21.2 (10.1, 44.2)	0.983
Urine albumin (mg/g of creatinine)		
Before	187 (52, 286)	0.402
6 h	111 (24, 272)	0.002
24 h	51 (2.5, 105)	0.004
Urine IgG(mg/g of creatinine)		
Before	16.1 (10.6, 32.7)	0.131
6 h	11.8 (4.9, 17.1)	0.221
24 h	6.3 (4.7, 11.5)	0.013

[a] Before, 6 h, and 24 h indicate before antibiotic administration and 6 and 24 h after antibiotic administration, respectively. Reference values: albumin, 1 to 15 mg/g of creatinine; IgG, 0.1 to 4.6 mg/g of creatinine; α_1-microglobulin, 0.1 to 10 mg/g of creatinine; β_2-microglobulin, 0.1 to 100 U/g of creatinine; NAG, 0.2 to 4.6 U/g of creatinine.

[b] For each RIM, the first entry is for the comparison of the values for before and 6 h after antibiotic administration, the second entry is for the comparison of the values for 6 and 24 h after antibiotic administration, and the third entry is for the comparison of the values for 24 h after and before antibiotic administration.

domly selected among patients admitted to the Infectious Diseases Service of a tertiary-care hospital between February 2000 and October 2001. Patients who had taken antibiotics in the previous week and those who were receiving immunosuppressants or nephrotoxic drugs, as well as diabetics and patients with known renal and/or urological diseases, were not included. Serum creatinine levels, the levels of inflammatory markers in serum (C-reactive protein [CRP], tumor necrosis factor alpha [TNF-α], and IL-6) and urine (IL-6 and IL-8), and the levels of RIMs in urine (albumin, IgG, NAG, α_1-microglobulin and β_2-microglobulin) were measured before the intravenous administration of 1 g of ceftriaxone and 6 and 24 h later, before administration of the next dose of ceftriaxone. Oral antibiotics were prescribed when the patients were afebrile, with the results of the antibiogram taken into account.

The levels of TNF-α (reference level, <15 pg/ml; Biosource, Europe, Nivelles, Belgium), IL-6 (reference level in serum, <5 pg/ml; Biosource, Europe), and IL-8 (reference level in urine, <126 pg/ml; Immunotech, Marseille, France) (12) were measured by a solid-phase enzyme immunoassay, which was performed on a microtiter plate with a monoclonal antibody directed against the corresponding cytokine. Finally, the amount of substrate turnover was determined colorimetrically by measuring the absorbance, which is proportional to the cytokine concentration. A standard curve was plotted, and the cytokine concentrations in the samples were determined by interpolation from the standard curve. The results for TNF-α, IL-6, and IL-8 are given in picograms per milliliter.

Serum CRP levels (reference levels, 0.1 to 0.8 mg/dl) and urine α_1-microglobulin (reference levels, 0.1 to 10 mg/g of creatinine), β_2-microglobulin (reference levels, 0.1 to 100 U/g of creatinine), and IgG (reference levels, 0.1 to 4.6 mg/g of creatinine) levels were assayed by immunonephelometric methods (Dade Behring, Marburg, Germany). The levels of NAG (reference levels, 0.2 to 4.6 U/g of creatinine) and albumin (reference levels, 0.1 to 15 mg/g of creatinine) in urine were measured by a spectrophotometric kinetic method with 3-cresolsulfonphthaleinyl-*N*-acetyl-β-D-glucosaminide sodium salt as the substrate for NAG and by an immunoturbidimetric assay for albumin in a Cobas Mira S analyzer by using kits from Roche Diagnostics (Basel, Switzerland). Levels in urine were normalized to the urine creatinine level. Measurement of the urine creatinine level was performed in the Cobas Mira S analyzer by an assay based on a modified Jaffe method (Roche Diagnostics).

Informed consent was obtained from all patients. The human experimentation guidelines of the Ethics Committee of the Hospital Clínic of Barcelona were followed.

Statistics. Quantitative data are reported as medians (interquartile ranges). Nonparametric tests were used for comparisons because the sample size was small. For each parameter differences among the three time points were estimated by the Wilcoxon signed-rank test: three pairwise comparisons were made, and the Bonferroni adjustment was used to correct the significance level. The correlation between inflammation markers and RIMs at each of the three time points was estimated by using the Spearman coefficient. In this case the level of significance required for each correlation coefficient to be different from zero was ≤0.05. Statistical analysis was performed with STATA statistical software (release 7.0, 1999; StataCorp., College Station, Tex.).

RESULTS

Twenty-two women with uncomplicated APN were studied. The mean (standard deviation) age was 49.4 (21.8) years. For

TABLE 3. Correlations between the levels and evolution of the inflammation markers and the RIMs

Marker	Spearman's rho value (*P*)[a]					
	Serum CRP			Serum TNF-α		
	Basal	6 h	24 h	Basal	6 h	24 h
Serum creatinine	0.13 (0.565)	0.31 (0.166)	0.12 (0.608)	0.08 (0.725)	−0.18 (0.415)	0.28 (0.249)
Urine NAG	**0.44 (0.038)**	**0.45 (0.037)**	**0.57 (0.011)**	0.20 (0.361)	0.25 (0.268)	−0.15 (0.550)
Urine α_1-microglobulin	**0.83 (<0.001)**	**0.70 (<0.001)**	**0.57 (0.012)**	0.21 (0.354)	0.17 (0.454)	−0.18 (0.459)
Urine β_2-microglobulin	**0.47 (0.029)**	**0.52 (0.013)**	**0.47 (0.042)**	−0.36 (0.097)	0.09 (0.700)	0.07 (0.772)
Urine albumin	0.36 (0.099)	**0.50 (0.019)**	**0.77 (<0.001)**	0.39 (0.074)	0.42 (0.050)	0.33 (0.170)
Urine IgG	0.36 (0.101)	0.21 (0.352)	0.38 (0.114)	0.38 (0.084)	**0.44 (0.039)**	0.14 (0.573)

[a] Bold face data indicate statistically significant correlations.

all but one of the patients the urine culture yielded *Escherichia coli*. Blood cultures were positive for four patients (18%), with *E. coli* growing in all of them. Patients were afebrile in a mean (standard deviation) of 1.64 (0.84) days after the onset of antibiotic therapy.

The median (interquartile range [IQR]) levels of inflammation markers in serum and urine before administration of the first dose of 1 g of ceftriaxone and 6 and 24 h later and the significance levels determined by the Wilcoxon signed-rank test are shown in Table 1. At the baseline, the levels of all inflammation markers were 2 to 19 times higher than the reference levels. Serum IL-6, urine IL-6, and urine IL-8 concentrations decreased significantly 24 h after antibiotic administration, with no changes at 6 h. Serum CRP and TNF-α levels did not change significantly and remained high during the first 6 and 24 h (Table 1).

Median urine RIM levels at the baseline and 6 and 24 h after the administration of 1 g of ceftriaxone and the significance level determined by the Wilcoxon signed-rank test are shown in Table 2. With the exception of urine NAG and serum creatinine levels, RIM levels were high at the baseline. After the onset of antibiotic therapy, the median α_1-microglobulin and β_2-microglobulin levels in urine at 6 h and the median NAG levels in urine at 24 h were higher than the baseline levels; however, the comparisons of the distributions were not statistically significant. Urine IgG levels decreased significantly after 24 h, with no change at 6 h, while urine albumin levels decreased significantly after 6 and 24 h from the onset of antibiotic treatment. Creatinine levels did not change significantly during the study period (Table 2).

Table 3 shows the levels of significance of the correlation between each inflammation marker and each RIM at the time points studied. A relation between CRP levels and the levels of tubular-injury markers was found at the three time points: an increment in the CRP level is related to an increment in the tubular-injury marker level. The same kind of relation was found between CRP and urine albumin levels at 6 and 24 h after the onset of treatment.

There was no correlation between TNF-α levels and the levels of any of the RIMs studied except for urine IgG levels at 6 h. Serum and urine IL-6 levels and urine IL-8 levels correlated well with urine albumin and IgG levels at the three time points studied and with urine α_1-microglobulin, β_2-microglobulin, and NAG levels at 24 h. Serum IL-6 levels also correlated with urine NAG levels at the baseline, with β_2-microglobulin levels at 6 h, and with α_1-microglobulin levels at the three time points studied (Table 3).

DISCUSSION

In the present study we analyzed the local and systemic inflammatory responses in patients with APN as well as the associated renal damage before and after the onset of effective antimicrobial therapy. The levels of the proinflammatory cytokines and CRP were elevated at the baseline. However, during the first 24 h of appropriate treatment a different evolutionary pattern emerged. On the one hand, no changes in TNF-α and CRP levels were detected throughout the study period; on the other hand, there was a significant decrease in IL-6 and IL-8 levels after the first 6-h period, during which they were stable. High levels of proinflammatory cytokines have previously been described in patients with upper UTIs (11); however, studies describing the evolving pattern of inflammation in adults with APN during antibiotic treatment are scarce. The rapid decline in serum IL-6 and urine IL-6 and IL-8 levels after the onset of antimicrobial therapy in this study suggests that in women with APN effective antimicrobial therapy significantly reduces the inflammatory process in 24 h. This has recently been reported in children with APN (13). The different patterns of CRP and interleukins can be explained in part by the longer half-life of CRP than those of the interleukins (8, 21) and because in patients with septic conditions the production of proinflammatory cytokines is down-regulated (21). We do not have a satisfactory explanation for the lack of a decline in TNF-α levels, but a similar evolutionary profile, despite significant decreases in the levels of other proinflammatory interleukins, has been described previously (21).

One interesting finding of our study is that the urine albumin and IgG levels followed the evolution of the IL-6 and IL-8 levels, respectively, with a significant correlation at the three time points of evaluation used in the study. Although urine IgG and albumin levels can be elevated by the urinary tract inflammatory response itself and could be considered markers of generalized urinary tract inflammation, they can also reflect glomerular damage (2, 23). The correlation between cytokine levels and renal and urinary tract injury may reflect in part the underlying link between bacterial infection, inflammation, and organ injury. In fact, in patients with renal scarring urine IL-8 levels are significantly higher than those in controls (10). Moreover, exogenous administration of cytokines has induced

TABLE 3—*Continued*

Spearman's rho value (*P*)[a]								
Serum IL-6			Urine IL-6			Urine IL-8		
Basal	6 h	24 h	Basal	6 h	24 h	Basal	6 h	24 h
0.03 (0.899)	−0.10 (0.645)	−0.20 (0.409)	−0.39 (0.079)	−0.39 (0.073)	−0.18 (0.456)	−0.18 (0.450)	0.12 (0.618)	0.15 (0.559)
0.46 (0.029)	0.24 (0.277)	**0.52 (0.022)**	0.32 (0.151)	0.24 (0.281)	**0.54 (0.017)**	0.42 (0.066)	0.40 (0.076)	**0.71 (<0.001)**
0.49 (0.021)	**0.45 (0.034)**	**0.56 (0.012)**	0.07 (0.749)	0.31 (0.165)	**0.49 (0.038)**	0.13 (0.596)	0.31 (0.171)	**0.81 (<0.001)**
0.29 (0.195)	**0.46 (0.030)**	**0.59 (0.009)**	0.20 (0.394)	0.29 (0.189)	**0.54 (0.018)**	0.24 (0.313)	0.39 (0.082)	**0.65 (0.003)**
0.60 (0.003)	**0.72 (<0.001)**	**0.77 (<0.001)**	**0.66 (0.001)**	**0.77 (<0.001)**	**0.75 (<0.001)**	**0.59 (0.006)**	**0.54 (0.011)**	**0.80 (<0.001)**
0.46 (0.030)	**0.44 (0.039)**	0.39 (0.097)	**0.80 (<0.001)**	**0.68 (<0.001)**	0.45 (0.053)	**0.58 (0.008)**	**0.48 (0.029)**	**0.56 (0.016)**

glomerular injury and enhanced renal damage in animals with glomerulonephritis (18). Thus, more renal damage occurs with a stronger inflammatory response. In this regard, it is striking that in our study, 24 h after the onset of effective antimicrobial therapy, IL-6 and IL-8 levels as well as the levels of glomerular and urinary tract-injury markers (urinary albumin and IgG) decreased significantly and nearly reached normal levels. This finding strengthens the importance of starting antimicrobial therapy as early as possible in patients with APN in order to prevent or limit renal injury. The early institution of antibiotic therapy has been shown in previous experiments with animals to mitigate the extent of renal scarring (9). On the other hand, because different antibiotics can induce a more intense or a less intense proinflammatory response (22), it is conceivable that the magnitude of organ injury could depend on the particular antibiotic used. In this regard, a previous study found a nonsignificant increase in proinflammatory cytokine levels 4 h after the administration of a selective PBP 3 binding antibiotic (ceftazidime) which was not observed after treatment with a specific PBP 2 inhibitor (imipenem) in patients with sepsis caused by gram-negative bacteria (21). In the present study we again found that the behavior of TNF-α was different from those of IL-6 and IL-8 6 h after the onset of treatment with ceftriaxone, another beta-lactam selective for PBP 3. At that time point, serum TNF-α levels tended to be higher than those at the baseline, whereas the interleukin levels were reduced. Although differences in sampling times (4 versus 6 h) can partly justify these discrepancies, we believe that the available data altogether cast some doubts about the real magnitude of the proinflammatory effect attributable to beta-lactams selective for PBP 3 in patients with APN. However, as in previous studies differences between antibiotics were detected (21). This is an area in which additional comparative studies are clearly needed. Another application of the findings of this study is complicated UTIs. It would be valuable to know if a lack of reduction in the levels of these markers of inflammation and renal dysfunction signify an unresolved infection, such as a renal abscess or an infection caused by a drug-resistant microorganism.

The levels of the tubular-injury markers α_1-microglobulin and β_2-microglobulin were elevated before the onset of antibiotic treatment and remained high during the first 24 h, with no significant changes. NAG levels were not elevated at the first two times of evaluation, but they were increased at 24 h. The elevations in the levels of the tubular-injury markers could be related to glomerular damage. It is known that patients with albuminuria also have elevated levels of α_1- and β_2-microglobulin excretion but that this situation improves as the albuminuria resolves (17). In our study, however, the levels of the tubular-injury markers did not change, despite the decrease in albuminuria, and correlated well with CRP levels at the three time points. Our findings indicate that in APN patients, tubular damage persists longer than glomerular injury, even if the infection is being controlled. We can only speculate that inflammation-mediated glomerular alteration seems functional in nature and hence improves as the level of inflammation decreases, whereas tubular injury could be structural, as the elevation of the levels of NAG (an intracellular enzyme) suggests. The persistence of tubular dysfunction after injury has previously been shown in patients with other pathologies, such as acute urine retention, in which it may linger for more than 6 months after the obstruction is relieved (17). The importance and consequences of this finding for adults with APN remains to be elucidated. Moreover, urine α_1- and β_2-microglobulin levels were somewhat higher at 6 h than before the onset of therapy, although not significantly. This could be related to the proinflammatory effect of the antibiotic used (21), although the small change observed together with the lack of a correlation of the tubular-injury marker levels with the levels of TNF-α (the only cytokine which showed some increment shortly after the start of treatment) does not support this hypothesis.

From a clinical point of view, our findings indicate the need for further studies aimed at investigating the effects of different classes of antibiotics on renal inflammation and injury when they are used to treat APN. Until this issue is clarified, our findings point to the importance of starting antimicrobial therapy as soon as possible in patients with pyelonephritis to promptly reduce the inflammatory response and, eventually, prevent or limit organ damage.

REFERENCES

1. **Baggliolini, M., A. Waltz, and S. L. Kunkel.** 1989. Neutrophil activating peptide-I/interleukin-8, a novel cytokine that activates neutrophils. J. Clin. Investig. **84:**1045–1047.
2. **Bergon, E., R. Granados, P. Fernandez-Segoviano, E. Miravalles, and M. Bergon.** 2002. Classification of renal proteinuria: a simple algorithm. Clin. Chem. Lab. Med. **40:**1143–1150.
3. **Chon, C. H., F. C. Lai, and L. M. Shortliffe.** 2001. Pediatric urinary tract infections. Pediatr. Clin. N. Am. **48:**1441–1459.
4. **Donaldson, M. D., R. E. Chamber, M. W. Woolridge, and J. T. Whicher.** 1990. α_1-Microglobulin, β_2-microglobulin and retinol binding protein in childhood febrile illness and renal disease. Pediatr. Nephrol. **4:**314–318.
5. **Everaert, K., J. Delanghe, C. Vande Wiele, P. Hoebeke, R. A. Dierckx, B. Clarysse, N. Lameire, and W. Oosterlinck.** 1998. Urinary alpha 1-microglobulin detects uropathy. A prospective study in 483 patients. Clin. Chem. Lab. Med. **36:**309–315.
6. **Everaert, K., A. Raes, O. Hoebeke, W. Samijn, J. Delanghe, C. Vande Wiele, and J. Vande Walle.** 1998. Combined use of urinary alpha1-microglobulin

and 99mTc DMSA scintigraphy in the diagnosis and follow-up of acute pyelonephritis and cystitis in children. Eur. Urol. **34:**486–491.
7. **Flynn, F. V.** 1990. Assessment of renal function: selected developments. Clin. Biochem. **23:**49–54.
8. **Gabay, C., and I. Kushner.** 1999. Acute-phase proteins and other systemic responses to inflammation. N. Engl. J. Med. **340:**448–454.
9. **Glauser, M. P., P. Meylan, and J. Billa.** 1987. The inflammatory response and tissue damage: the example of renal scars following acute renal infection. Pediatr. Nephrol. **1:**615–622.
10. **Haraoka, M., K. Senoh, N. Ogata, M. Furukawa, T. Matsumoto, and J. Kumazawa.** 1996. Elevated interleukin-8 levels in the urine of children with renal scarring and/or vesicoureteral reflux. J. Urol. **155:**678–680.
11. **Hedges, S., K. Stenqvist, G. Lidin-Janson, J. Martinell, T. Sandberg, and C. Svanborg.** 1992. Comparison of urine and serum concentrations of interleukin-6 in women with acute pyelonephritis or asymptomatic bacteriuria. J. Infect. Dis. **166:**653–656.
12. **Jacobson, S. H., B. Hylander, B. Wretlind, and A. Brauner.** 1994. Interleukin-6 and interleukin-8 in serum and urine in patients with acute pyelonephritis in relation to bacterial-virulence-associated traits and renal function. Nephron **67:**172–179.
13. **Kassir, K., O. Vargas-Shiraishi, F. Zaldivar, M. Berman, J. Singh, and A. Arrieta.** 2001. Cytokine profiles of pediatric patients treated with antibiotics for pyelonephritis: potential therapeutic impact. Clin. Diagn. Lab. Immunol. **8:**1060–1063.
14. **Kishimoto, T.** 1989. The biology of interleukin-6. Blood **74:**1–10.
15. **Ko, Y. C., N. Mukaida, S. Ishiyama, A. Tokue, T. Kawai, K. Matsushima, and T. Kasahara.** 1993. Elevated interleukin-8 levels in the urine of patients with urinary tract infections. Infect. Immun. **61:**1307–1314.
16. **Mock, C. N., G. J. Jurkovich, D. J. Dries, and R. V. Maier.** 1995. Clinical significance of antibiotic endotoxin-releasing properties in trauma patients. Arch. Surg. **130:**1234–1241.
17. **Mustonen, S., I. Ala-Houhala, and T. L. J. Tammela.** 1999. Proteinuria and renal function during and after acute urinary retention. J. Urol. **161:**1781–1785.
18. **Ortiz, A., M. Gomez-Chiarri, J. Alonso, et al.** 1994. The potential role of inflammatory and fibrogenic cytokines in glomerular diseases. J. Lipid Mediat. Cell Signal **9:**55–74.
19. **Otto, G., J. Braconier, A. Andreasson, and C. Svanborg.** 1999. Interleukin-6 and disease severity in patients with bacteremic and nonbacteremic febrile urinary tract infection. J. Infect. Dis. **179:**172–179.
20. **Price, R. G.** 1992. The role of NAG (*N*-acetyl-beta-D-glucosaminidase) in the diagnosis of kidney disease including the monitoring of nephrotoxicity. Clin. Nephrol. **38**(Suppl.)**:**14–19.
21. **Prins, J. M., M. A. van Agtmael, E. J. Kuijper, S. J. H. van Deventer, and P. Speelman.** 1995. Antibiotic-induced endotoxin release in patients with gram-negative urosepsis: a double-blind study comparing imipenem and ceftazidime. J. Infect. Dis. **172:**886–891.
22. **Prins, J. M., S. J. H. van Deventer, E. D. J. Kuijper, and P. Speelman.** 1994. Clinical relevance of antibiotic-induced endotoxin release. Antimicrob. Agents Chemother. **38:**1211–1218.
23. **Recker, F., W. Hofmann, A. Bex, and R. Tscholl.** 1992. Quantitative determination of urinary marker proteins: a model to detect intrarenal bioeffects after extracorporeal lithotripsy. J. Urol. **148:**1000–1006.
24. **Roilides, E., F. Papachristou, E. Gioulekas, S. Tsaparidou, N. Karatzas, J. Sotiriou, and J. Tsiouris.** 1999. Increased urine interleukin-6 concentrations correlate with pyelonephritic changes on 99mTc-diercaptosuccinic acid scans in neonates with urinary tract infections. J. Infect. Dis. **180:**904–907.

JOURNAL OF CLINICAL MICROBIOLOGY, June 2005, p. 2962–2964 Vol. 43, No. 6
0095-1137/05/$08.00+0 doi:10.1128/JCM.43.6.2962–2964.2005

Quinolone-Resistant Uropathogenic *Escherichia coli* Strains from Phylogenetic Group B2 Have Fewer Virulence Factors than Their Susceptible Counterparts

Juan P. Horcajada,[1]* Sara Soto,[2] Abby Gajewski,[3]† Alex Smithson,[1] M. Teresa Jiménez de Anta,[2] Josep Mensa,[1] Jordi Vila,[2] and James R. Johnson[3]

Service of Infectious Diseases[1] and Service of Microbiology,[2] Hospital Clínic Universitari-IDIBAPS, Barcelona, Spain, and Medical Service, VA Medical Center, Minneapolis, Minnesota, and Department of Medicine, University of Minnesota, Minneapolis, Minnesota[3]

Received 2 October 2004/Returned for modification 6 December 2004/Accepted 4 February 2005

The prevalence of 31 virulence factors was analyzed among nalidixic acid-susceptible and -resistant *Escherichia coli* strains from phylogenetic group B2. Hemolysin, cytotoxic necrotizing factor 1, and S and F1C fimbriae genes were less prevalent among nalidixic acid-resistant *E. coli* strains. Quinolone resistance may be associated with a decrease in the presence of some virulence factors.

Extraintestinal pathogenic *Escherichia coli* (ExPEC) strains have multiple virulence factors (VFs) that confer the potential for pathogenicity (6). Recently, extended virulence genotypes have been reported for ExPEC isolates from patients with diverse extraintestinal syndromes (9). *E. coli* strains derive from different phylogenetic groups (5). Pathogenic *E. coli* strains derive mainly from the more virulent phylogenetic group B2 (3, 7, 13).

Recent data suggest that quinolone-resistant ExPEC are less able to cause upper urinary tract infection and have fewer VFs than quinolone-susceptible *E. coli* (14, 15). Some studies have related quinolone resistance and low virulence with phylogenetic origin (8). However, in vitro studies (unpublished data) suggest a decreased pathogenicity of *E. coli* associated with the acquisition of quinolone resistance itself. To study whether the absence of VFs is associated with resistance specifically within phylogenetic group B2, we investigated the prevalence of 31 VFs among quinolone-resistant versus quinolone-susceptible *E. coli* urinary tract infection (UTI) isolates, all belonging to phylogenetic group B2 (the most virulent; not intrinsically related to quinolone resistance, as shown for phylogenetic group A) (10). The prevalence of the studied VFs according to susceptibility to ampicillin, cotrimoxazole, and gentamicin was also assessed.

E. coli strains isolated from urine from patients with acute pyelonephritis, acute cystitis, or acute prostatitis who presented at our department were identified by conventional biochemical tests. Cystitis, acute pyelonephritis, and acute prostatitis were defined as they were defined previously elsewhere (12). Fifty-three *E. coli* isolates causing acute pyelonephritis in women, 19 causing cystitis in women, and 13 causing prostatitis in men were analyzed.

Susceptibilities to nalidixic acid, ciprofloxacin, ampicillin, cotrimoxazole, and gentamicin were tested by the E-test method (AB Biodisk, Sölna, Sweden). All isolates were assigned to phylogenetic group B2 with the use of the multiplex PCR-based method (1), and all isolates belonged to different clones by Rep-PCR. Extended virulence genotypes, including 31 individual VFs and *papA* alleles, were determined by multiplex PCR assays and dot blot hybridization, as previously described (7). In addition, *sat* (secreted autotransporter toxin) was detected using previously described PCR conditions and primers (15). Each isolate was tested in duplicate, in parallel with appropriate positive and negative controls.

Statistical analyses were performed by using Fisher's exact and chi-square tests. Stratified analysis was performed by means of Mantel-Haenszel test. A P value of <0.05 was considered statistically significant.

The population studied included 64 nalidixic-susceptible isolates (cystitis, $n = 14$; pyelonephritis, $n = 38$; prostatitis, $n = 12$) and 21 nalidixic acid-resistant isolates (cystitis, $n = 5$; pyelonephritis, $n = 15$; prostatitis, $n = 1$). Among the 85 *E. coli* UTI isolates, each of the four drug resistance phenotypes studied was associated with a statistically significant shift in the prevalence of one or more of the studied VFs (Table 1). The greatest number of these shifts was observed with nalidixic acid resistance. Nalidixic acid resistance was associated with a significantly decreased prevalence of three factors, i.e., *sfa*/*foc* (S and F1C fimbriae), *hlyD* (hemolysin), and *cnf1* (cytotoxic necrotizing factor 1), and a significantly increased prevalence of six factors, *bmaE* (M fimbriae), *gafD* (G fimbriae), *iutA* (aerobactin system), *ireA* (siderophore receptor), *cvaC* (microcin V), and *iss* (increased serum survival) (Table 1).

Because of the disproportionate distribution of prostatitis isolates in the study population in relation to quinolone resistance and the known associations of clinical syndrome with both quinolone resistance and virulence (12, 14), stratified analysis was used to simultaneously assess the associations of resistance phenotype and clinical syndrome with virulence. This analysis showed a tendency to a lower prevalence of *hly*

* Corresponding author. Mailing address: Service of Infectious Diseases, Hospital Clínic Universitari-IDIBAPS, Villaroel 170, 08036 Barcelona, Spain. Phone: 34 932 275708. Fax: 34 934 514438. E-mail: jhorcaja@yahoo.es.

† Current address: Aerotek Scientific, St. Paul, MN.

TABLE 1. Distribution of virulence factors according to antimicrobial resistance phenotype among 85 *E. coli* urine isolates from phylogenetic group B2

Drug (no. susceptible, no. resistant)[a]	Virulence factor[b]	No. (%) of isolates that were:[c]		*P* value[d]
		Susceptible	Resistant	
Nalidixic acid (64, 21)	*sfa/focDE*	46 (72)	7 (33)	(0.0015)
	bmaE	3 (5)	5 (24)	0.009
	gafD	3 (5)	5 (24)	0.009
	hlyD	42 (66)	7 (33)	(0.009)
	cnf1	39 (61)	7 (33)	(0.02)
	iutA	21 (33)	15 (71)	0.006
	ireA	15 (23)	11 (52)	0.012
	cvaC	10 (16)	9 (43)	0.009
	iss	9 (14)	14 (66)	<0.001
Ampicillin (30, 55)	K1 *kpsM*	13 (43)	7 (13)	(0.001)
Cotrimoxazole (59, 26)	*bmaE*	3 (5)	5 (19)	0.03
	gafD	3 (5)	5 (19)	0.03
	iroN	46 (78)	10 (38)	(<0.001)
	malX	53 (89)	19 (73)	(0.047)
Gentamicin (81, 4)	*fimH*	78 (96)	3 (75)	(0.04)
	iutA	32 (39)	4 (100)	0.01

[a] Susceptibility and resistance are as defined by E-test.

[b] Only those virulence factors that yielded a *P* value of <0.05 are shown. *sfa/focDE*, S and F1C fimbriae; *bmaE*, M fimbriae; *gafD*, G fimbriae; *fimH*, type 1 fimbriae; *hlyD*, hemolysin; *cnf1*, cytotoxic necrotizing factor 1; *iutA*, aerobactin receptor; *iroN* and *ireA*, novel siderophore receptors; *cvaC*, colicin (microcin) V; *iss*, increased serum survival; K1 *kpsM*, group 2 capsule (variant K1); and *malX*, pathogenicity island marker. No statistically significant association with resistance was noted for the following virulence factors: *papA*, P fimbriae structural subunit; *papC*, P fimbriae assembly; *papEF*, P fimbriae tip pilis; *papG*, P fimbriae adhesin (and alleles II and III); *sfaS*, S fimbriae; *focG*, F1C fimbriae; *afa/draBC*, Dr-binding adhesins; *iha*, putative adhesin-siderophore; *cdtB*, cytolethal distending toxin; *sat*, secreted autotransporter toxin; *fyuA*, yersiniabactin receptor; *kpsM* II, group 2 capsule (variant K2); *kpsMT* III, group 3 capsule; *rfc*, O4 lipopolysaccharide; *ibeA*, invasion of brain endothelium; *ompT*, outer membrane protease T; and *fliC*, flagellin.

[c] Numbers of isolates that were susceptible or resistant to drugs in column 1 are shown. Comparison groups varied by drug.

[d] *P* values (by Fisher's exact test or χ^2 test) are for comparisons of isolates susceptible and resistant to the indicated drug. Parentheses indicate negative associations of virulence factor with resistance.

and *cnf* in quinolone-resistant *E. coli* from cystitis (20%) and pyelonephritis (49%) than in their susceptible counterparts (40% and 69%, respectively; the *P* value was 0.08 between cystitis isolates and 0.23 for pyelonephritis isolates). Mantel-Haenszel stratified analysis showed that clinical syndrome was not a confounding factor (crude relative risk, 0.51 [95% confidence interval, 0.27 to 0.95]; adjusted relative risk, 0.52 [95% confidence interval, 0.27 to 0.99]). This fact suggests that quinolone resistance could be directly associated with virulence loss, as suggested in a previous study (15). Other studies have also found that resistance to quinolones is significantly more frequent among nonhemolytic *E. coli* isolates (11), although the mechanism is unknown. The *hly*, *cnf*, and *sfa* genes have been found in pathogenicity islands (PAI). PAI can be easily deleted from the chromosome, leading to mutants with reduced virulence (4). During the development of quinolone resistance, deletion and transposition of DNA regions may occur with the loss of PAI.

Recent studies have demonstrated a relationship between phylogenetic origin and antibiotic resistance and low prevalence of VFs (8, 10). In a previous study, phylotypes were not taken into account, and the presence of some low-virulence phylotypes, such as phylotype A, may explain the lower prevalence of VFs found among quinolone-resistant *E. coli* strains in our series. To test the hypothesis of the association between quinolone resistance and low virulence, independently of the phylogenetic origin and without its possible confounding effect on this association, we analyzed the prevalence of several VFs in quinolone-resistant and susceptible *E. coli* strains of phylotype B2 exclusively. The results suggested that quinolone resistance may be directly associated with virulence loss. However, a previous study showing that spontaneous quinolone-resistant mutants obtained from hemolytic quinolone-susceptible strains still produce hemolysin could suggest otherwise, although the authors used two mutants and a one-step selection method which does not mimic "in vivo" selection of mutants, which would have made the findings conclusive (11).

In countries with a higher prevalence of quinolone resistance, it is possible to find phylotype B2 *E. coli* strains resistant to quinolones in clinical settings. The higher prevalence of quinolone resistance in these countries has been related to the higher use of quinolones (2). Therefore, it is feasible that although phylotype B2 strains have harbored some VFs during their evolutionary history, in the presence of quinolones, they develop genetic changes leading to quinolone resistance and to a loss of VFs.

We performed statistical analyses by syndrome and demonstrated a tendency toward a lower prevalence of *hly* and *cnf* in quinolone-resistant *E. coli* from cystitis and pyelonephritis than in their susceptible counterparts. Isolates from prostatitis were not taken into consideration, since the majority of isolates were quinolone susceptible. However, the limited number of isolates by syndrome does not permit us to be conclusive regarding the possible association between quinolone resistance and fewer VFs confounded by syndrome.

To assess the possible relationship between fewer VFs in ExPEC and resistance to other antimicrobial agents, the prevalence of the studied VFs was also assessed according to susceptibility to ampicillin, cotrimoxazole, and gentamicin, with few differences among susceptible and resistant isolates to these antibiotics; the exceptions were the *iroN* and *malX* genes, which were less prevalent in cotrimoxazole-resistant strains.

Another interesting finding is that in quinolone-resistant *E. coli* strains, the aerobactin receptor gene *iutA* was significantly more prevalent than in their quinolone-susceptible counterparts. The same result occurred with other, less prevalent VFs, such as the *cva*, *bma*, *gaf*, *iss*, and *ire* genes, and it has been previously shown with the aerobactin gene (8). It is difficult to explain these findings, and further studies are needed to investigate the basis for their occurrence.

In conclusion, the study with the phylogenetic group B2 suggests that quinolone-resistant *E. coli* could be directly associated with low prevalences of hemolysin and *cnf1*.

Grant support was provided by the Office of Research and Development, Medical Research Service, Department of Veterans Affairs, and the National Research Initiative (NRI) Competitive Grants Program/U.S. Department of Agriculture grant 00-35212-9408 (to J.R.J.) and grants FIS 02/0327 (Ministry of Health, Spain) and 2002

SGR00121 (Department d'Universitats, Recerca I Societat de la Informació de la Generalitat de Catalunya, Spain) (to J.V.).

J.R. has previously received grant support and/or honoraria from Bayer, Wyeth-Ayerst, Ortho-McNeil, Merck, and Rochester Medical. All other potential conflicts of interest have been disclosed.

REFERENCES

1. **Clermont, O., S. Bonacorsi, and E. Bingen.** 2000. Rapid and simple determination of the *Escherichia coli* phylogenetic group. Appl. Environ. Microbiol. **66:**4555–4558.
2. **Garau, J., M. Xercavins, M. Rodríguez-Caballeira, J. R. Gomez-Vera, I. Coll, D. Vidal, T. Llovet, and A. Ruiz-Bremon.** 1999. Emergence and dissemination of quinolone-resistant *Escherichia coli* in the community. Antimicrob. Agents Chemother. **43:**2736–2741.
3. **Goullet, P., and B. Picard.** 1990. Electrophoretic type B2 of carboxylesterase B for characterization of highly pathogenic *Escherichia coli* strains from extraintestinal infections. J. Med. Microbiol. **33:**11–16.
4. **Hacker, J., G. Blum-Oehler, I. Mühldorfer, and H. Tschäpe.** 1997. Pathogenicity islands of virulent bacteria: structure, function and impact on microbial evolution. Mol. Microbiol. **23:**1089–1097.
5. **Herzer, P. J., S. Inouye, M. Inouye, and T. S. Whittam.** 1990. Phylogenetic distribution of branched RNS-linked multicopy single-stranded DNA among natural isolates of *Escherichia coli*. J. Bacteriol. **172:**6175–6181.
6. **Johnson, J. R.** 2003. Microbial virulence determinants and the pathogenesis of urinary tract infection. Infect. Dis. Clin. N. Am. **17:**261–278.
7. **Johnson, J. R., M. A. Kuskowsky, T. T. O'Bryan, and J. N. Maslow.** 2002. Epidemiological correlates of virulence genotype and phylogenetic background among *Escherichia coli* blood isolates from adults with diverse-source bacteremia. J. Infect. Dis. **185:**1439–1447.
8. **Johnson, J. R., M. A. Kuskowski, K. Owens, A. Gajewski, and P. L. Winokur.** 2003. Phylogenetic origin and virulence genotype in relation to resistance to fluoroquinolones and/or extended-spectrum cephalosporins and cephamycins among *Escherichia coli* isolates from animals and humans. J. Infect. Dis. **188:**759–768.
9. **Johnson, J. R., and A. L. Stell.** 2000. Extended virulence genotypes of *Escherichia coli* strains from patients with urosepsis in relation to phylogeny and host compromise. J. Infect. Dis. **181:**261–272.
10. **Johnson, J. R., C. van der Schee, M. A. Kuskowski, W. Goessens, and A. van Belkum.** 2002. Phylogenetic background and virulence profiles of fluoroquinolone-resistant clinical *Escherichia coli* isolates from The Netherlands. J. Infect. Dis. **186:**1852–1856.
11. **Martínez-Martínez, L., F. Fernandez, and E. J. Perea.** 1999. Relationship between haemolysis production and resistance to fluoroquinolones among clinical isolates of *Escherichia coli*. J. Antimicrob. Chemother. **43:**277–279.
12. **Ruiz, J., K. Simon, J. P. Horcajada, M. Velasco, M. Barranco, G. Roig, A. Moreno-Martinez, J. A. Martinez, T. Jimenez de Anta, J. Mensa, and J. Vila.** 2002. Differences in virulence factors among clinical isolates of *Escherichia coli* causing cystitis and pyelonephritis in women and prostatitis in men. J. Clin. Microbiol. **40:**4445–4449.
13. **Russo, T. A., and J. R. Johnson.** 2000. Proposal for a new inclusive designation for extraintestinal pathogenic isolates of *Escherichia coli*: ExPEC. J. Infect. Dis. **181:**1753–1754.
14. **Velasco, M., J. P. Horcajada, J. Mensa, A. Moreno-Martinez, J. Vila, J. A. Martinez, J. Ruiz, M. Barranco, G. Roig, and E. Soriano.** 2001. Decreased invasive capacity of quinolone-resistant *Escherichia coli* in patients with urinary tract infections. Clin. Infect. Dis. **33:**1682–1686.
15. **Vila, J., K. Simon, J. Ruiz, J. P. Horcajada, M. Velasco, M. Barranco, A. Moreno, and J. Mensa.** 2002. Are quinolone-resistant uropathogenic *Escherichia coli* less virulent? J. Infect. Dis. **186:**1039–1042.

IS MANNOSE-BINDING LECTIN (MBL) DEFICIENCY ASSOCIATED WITH GRAM POSITIVE INFECTIONS?

Alex Smithson[1*], Rafael Perelló[1], Juan Pablo Horcajada[4], Josep-Maria Nicolas[2] and Francisco Lozano[3]. Infectious Diseases Unit[1], Medical Intensive Care Unit[2], Department of Immunology[3], Hospital Clínic, IDIBAPS, University of Barcelona, Spain. Service of Internal Medicine and Infectious Diseases[4]. Hospital del Mar. Barcelona.

SIR- We have read with great interest the article by Eisen *et al*. (1) in which the authors perform an ambitious multivariable analysis of several of the published articles on the implication of MBL deficiency in the outcome of severe bacterial infections and sepsis. Although genetic polymorphisms of different molecules of the innate immune system, have been associated with an increased mortality in patients with severe sepsis and septic shock (2, 3, 4) the association of MBL with death in intensive care unit (ICU) patients has yielded conflicting results (5, 6). In fact, in the study by Eisen *et al* (1), only a trend towards an increased risk of death among MBL deficient ICU patients was observed which could be due to the high heterogenicity of the patients included in these studies. Similar conflicting results are observed when the relationship between a particular or a group of microorganism and MBL deficiency is analyzed. While some of the published studies have found an association between Gram positive infections and MBL deficiency (6), others have observed a link between Gram negative infections and MBL deficiency (7). Regarding this question, in the study by Eisen *et al* (1), an increased risk of death was observed in patients with low serum levels of MBL and pneumococcal infection. It is worth mentioning that most of the published articles that have focused on this topic have not been capable to establish an association between the incidence or outcome of *S. pneumoniae* infections and the existence of MBL deficiency. In fact, in vitro studies have shown that *S. pneumoniae* has a low binding capacity to MBL (8). Additionally, the level of expression of the capsule of *S. pneumoniae,* which has a negative impact on MBL binding, may vary during different phases (9). Finally, minor differences in the sugar array composition of the membranes of *S. pneumoniae*,

responsible for the different serotypes of the bacteria, may account for additional differences in the binding ability of *S. pneumoniae* to MBL.

In the same study by Eisen DP *et al* (1), no significant association was found regarding MBL deficiency and death in patients with *S. aureus* infection, probably due to the small number of patients with this condition included in the analysis. In vitro studies have demonstrated that *S. aureus* binds with high affinity to MBL (8), and therefore MBL deficiency should be related to high mortality. In the 2007 ESCMID, we addressed this issue by analyzing the *MBL2* genotypes of 49 Caucasoid patients with *S. aureus* bacteremia (10). The study was conducted with the approval of the hospital Ethics Committee and the informed consent of the patients. No significant differences were observed regarding the frequencies for low expression *MBL2* genotypes (LXA/O and O/O) between a group of healthy controls and the patients with *S. aureus* bacteremia. In our study death was not related to the existence of low expression *MBL* genotypes. We did not analyze the effect of the serotypes of *S. aureus* in the clinical outcomes of the patients. Analyzing all serotypes together could be masking the influence of serotypes in MBL binding. In conclusion, in order to properly evaluate the role of MBL in different infectious settings it is fundamental to focus not only in the host (quantity of MBL) but also on the bacterial characteristics, particularly the binding capacity of MBL to different strains or serotypes.

Acknowledgements

The work by Francisco Lozano is supported by the Spanish Research Network on Infectious Diseases (REIPI, RD 06/008/1013) from Instituto de Salud Carlos III.

Potential conflicts of interest

All authors: no conflicts.

References

1. Eisen DP, Dean MM, Boermeester MA, et al. Low serum mannose-binding lectin levels increases the risk of death due to pneumococcal infection. Clin Infect Dis 2008; 47: 510-6.
2. Mira JP, Cariou A, Grall F, et al. Association of TNF2, a TNF-alpha promoter polymorphisms, with septic shock susceptibility and mortality: a multicenter study. JAMA 1999; 282:561-8.
3. Arnalich F, Lopez-Maderuelo D, Codoceo R, et al. Interleukin-1 receptor antagonist gene polymorphism and mortality in patients with severe sepsis. Clin Exp Immunol 2002; 127: 331-6.
4. García-Segarra G, Espinosa G, Tassies D, et al. Increased mortality in septic shock with the 4G/4G genotype of plasminogen activator inhibitor 1 in patients of white descent. Intensive Care Med 2007; 33: 1354-62.
5. Gordon A, Waheed U, Hansen T, et al. Mannose-binding lectin polymorphisms in severe sepsis: relationship to levels, incidence and outcome. Shock 2006; 25:88-93.
6. Hellemann D, Larsson A, Madsen HO, et al. Heterozygosity of mannose-binding lectin (*MBL2*) in relation to fatal outcome in intensive care patients. Hum Mol Gene 2007; 16: 3071-80.
7. Smithson A, Muñoz A, Suarez B, et al. Association between mannose-binding lectin deficicieny and septic shock following acute pyelonephritis

due to *Escherichia coli*. Clin Vacccine Immunol 2007; 14: 256-61.

8. Neth O, Jack DL, Dodds AW, et al. Mannose-binding lectin binds to a range of clinically relevant microorganism and promotes complement deposition. Infect Immun 2000; 68: 688-93.
9. Krarup A, Sorensen UB, Matsushita M, et al. Effect of capsulation of opportunistic pathogenic bacteria on binding of the pattern recognition molecules mannan-binding lectin, L-ficolin, and H-ficolin. Infect Immun 2005; 73: 1052-60.
10. Smithson A, Muñoz A, Suarez B, et al. “Mortality and time for detection of blood growth in patients with Sthapyloccoccus aureus bacteremia; implication of the mannose-binding lectin pathway”. In: Program and abstracts of the 17th European Society of Clinical Microbiology and Infectious Diseases (Munich). ESCMID 2007, 1753.