Consecuencias clínicas de la persistencia de actividad inflamatoria en la arteritis de células gigantes. Estudio de factores implicados

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CONSECUENCIAS CLÍNICAS DE LA PERSISTENCIA

DE ACTIVIDAD INFLAMATORIA EN

LA ARTERITIS DE CÉLULAS GIGANTES.

ESTUDIO DE FACTORES IMPLICADOS

**Memoria presentada por Ana García Martínez para optar
al grado de Doctora por la Universitat de Barcelona**

**Tesis dirigida por
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Barcelona, 9 de julio de 2010

AGRADECIMIENTOS

A la Marta, l'Ester i la Montse, pel seu ajut i col·laboració a la feina del laboratori.

A la Mariona, perquè treballar amb ella és un regal. A més d'excel·lent professional, és honesta i generosa amb els companys i sap guanyar-se el respecte i l'estimació dels pacients. Gràcies per ensenyar-me tant i per fer-ho amb tanta il·lusió.

Als meus pares i la meva germana, per ser al meu costat sempre.

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I. INTRODUCCIÓN

1. Consideraciones generales sobre la arteritis de células gigantes.

1.1. Epidemiología, manifestaciones clínicas y diagnóstico.

La primera descripción clínica de la arteritis de células gigantes (ACG) data de 1890, aunque fue en 1932, cuando Horton publicó los dos primeros casos documentados con biopsia de arteria temporal y describió las características anatomopatológicas típicas de esta vasculitis (*Horton, 1932; Hunder, 2006*).

En la actualidad la ACG es la vasculitis sistémica más frecuente en nuestro medio, con una incidencia que oscila entre 6-25 casos/100000 habitantes/año entre la población mayor de 50 años. La enfermedad es más frecuente entre personas de edad avanzada y sexo femenino, con una ratio mujer/hombre de 2-3/1 (*Salvarani et al, 2002; Weyand y Goronzy, 2003*). También es más frecuente en poblaciones originarias de países situados en latitudes nórdicas mientras que raramente afecta a individuos de raza negra. En España se ha descrito una incidencia de 10.2 casos/100000 habitantes/año (*González-Gay et al, 2009*). Estas diferencias geográficas podrían justificarse por la participación de algún factor ambiental o por la variabilidad genética existente entre las distintas poblaciones. Ocasionalmente se han descrito casos familiares, aunque de entre los factores inmunogenéticos estudiados, únicamente los alelos HLA-DRB1*04 han demostrado cierta relación con el desarrollo de la enfermedad. (*Cid et al, 1988; González-Gay et al, 2003*). Por otra parte, algunos estudios

epidemiológicos han demostrado que la ACG se presenta de forma cíclica, con aumentos puntuales de la incidencia que tienen lugar cada 5-6 años, lo que sugiere que algún agente infeccioso podría dar lugar a la activación de la respuesta inmune y actuar como detonante de la enfermedad (*Salvarani et al, 1995; 2004*). No sabemos, si los síntomas inespecíficos que presentan algunos pacientes al inicio de la enfermedad corresponden a una infección vírica banal o forman parte de los síntomas sistémicos de esta vasculitis. Se han estudiado diversos microorganismos, entre ellos *Parvovirus B19*, *Chlamydia pneumoniae* y virus de la familia herpes, aunque hasta la fecha ninguno de ellos ha demostrado jugar un papel determinante en el desarrollo de la enfermedad (*Salvarani et al, 2002*).

La inflamación de los vasos del área craneofacial da lugar a los síntomas guía de esta vasculitis (cefalea, hiperestesia del cuero cabelludo, claudicación mandibular y diversas algias faciales). La mayoría de pacientes también presentan síntomas sistémicos en forma de astenia, anorexia o pérdida de peso. En la mitad de los casos se constata fiebre y en un 40-50% polimialgia reumática. Los síntomas se acompañan de una respuesta de fase aguda exuberante, con elevación marcada de la VSG y de las proteínas de fase aguda (PCR, haptoglobina, fibrinógeno) y de la presencia de anemia normocítica-normocrómica debido a alteraciones en la eritropoyesis (*Keel and Abkowitz, 2009*). Las complicaciones isquémicas aparecen en el 20% de los pacientes y constituyen la principal causa de morbilidad. La más frecuente es la neuritis óptica isquémica anterior que se produce como consecuencia de la arteritis en las arterias ciliares posteriores

o con menor frecuencia, por afectación de la arteria central de la retina. Esporádicamente aparecen eventos isquémicos en otros territorios vasculares en forma de accidente vascular cerebral vertebro-basilar o carotideo, isquemia del cuero cabelludo o isquemia lingual. Los síntomas isquémicos suelen ser irreversibles y únicamente, el inicio precoz del tratamiento durante las primeras 24 horas desde su aparición, puede mejorar su pronóstico. Una vez iniciado el tratamiento corticoideo el riesgo de presentar nuevos eventos isquémicos disminuye de forma drástica (*Salvarani et al, 2002; Weyand y Goronzy, 2003*).

A pesar de esta aparente predilección por la circulación craneal, la ACG es una vasculitis sistémica que afecta otros territorios vasculares como la aorta y sus ramas. La inflamación del territorio extracraneal, aunque frecuente, suele ser asintomática y pasar desapercibida. No obstante, entre un 9.5 y 18% de pacientes desarrolla aneurismas y/o disección aórticos durante el seguimiento (*Evans, O'Fallon y Hunder, 1995; Nuenninghof et al, 2003; González-Gay et al, 2004*). La arteritis a nivel de ramas de la aorta da lugar a una disminución de la luz vascular y la aparición de síntomas isquémicos en aproximadamente un 14% de los pacientes. Aparentemente, las arterias que derivan del cayado aórtico son las que se afectan con mayor frecuencia (*Klein et al, 1975; Caselli, Hunder y Whisnant, 1988; Nuenninghof et al, 2003*). Los pacientes pueden presentar claudicación de extremidades, isquemia transitoria similar al fenómeno de Raynaud, parestesias periféricas y en casos graves gangrena tisular. La asimetría en la intensidad de los pulsos y en la presión arterial entre ambos brazos, así como la

presencia de soplos vasculares debe hacernos sospechar la existencia de una estenosis arterial. La prevalencia de vasculitis en arterias viscerales no se conoce, aunque en la literatura se encuentran múltiples descripciones de pacientes que han padecido eventos isquémicos en diversas localizaciones como consecuencia de una ACG (*Lie, Falloni y Davis, 1986; Säve-Söderbergh et al, 1986*).

La prueba de elección para el diagnóstico de la enfermedad es la biopsia de arteria temporal. Es recomendable que tenga una longitud de 2 a 3 cm ya que las lesiones inflamatorias se distribuyen de forma parcheada. No obstante, sólo el 1-3% de los pacientes con biopsia negativa tienen signos de ACG en la biopsia contralateral. Por tanto, únicamente los pacientes con síntomas muy sugestivos de ACG deberían ser sometidos a una segunda biopsia en el caso de que la primera no haya sido diagnóstica (*Boyev, Miller y Green, 1999; Hall et al, 2003*). A falta de biopsia, el diagnóstico de ACG es altamente probable cuando se cumplen los criterios propuestos por el American College of Rheumatology (ACR) para la clasificación de las vasculitis sistémicas.

Estos criterios se establecieron con el objetivo de clasificar aquellos pacientes que padecían una vasculitis sistémica aunque, en centros donde no se tiene un acceso rápido a la biopsia temporal, suelen ser la base del diagnóstico.

Criterios del ACR para la clasificación de las vasculitis sistémicas (1990)

- ✓ Edad superior a 50 años.
- ✓ Cefalea de nueva aparición.
- ✓ Alteraciones a nivel de la AT.
- ✓ VSG ≥ 50 mmHg/hora
- ✓ Cambios inflamatorios típicos en la biopsia de AT.

La presencia de ≥ 3 criterios tiene una sensibilidad de 93.5 % y una especificidad del 91.2 % para el diagnóstico de ACG. AT: arteria temporal.

La tasa de mortalidad en pacientes con ACG es similar a la de la población general de la misma edad y sexo, excepto en aquellos pacientes que desarrollan complicaciones aórticas, en los que la mortalidad aumenta (*Matteson et al, 1996; Nuenninghoff et al, 2003*). Un estudio previo describió un aumento de la mortalidad por causas cardiovasculares en fases iniciales de la enfermedad, que se equilibraba pocos meses después de iniciado el tratamiento. Estas muertes precoces se produjeron en pacientes con enfermedad activa, que continuaban presentando lesiones inflamatorias en la pared arterial (*Nordborg y Bengtsson, 1989*).

1.2. Mecanismos patogenéticos.

A nivel anatomopatológico la ACG se caracteriza por la presencia de un infiltrado inflamatorio compuesto por linfocitos T y monocitos/macrófagos, que penetra a través de la pared arterial llegando a

invadir todas las capas (adventicia, media e íntima). A nivel de la media las células T activadas y los macrófagos se organizan y pueden agruparse formando granulomas. Aproximadamente, en la mitad de los pacientes se identifican células gigantes multinucleadas en proximidad a la lámina elástica interna que aparece fragmentada. Con frecuencia se produce la hiperplasia de la capa íntima, que da lugar a una disminución de la luz arterial responsable de la aparición de fenómenos isquémicos (Figura 1) (*Weyand y Goronzy, 2003*).

Figura 1. Biopsia de arteria temporal de un paciente con ACG
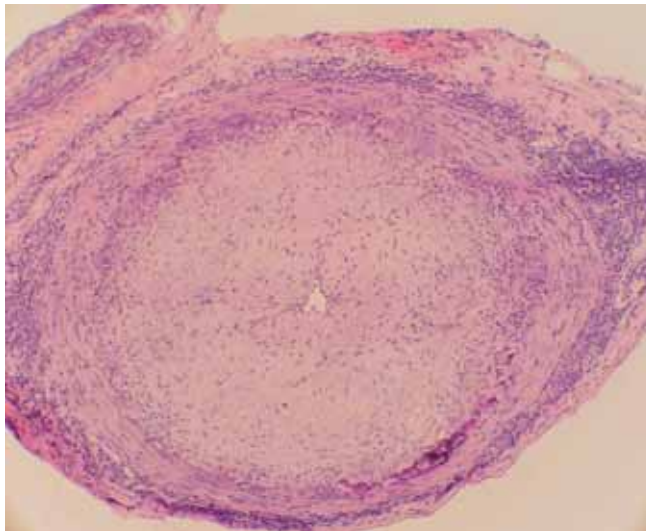
Los mecanismos que intervienen en esta respuesta inflamatoria arterial no se conocen. El modelo patogenético más aceptado en la actualidad fue descrito por Weyand hace unos años (Figura 2) (*Weyand y Goronzy, 1999; 2003*). Considera que el proceso inflamatorio se origina en la

adventicia desde donde progresa hacia el interior del vaso. Se desencadena una respuesta inmune dirigida contra antígenos presentes en la pared arterial, que son procesados a nivel advencial por las células dendríticas residentes, principales presentadoras de antígenos. Las células dendríticas activadas producen citocinas inflamatorias como interleucina (IL)-6 e IL-18 y expresan CD86, correceptor necesario para una correcta interacción entre los linfocitos T y las células dendríticas. Se produce la estimulación de linfocitos T CD4+ que sufren una expansión oligoclonal, como sugiere la identificación de receptores antigénicos idénticos en linfocitos aislados de distintas lesiones vasculares (*Weyand et al, 1994*). Las células dendríticas activadas producen las quimiocinas CCL19 y CCL21 con funciones quimiotácticas sobre las células inflamatorias que alcanzarían la arteria a través de los *vasa vasorum*. Además, estas quimiocinas también se unen al receptor CCR7 expresado por las propias células dendríticas activadas lo que las mantiene atrapadas en el propio tejido arterial. Las células endoteliales de los *vasa vasorum* expresan moléculas de adhesión, facilitando la migración de células inflamatorias hacia el interior de la pared arterial. Los linfocitos T CD4+ activados sufren una diferenciación funcional de tipo Th1 con importante producción de interferón (IFN)-γ que tiene un papel fundamental en la activación, diferenciación y migración de los macrófagos, en la producción de células gigantes y en la formación del granuloma.

Los macrófagos activados producen gran cantidad de citocinas inflamatorias, especialmente factor de necrosis tumoral (TNF) α, IL-1 e IL-6,

metaloproteasas (MMP) y productos derivados de reacciones de estrés oxidativo, dando lugar a la destrucción de la pared arterial. Las células musculares lisas sufren apoptosis y se produce la fragmentación de la lámina elástica interna. Paralelamente se ponen en marcha mecanismos reparadores del daño vascular. Se produce la movilización, migración y proliferación de miofibroblastos hacia la íntima y el depósito de proteínas de matriz extracelular que da lugar a la hiperplasia intimal. Estos cambios se acompañan de la formación de neovasos en el interior de la pared arterial que, en condiciones normales, es una estructura avascular. Los macrófagos y células gigantes localizados a nivel de la media son los principales secretores de factor de crecimiento derivado de las plaquetas (PDGF) y factor de crecimiento del endotelio vascular (VEGF), ambos relacionados con la formación de la hiperplasia intimal y la neovascularización respectivamente. Las células musculares lisas de la pared arterial también producen PDGF. La expresión de PDGF en las lesiones vasculares de los pacientes con ACG se ha relacionado con el grado de oclusión de la luz vascular y con la presencia de manifestaciones isquémicas (*Kaiser M, 1998*). Estudios funcionales también sugieren un importante papel del PDGF en el remodelado vascular. La incubación de células miointimales con PDGF estimula la proliferación y migración de estas células así como, la secreción de proteínas de matriz extracelular, moléculas proinflamatorias y angiogénicas (CCL-2 y angiogenina) (*Lozano et al, 2008*).

Figura 2. Esquema de los mecanismos patogenéticos en la ACG

· Activación células dendríticas
· Reclutamiento células T y macrófagos
· Activación células T
　　　　Expansión clonal
　　　　Producción de IFNγ
· Activación macrófagos por IFNγ
　　　　Producción IL-1, IL-6 y TNFα
　　　　Producción MMPs ➤ Digestión MEC
　　　　Producción IEO ➤ Daño tisular
　　　　Producción de PDGF y VEGF
　　　　　　Crecimiento miofibroblastos
　　　　　　Neoangiogénesis
· Hiperplasia íntima ➤ Isquemia
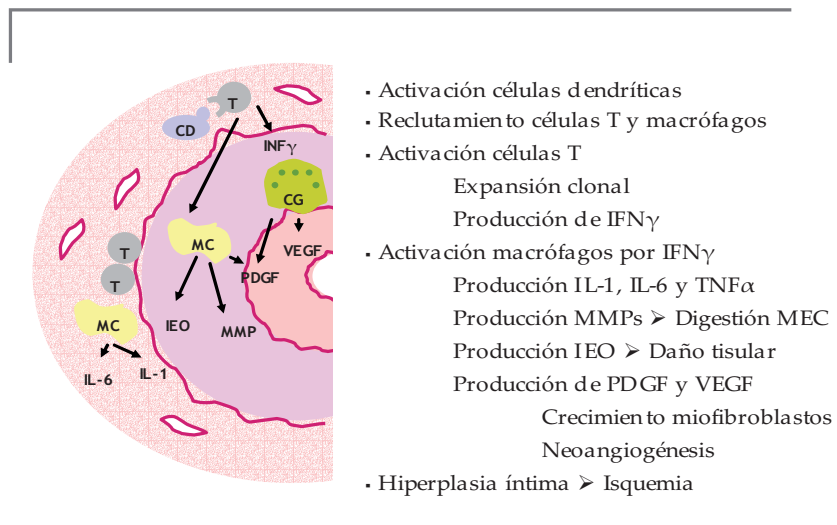
CD: célula dendrítica, T: célula T, MC: macrófago, CG: célula gigante,
MEC: matriz extracelular, IEO: intermediarios estrés oxidativo

El antígeno responsable de activar esta respuesta inflamatoria se desconoce. Se ha detectado RNA de *Parvovirus B19* o de *Chlamydia pneumoniae* en la pared arterial de pacientes con ACG, aunque no se ha podido demostrar que tengan una relación causal con la enfermedad. También se ha sugerido que elementos intrínsecos de la propia pared arterial podrían actuar como antígenos detonantes de la activación del sistema inmune. Tanto la estructura, como los elementos constitutivos del vaso varían en función del territorio vascular estudiado, lo que podría explicar el tropismo de la enfermedad por ciertos territorios. En la ACG se afectan arterias musculares de mediano y gran calibre, caracterizadas por

tener una lámina elástica interna prominente y *vasa vasorum* a nivel adventicial. A medida que se reduce el diámetro de las arterias, el contenido arterial de fibras elásticas y *vasa vasorum* disminuye. De hecho, las arterias intracraneales, que carecen de lámina elástica y *vasa vasorum*, raramente se ven afectadas en esta enfermedad (*Salvarani et al, 2006*). Puesto que la ACG aparece en edades avanzadas de la vida, se ha sugerido que el proceso de envejecimiento vascular podría dar lugar a cambios en la pared arterial con la exposición de elementos antigénicos previamente no expuestos al sistema inmune. Por otra parte, el sistema inmunitario sufre cambios a lo largo de los años, por lo que también es posible que se modifique el grado de inmunotolerancia. De esta manera, se favorecería el reconocimiento antigénico de elementos estructurales vasculares previamente reconocidos como propios (*Hoffman, 2003; 2005; 2008*). La identificación del antígeno responsable de la enfermedad sería importante ya que permitiría el desarrollo de tratamientos específicos como las vacunas.

2. La respuesta inflamatoria sistémica

2.1. Características de la respuesta inflamatoria sistémica.

En las lesiones inflamatorias vasculares de los pacientes con ACG se produce una importante secreción de las citocinas proinflamatorias IL-6, TNFα e IL-1. Estas citocinas se liberan a la circulación y ejercen funciones a distancia sobre diversos órganos. Se producen manifestaciones clínicas

sistémicas (fiebre, anorexia, pérdida de peso), alteraciones hematológicas (anemia y trombocitosis), alteraciones bioquímicas (síntesis de proteínas de fase aguda) y cambios metabólicos (aumento de la lipólisis y pérdida de masa muscular). Los mecanismos que regulan esta respuesta de fase aguda son complejos. El hígado es un órgano fundamental en el mantenimiento y amplificación de esta respuesta ya que los hepatocitos, principales productores de proteínas plasmáticas, expresan receptores para diversas citocinas proinflamatorias. Durante la respuesta inflamatoria, los niveles plasmáticos de algunas de estas proteínas aumentan (ej. PCR, haptoglobina, fibrinógeno) mientras que en otros casos (ej. albúmina) disminuyen. Por otro lado, la activación de las células de Kupffer o macrófagos residentes hepáticos también aumenta la secreción de citocinas proinflamatorias, contribuyendo a la amplificación de la respuesta inflamatoria sistémica (RIS) (*Baumann y Gauldie, 1994; Gabay y Kushner, 1999; Gabay, 2006; Ramadori y Armbrust, 2001*).

La intensidad de la RIS es muy variable entre pacientes. La elevación de la VSG es uno de los datos más característicos de esta enfermedad y forma parte de los criterios diagnósticos. Sin embargo, hasta una cuarta parte de los pacientes presenta valores de VSG poco elevados o incluso normales en el momento del diagnóstico (*Salvarani y Hunder, 2001*). En algunos pacientes predominan los síntomas craneales mientras que en otros predominan las manifestaciones sistémicas. En otras ocasiones la enfermedad cursa de forma tan poco sintomática que incluso puede pasar desapercibida. En estudios necrópsicos se han descrito pacientes con

lesiones vasculares sugestivas de ACG "curada" que no habían presentado síntomas o, si los presentaron, éstos fueron leves y no motivaron su estudio (*Lie, Brown y Carter, 1970*). En la literatura existen múltiples descripciones de pacientes con "arteritis temporal oculta", haciendo referencia a casos de ACG paucisintomáticos que pasan desapercibidos hasta que el paciente consulta por un fenómeno vascular isquémico (*Gutrecht, 1970*)

Por otra parte, la evolución de la enfermedad también es muy variable. De media, los pacientes necesitan de 2 a 3 años de tratamiento antes de poder suspenderlo completamente. Más de la mitad presentan recidivas clínicas al intentar reducir la dosis de corticoides, siendo en ocasiones necesario mantener dosis bajas de forma indefinida e incluso asociar otro tratamiento inmunorregulador. Otros pacientes, en cambio, presentan un curso clínico favorable, con pocas recidivas y son capaces de suspender el tratamiento corticoideo en pocos meses. Se han descrito incluso, pacientes en los que la enfermedad remite de forma espontánea sin necesidad de tratamiento (*Hernández-Rodríguez et al, 2006*).

Esta variabilidad, tanto en la forma de presentación como en la evolución de la enfermedad, está íntimamente relacionada con la intensidad de la RIS. Diversos estudios han dado lugar a la descripción de subgrupos de pacientes que presentan unas características clínicas claramente diferenciadas, con un pronóstico y unas necesidades terapéuticas distintas.

2.2. La respuesta inflamatoria sistémica como factor pronóstico.

Diversos estudios publicados en los últimos años demuestran una relación inversa entre la intensidad de la RIS y la presencia de manifestaciones isquémicas. (*Cid et al, 1998; González-Gay et al, 2000; Salvarani et al, 2005*). Las causas de esta relación no se conocen aunque existen algunas hipótesis. Los neovasos formados en las lesiones inflamatorias expresan gran cantidad de moléculas de adhesión leucocitarias y liberan quimiocinas que facilitan el reclutamiento de nuevos leucocitos a las lesiones, lo que puede amplificar y perpetuar el fenómeno inflamatorio (*Cid et al, 2000*). De este modo, los neovasos compensarían la oclusión vascular y la aparición de eventos isquémicos a la vez que potenciarían la actividad inflamatoria. En estudios previos, las manifestaciones isquémicas fueron más frecuentes en pacientes que presentaban una RIS débil y menor formación de neovasos en la biopsia de arteria temporal. Además, se comprobó que el suero de estos pacientes no era tan eficaz estimulando respuestas angiogénicas en comparación con el suero de pacientes que no presentaban eventos isquémicos. Los pacientes con manifestaciones isquémicas presentaban una menor expresión de IL-6 en arteria temporal y unos niveles circulantes de IL-6 inferiores que los pacientes sin eventos isquémicos. La IL-6 estimula la proliferación de células endoteliales y su diferenciación a estructuras capilares y es capaz de inducir actividad angiogénica en modelos de angiogénesis (anillo aórtico y membrana corioalantoidea del embrión de pollo). Los resultados de estos estudios sugerían que la IL-6, además de ser uno de los principales

inductores de la RIS, podría estimular la neovascularización en las lesiones inflamatorias y de esa manera prevenir la aparición de eventos isquémicos (*Cid et al, 2002; Hernández-Rodríguez et al, 2003*).

Además de esta relación inversa con la isquemia, la intensidad de la RIS se correlaciona con el pronóstico de la enfermedad. La identificación de factores que permitan predecir el pronóstico de la enfermedad y realizar pautas de tratamiento individualizadas, ha sido uno de los principales objetivos de los últimos años. En un estudio previo se observó que la intensidad de la RIS en el momento del diagnóstico se relacionaba con el número de recidivas durante el seguimiento y las necesidades terapéuticas (*Hernández-Rodríguez et al, 2002*). Este trabajo constituye el punto de partida de esta tesis doctoral por lo que el artículo completo se adjunta al final de la introducción.

Se estudiaron 75 pacientes con ACG seguidos regularmente en nuestro centro y tratados de manera uniforme. La intensidad de la RIS se definió en base a cuatro parámetros que habían demostrado utilidad a la hora de discriminar entre pacientes con riesgo elevado o bajo de desarrollar complicaciones isquémicas: fiebre, pérdida de peso, VSG ≥ 85 mm/hora y hemoglobina < 110 gr/l antes del inicio del tratamiento (*Cid et al, 1998*). Se consideró que la RIS era débil cuando los pacientes presentaban de 0 a 2 parámetros e intensa cuando presentaban más de dos. En definitiva, se incluyeron 40 pacientes con una RIS débil y 35 con una RIS intensa.

Los pacientes que presentaban una RIS intensa, presentaron más recidivas durante el seguimiento y necesitaron dosis más elevadas de prednisona que los pacientes con una RIS débil. Los primeros tardaron más tiempo en poder alcanzar una dosis de mantenimiento de prednisona inferior a 10 mg/día de forma estable. La dosis de prednisona acumulada hasta ese momento también fue significativamente superior en los pacientes con una RIS intensa.

Los niveles de IL-6 y TNFα en suero fueron significativamente más elevados en pacientes que en el grupo de controles sanos, especialmente en aquellos que tenían una RIS intensa en el momento del diagnóstico (Figura 3). Estos datos sugerían el posible papel de estas citocinas en la regulación de la respuesta inflamatoria, lo que las convertía en posibles dianas terapéuticas.

Fig 3. Diferencias en los niveles de citocinas (TNFα e IL-6) entre controles, pacientes con una RIS débil y pacientes con una RIS intensa.
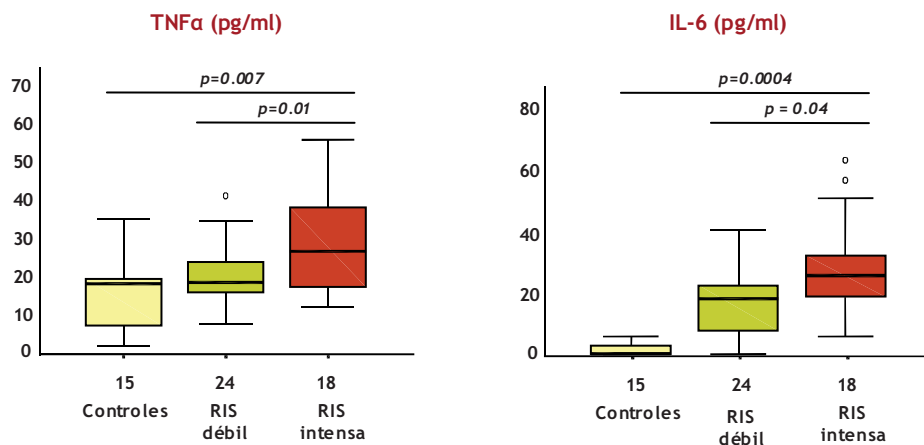
2.3. Evolución a largo plazo de la respuesta inflamatoria sistémica.

La evolución de los parámetros de fase aguda a lo largo del tiempo suele servir de guía para monitorizar la respuesta al tratamiento. La administración de corticoides produce una serie de cambios funcionales precoces que dan lugar a una disminución de la secreción de citocinas proinflamatorias y reactantes de fase aguda, así como una mejoría de los síntomas durante las primeras 24-48 horas.

En pacientes con polimialgia reumática, la administración de corticoides produce un descenso brusco de los niveles circulantes de IL-6 seguido de la mejoría sintomática. La suspensión transitoria del tratamiento provoca de nuevo una rápida elevación de los niveles de IL-6 y la reaparición de los síntomas (*Roche et al, 1993*). En la ACG, algunos mediadores inflamatorios se mantienen elevados durante periodos de tiempo más o menos prolongados tras el inicio del tratamiento, e incluso en pacientes que están asintomáticos. Tal es el caso del factor vonWillebrand, que permanece moderadamente elevado durante los tres primeros años, sugiriendo la persistencia de cierto grado de actividad inflamatoria vascular (*Cid et al, 1996*). Weyand y cols monitorizaron los niveles plasmáticos de IL-6 durante el primer año y medio de seguimiento en un grupo de pacientes con ACG tratados de manera uniforme (Figura 4). Al finalizar el estudio, un 69% de los pacientes que alcanzaron la remisión y pudieron suspender el tratamiento, continuaba presentando niveles de IL-6 superiores a la normalidad (*Weyand et al, 2000*).

Figura 4. Evolución de los distintos parámetros inflamatorios durante el seguimiento
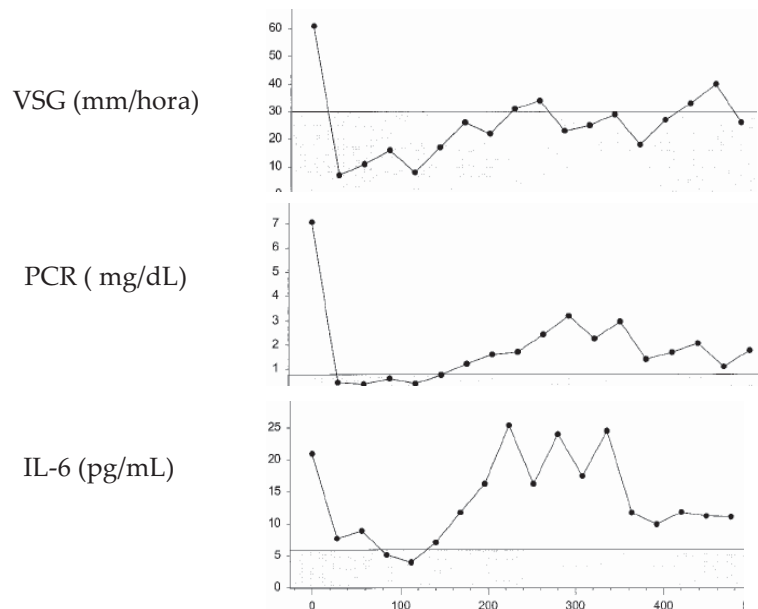
A pesar de que el tratamiento corticoideo reducía los niveles circulantes de IL-6, éstos no llegaban a alcanzar los valores de normalidad que presentaban los individuos sanos y se mantenían discretamente elevados durante periodos de tiempo prolongados, incluso en pacientes que habían alcanzado la remisión clínica. Los corticoides, parecen modular la intensidad de la respuesta inflamatoria pero no son capaces de abortarla por completo.

Se desconoce el origen de esta actividad inflamatoria crónica en pacientes que están en remisión clínica. El estudio de necropsias y

especimenes quirúrgicos ha demostrado que el infiltrado inflamatorio persiste en la pared arterial, aún después de haber realizado tratamiento corticoideo durante periodos de tiempo más o menos prolongados (*Achkar et al, 1994; Lie, 1995*). En base a estos hallazgos, se ha sugerido que la persistencia de pequeños focos de inflamación a nivel de la pared de los vasos podría ser el origen de esta actividad inflamatoria subclínica.

Brack y cols desarrollaron un modelo de xenotransplante que permitía estudiar los cambios que se producen a nivel arterial después de distintas intervenciones terapéuticas. El modelo consiste en implantar un segmento de arteria temporal proveniente de un paciente con ACG en ratones con inmunodeficiencia combinada severa. La sección arterial se implanta a nivel subcutáneo lo que permite mantener la viabilidad de la arteria, que conserva el infiltrado inflamatorio y la capacidad de producir citocinas y factores de crecimiento (IL-2, IFN-γ, IL-6, IL-1β y TGF-β1) (*Brack et al, 1995*). La administración de dosis elevadas de dexametasona durante una semana produjo la disminución de la síntesis de citocinas aunque no llegó a inhibirla por completo. La síntesis de IL-2, IFN-γ e IL-1β mRNA disminuyó de forma significativa. La producción de IL-6 mRNA, aunque disminuyó de manera significativa, se mantuvo a niveles inferiores mientras que, la síntesis de TGF-β1 mRNA no se modificó. A pesar del tratamiento, el infiltrado inflamatorio persistía a nivel arterial y los macrófagos infiltrantes conservaban cierto grado de activación, como sugería la inhibición incompleta de la síntesis de IL-6. Estos hallazgos

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podrían justificar la rapidez con que se produce la reactivación de la enfermedad tras la suspensión del tratamiento (*Brack et al, 1997*).

La persistencia de actividad inflamatoria moderada de forma crónica tiene efectos perjudiciales sobre el paciente. Enfermedades como la artritis reumatoide y el lupus eritematoso sistémico se asocian a una mayor prevalencia de enfermedad cardiovascular por arteriosclerosis acelerada (*Roman MJ et al, 2003, 2006; Van Leuven et al, 2006*). Es más, niveles moderadamente elevados de IL-6 y PCR también se han asociado a un mayor riesgo de eventos cardiovasculares en la población general (*Tzoulaki et al, 2005; Ridker et al, 2008*). Aunque este riesgo no se ha confirmado en la ACG, algunos pacientes desarrollan complicaciones en forma de estenosis vasculares y/o aneurismas o disección aórticos durante el seguimiento (*González-Juanatey et al, 2007*). Las causas de estas complicaciones no se conocen pero se ha sugerido que la persistencia de actividad inflamatoria vascular a lo largo del tiempo podría favorecer su desarrollo. Aunque sea asintomática, la persistencia de actividad inflamatoria a lo largo del tiempo podría llegar a ser preocupante, tanto por el riesgo potencial de complicaciones vasculares propias de la enfermedad (aneurisma/disección aórticos), como complicaciones secundarias a arteriosclerosis acelerada. Por otra parte, la activación continuada de la RIS podría favorecer la pérdida de masa ósea inducida por algunas citocinas proinflamatorias.

Uno de los principales objetivos de esta tesis consiste en determinar si existe relación entre la intensidad o persistencia a lo largo del tiempo de

actividad inflamatoria y el desarrollo de complicaciones clínicas. La demostración de que la persistencia de actividad inflamatoria es perjudicial para el paciente podría dar lugar a cambios en los objetivos terapéuticos, ya que no bastaría con obtener la remisión clínica sino que deberíamos perseguir también la normalización de los parámetros biológicos. Sería importante valorar detenidamente el riesgo/beneficio de mantener un tratamiento que presenta importantes efectos secundarios, sobretodo en pacientes de edad avanzada.

3. Afectación extracraneal y desarrollo de complicaciones aórticas

3.1. Afectación y complicaciones extracraneales

En 1938 Jennings apuntó por primera vez al carácter sistémico de la ACG al describir un paciente que presentaba asimetría en los valores de presión arterial entre ambas extremidades (*Jennings, 1938*). En 1941 Gilmour describió la existencia de vasculitis extracraneal al realizar la necropsia de tres pacientes que habían presentado síntomas sugestivos de ACG y por primera vez relacionó esta arteritis generalizada con la "arteritis temporal" (*Hunder, 2006*). Desde entonces, en la literatura se encuentran múltiples descripciones de pacientes con complicaciones derivadas de la presencia de vasculitis a nivel extracraneal.

La ACG afecta con frecuencia a la aorta y sus ramas primarias y secundarias. En la mayoría de pacientes, esta afectación es asintomática y

pasa desapercibida, aunque un número no despreciable de pacientes pueden presentar síntomas como consecuencia de la estenosis o dilatación de los vasos. Entre un 9.5-18% de pacientes desarrolla aneurisma o disección aórticos, o insuficiencia valvular secundaria a la dilatación de la raíz aórtica (*Evans, O'Fallon y Hunder, 1995; Nuenninghof et al, 2003; González-Gay et al, 2004*). En el resto de vasos, la hiperplasia intimal puede dar lugar a la aparición de estenosis vasculares y síntomas isquémicos hasta en un 14% de los pacientes (*Klein et al, 1975; Caselli, Hunder y Whisnant, 1988; Nuenninghof et al, 2003*). El síndrome del arco aórtico representa, tras las complicaciones aórticas, la manifestación clínica extracraneal más frecuente de estos pacientes. Los pacientes presentan síntomas por isquemia a nivel de las extremidades superiores y puede objetivarse asimetría de pulsos y de la presión arterial. La afectación de los vasos cervicales (arterias carótidas, vertebrales y basilar) puede dar lugar a la aparición de accidentes vasculares cerebrales. La prevalencia de vasculitis en otros territorios y sus consecuencias clínicas prácticamente no ha sido estudiada. Menos del 1% de los pacientes presenta claudicación intermitente a nivel de extremidades inferiores, aunque es probable que esta cifra esté infravalorada. La elevada frecuencia de arteriosclerosis a nivel de arterias ilíacas, femorales y/o femoro-poplíteas en pacientes de edad avanzada puede enmascarar la contribución de la vasculitis en el desarrollo de los síntomas (*Nuenninghoff et al, 2003*). La enfermedad puede afectar otros territorios (arterias coronarias, renales, mesentéricas, pancreáticas y pulmonares), en ocasiones con consecuencias graves, como se ha descrito de forma anecdótica en necropsias de pacientes con ACG. En

algunos pacientes predominan las manifestaciones extracraneales sobre los síntomas craneales típicos. En estos casos, la biopsia de arteria temporal puede ser negativa hasta en un 40% de los pacientes y el diagnóstico de ACG suele requerir la realización de pruebas de imagen que demuestren alteraciones sugestivas de vasculitis (*Brack et al, 1999*) Los síntomas isquémicos suelen mejorar tras el inicio del tratamiento corticoideo aunque si persisten, puede ser necesario realizar una revascularización percutánea o quirúrgica. El lento desarrollo de las lesiones estenóticas favorece la aparición de circulación colateral que compensa las necesidades vasculares y minimiza los síntomas, aún en pacientes con estenosis significativas (*Klein et al, 1975; Both et al, 2006*).

La afectación extracraneal en la ACG es frecuente, aunque se desconoce su prevalencia exacta ya que en la mayoría de pacientes pasa desapercibida. El estudio histológico de los vasos extracraneales permite obtener la máxima sensibilidad y especificidad en el diagnóstico. La mayoría de series publicadas que describen la presencia de vasculitis extracraneal en base a datos histológicos, son retrospectivas y proceden del estudio de necropsias o especímenes quirúrgicos. Una revisión de cuatro estudios publicados entre los años 1951-54 describió los hallazgos de 18 necropsias de pacientes con ACG. En la tabla 1 se muestra el número de pacientes que presentaba vasculitis en los distintos territorios (*Hamrin, 1972*).

Tabla 1. Afectación vascular extracraneal en necropsias de pacientes con ACG

Aorta	17	Extremidades inferiores	
Pulmonar	3	Ilíaca	8
Cervicales/craneales		Femoral	4
Carótidas	14	Poplítea	2
Vertebrales	3	Viscerales	
Basilar	1	Coronarias	6
Extremidades superiores		Celíaca	4
Tronco braquiocefálico	6	Mesentéricas	3
Subclavia	8	Renales	3
Axilar	2	Hipogástrica	2
Radial	1	Ovárica	1

La mayoría de los casos presentaba afectación aórtica. La información respecto a otros territorios fue incompleta, ya que no se examinaron de forma sistemática, aunque el estudio demuestra que la presencia de arteritis es frecuente. En otro estudio, Ostberg realizó un análisis sistemático de los distintos territorios vasculares en 13 necropsias de pacientes con ACG. Encontró aortitis en 12 de los 13 pacientes estudiados y arteritis a nivel de carótidas, extremidades superiores y extremidades inferiores en 11, 12 y 10 casos respectivamente. Un elevado porcentaje de los pacientes incluidos en estos estudios había fallecido como consecuencia de complicaciones vasculares por lo que es posible que exista un sesgo debido a la inclusión de pacientes más graves o con una enfermedad más extensa (*Ostberg, 1972*). La confirmación histológica de

arteritis a nivel extracraneal sólo es posible en pacientes que fallecen o que precisan cirugía reparativa por complicaciones vasculares. En el resto, el territorio vascular extracraneal es inaccesible, por lo que es estudio histológico queda limitado a los casos más graves.

En los últimos años hemos asistido a un importante desarrollo de las técnicas de imagen que permiten detectar enfermedad a nivel extracraneal en pacientes vivos y en fases precoces, cuando dicha afectación es asintomática. Schmidt observó signos ecográficos de vasculitis a nivel de extremidades superiores en el 30% de los pacientes con ACG en el momento del diagnóstico, aunque sólo un 6% de ellos presentaba síntomas isquémicos (*Schmidt et al, 2008*). En 35 pacientes con ACG, la realización de una tomografía por emisión de positrones (PET) en el momento del diagnóstico puso de manifiesto un aumento de la captación de ^{18}F-FDG en aorta torácica (51%), aorta abdominal (54%) arterias subclavias (70%), axilares (40%), carótidas (40%), ilíacas (37%) y femorales (37%) sugestivo de actividad inflamatoria a esos niveles (*Blockmans D, 2006*). Recientemente, en una serie de 30 pacientes con ACG de nuevo diagnóstico, la realización de una angio-TC antes del inicio del tratamiento demostró datos sugestivos de aortitis en el 73% de los pacientes mientras que sólo el 14% de ellos mostraba dilatación aórtica (definida como un diámetro a nivel de aorta ascendente > 4 mm) (*Prieto et al, 2009*).

Las distintas técnicas de imagen disponibles en la actualidad pueden detectar de forma no invasiva datos sugestivos de vasculitis en

fases iniciales, antes de que se produzcan alteraciones significativas en la estructura del vaso que puedan dar lugar a manifestaciones clínicas. Esto puede suponer una auténtica revolución en el estudio de las vasculitis de gran vaso, aunque por el momento será necesario unificar los criterios que se utilizan al realizar estos estudios y así poder determinar la sensibilidad y especificidad de los mismos en la detección de enfermedad. Debe valorarse también si es necesario realizar un estudio de extensión de la enfermedad a todos los pacientes de forma sistemática, teniendo en cuenta que no todos los que presentan vasculitis extracraneal desarrollarán complicaciones clínicas. Para ello será importante conocer la historia natural de estas complicaciones. Las técnicas de imagen pueden ayudar a realizar un seguimiento prospectivo de los pacientes después del diagnóstico para conocer qué porcentaje de ellos desarrolla complicaciones significativas y en qué momento del seguimiento (*Tatò y Hoffmann, 2008*).

3.2. Afectación y complicaciones aórticas.

Ya hemos mencionado que la prevalencia de aortitis es elevada (>90% en estudios necrópsicos y >50% e incluso >70% según la técnica de imagen utilizada). Sin embargo, la prevalencia de complicaciones aórticas oscila entre el 9.5 y el 18% según las series (*Evans, O'Fallon y Hunder, 1995; Nuenninghoff et al, 2003; González-Gay et al, 2004*). Normalmente estas complicaciones se manifiestan años después del diagnóstico, aunque en algunos pacientes pueden aparecer en fases precoces e incluso constituir el evento inicial que da lugar al diagnóstico de la vasculitis. En la serie

publicada por Ostberg, 2 de los 13 pacientes falleció como consecuencia de una rotura de aorta, siendo éste el evento inicial que motivó el diagnóstico de la enfermedad (*Ostberg, 1972*). Lie publicó una serie de 72 pacientes con ACG extracraneal demostrada en el estudio histológico de necropsias o especimenes quirúrgicos. Dieciocho de estos pacientes habían fallecido como consecuencia de una complicación de la enfermedad sin que hubieran sido diagnosticados previamente de ACG. Las causas de la muerte fueron: rotura de aneurisma aórtico (6 pacientes), disección aórtica (6 pacientes), infarto agudo de miocardio (3 pacientes) y accidente vascular cerebral (3 pacientes). El examen histológico de las arterias temporales de estos pacientes demostró ACG en todos ellos (*Lie, 1995*). En una serie de más de 20000 autopsias consecutivas, Ostberg encontró 79 casos con aortitis y presencia de células gigantes, aunque el 52% de estos pacientes no tenía el diagnóstico previo de ACG. Veintinueve de estos pacientes presentaban rotura de la aorta (*Ostberg, 1973*). Una revisión de 24 casos con disección aórtica secundaria a ACG puso de manifiesto que, en casi la mitad de los pacientes (11/24), la complicación aórtica fue el evento que motivó el diagnóstico de la vasculitis (*Liu, Shupak y Chiu, 1995*).

Al margen de estos casos catastróficos que tienen lugar de forma más o menos precoz, en la mayoría de pacientes las complicaciones aórticas se diagnostican en fases tardías de la enfermedad y con frecuencia, en pacientes que están en remisión clínica y no precisan tratamiento. En un primer estudio publicado en 1995, Evans y cols observaron que la aparición de aneurismas a nivel de aorta torácica y abdominal era 17.3 y 2.4 veces

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más frecuente respectivamente en pacientes con ACG que entre la población general de la misma edad y sexo. El tiempo de seguimiento de los pacientes incluidos fue muy dispar (rango de 1 mes hasta 28 años, con una mediana de 8.6 años) así como la duración del tratamiento corticoideo (rango < 1 mes hasta 4.2 años con una mediana de un año). El tiempo de seguimiento desde el diagnóstico de la vasculitis hasta la aparición del evento a nivel de aorta torácica fue de 5.8 años (rango de 2.5 meses a 20 años) (*Evans, O'Fallon y Hunder, 1995*). En otro estudio posterior realizado por el mismo grupo, se incluyeron 168 pacientes con una mediana de seguimiento de 7.6 años (rango 3.9 a 13.5 años) y se observó que el 18% de los pacientes desarrollaba complicaciones en forma de aneurisma y/o disección aórticos (18 pacientes a nivel de aorta torácica y 16 a nivel de aorta abdominal). Los casos de disección aórtica se presentaron de forma más precoz en el tiempo que los casos de aneurisma (1.1 años, rango 0.2-2.1 en el caso de disección a nivel de aorta torácica y 10.9 años, rango 4.5-13.3 en el caso de aneurisma a nivel de aorta torácica) (*Nuenninghoff et al, 2003*). Un estudio similar realizado en España sobre una serie de 210 pacientes mostró la presencia de aneurismas y/o disección aórticos en el 9.5% de los casos. El tiempo de seguimiento desde el diagnóstico de la vasculitis hasta la detección del evento aórtico fue de 3.2 años (rango 0-13.5 años). En este estudio, tres pacientes presentaban un aneurisma aórtico en el momento del diagnóstico de la enfermedad, aunque en la mayoría de los casos el aneurisma se diagnosticó en fases tardías (*González-Gay et al, 2004*).

Entre las limitaciones de estos estudios se incluye un diseño retrospectivo, que no permite conocer la prevalencia real de complicaciones aórticas ya que, pueden existir casos que hayan pasado desapercibidos. Por otra parte, en todos ellos se incluyen pacientes que fueron diagnosticados durante periodos de tiempo muy largos, entre 20 y 50 años, lo que dificulta la homogeneidad de la muestra. A lo largo de esos años probablemente fueron cambiando tanto las pautas de tratamiento, como la accesibilidad a las técnicas de imagen que permiten la detección precoz de estas alteraciones. No obstante, todos ellos coinciden en una mayor frecuencia de complicaciones a nivel de la aorta torácica, que contrasta con los aneurismas de origen arteriosclerótico que se desarrollan con mayor frecuencia a nivel de la aorta abdominal. La aparición de aneurismas aórticos suele ser un evento tardío en el curso de la enfermedad, a diferencia de la disección aórtica que suele aparecer de forma más precoz, causando la muerte del paciente en la mayoría de ocasiones.

3.3. Factores que determinan el desarrollo de complicaciones aórticas.

Aún no se conocen los factores que favorecen el desarrollo de alteraciones estructurales aórticas en pacientes con ACG. Nuenninghoff observó que la formación de aneurismas y/o disección aórticos se asociaba a una mayor frecuencia de hiperlipidemia y cardiopatía isquémica (*Nuenninghoff et al, 2003*). En nuestro país, González-Gay encontró cierta relación entre el desarrollo de aneurismas aórticos y la presencia de hipertensión arterial o una respuesta inflamatoria sistémica intensa,

definida en ese estudio por la presencia de PMR y alteración en los parámetros de fase aguda (VSG, hemoglobina, recuento plaquetario) (*González-Gay et al, 2004*).

Históricamente se ha sugerido que el desarrollo de alteraciones estructurales aórticas se relaciona con la persistencia de actividad inflamatoria en la pared arterial. Esta idea se sustenta en los hallazgos histológicos de especimenes quirúrgicos o de necropsias de pacientes con ACG, que muestran la persistencia del infiltrado inflamatorio a nivel de la pared aórtica, incluso después de recibir tratamiento durante periodos de tiempo más o menos prolongados. Las características del infiltrado inflamatorio varían en función del tiempo de evolución de la enfermedad y la existencia o no de síntomas de actividad. En pacientes con enfermedad activa o que han recibido un tratamiento insuficiente se observa la típica arteritis granulomatosa, con presencia de células mononucleares (linfocitos T y macrófagos) y células gigantes multinucleadas. Por el contrario, las muestras de pacientes que no presentan síntomas de actividad o que han sido tratados durante periodos de tiempo más largos muestran un infiltrado inflamatorio menos florido (*Lie, Failoni y Davis, 1986; Klein et al, 1975; Lie, 1995; Liu, Shupak y Chiu, 1995*). Entre los datos clínicos que apoyan esta hipótesis se encuentra el hecho de que existen pacientes que presentan una enfermedad refractaria y requieren tratamiento durante periodos de tiempo prolongados e incluso de forma indefinida. Este curso desfavorable se ha relacionado con la posible persistencia de focos de inflamación a nivel aórtico, que con el tiempo favorecerían el desarrollo de alteraciones

estructurales. Igualmente, la elevación moderada de los parámetros de fase aguda y citocinas proinflamatorias de forma crónica en pacientes que han alcanzado la remisión, también podría reflejar la persistencia de focos de inflamación a nivel aórtico.

El segundo trabajo de esta tesis tiene como objetivo investigar, de manera prospectiva, la prevalencia y distribución de alteraciones estructurales aórticas clínicamente significativas en un grupo de pacientes con ACG que tienen un seguimiento mínimo de cuatro años. Además, se intentarán identificar posibles factores relacionados con el desarrollo de dichas complicaciones, principalmente si existe relación con la persistencia de actividad inflamatoria.

4. Búsqueda de dianas terapéuticas.

El tratamiento con corticoides mejora los síntomas de forma espectacular y modifica el curso de la enfermedad, reduciendo el riesgo de padecer complicaciones isquémicas. Los corticoides producen un aumento de la síntesis de IκBα que inhibe la traslocación del NFκB hacia el núcleo, lo que disminuye la transcripción de genes regulados por el NFκB, como es el caso de algunas citocinas y mediadores de la respuesta inflamatoria. Sin embargo, en muchos pacientes los síntomas recidivan al reducir la dosis de corticoides y hasta en la mitad de los casos es necesario mantener el tratamiento durante más de 2 ó 3 años, e incluso de forma indefinida, a pesar de sus importantes efectos secundarios. Se ha observado que los

pacientes con ACG tienen un mayor riesgo de fracturas y de diabetes inducidas por corticoides que las personas de la misma edad y sexo. Hasta un 80% de pacientes con ACG presentará por lo menos una complicación secundaria al tratamiento corticoideo y el 60% de ellos presentará dos o más eventos adversos (*Proven et al, 2003*). Algunos pacientes son especialmente susceptibles al desarrollo de complicaciones, como es el caso de aquellos que presentan osteopenia o síndrome metabólico en el momento del diagnóstico (*Queralt et al, 2000; Uzu et al, 2007*).

Esta elevada tasa de efectos secundarios ha motivado la búsqueda de tratamientos coadyuvantes que permitan disminuir la exposición a los corticoides en pacientes con ACG. Aunque se han publicado algunos estudios en este sentido, hasta la fecha los únicos agentes que se han probado de forma controlada son metotrexato e infliximab. El primero se utilizó en 3 estudios que dieron lugar a conclusiones dispares (*Hoffman et al, 2002; Jover et al, 2001; Spiera et al, 2001*). Sin embargo, un metanálisis posterior sugirió que el metotrexato reducía modestamente la probabilidad de recidivas en comparación con el placebo, por lo que en la actualidad, es el fármaco coadyuvante de elección en pacientes con una enfermedad refractaria o en aquellos que presentan importantes efectos secundarios derivados del tratamiento con corticoides (*Mahr et al, 2007*).

Las estatinas, además del efecto hipolipemiante poseen propiedades anti-inflamatorias, por lo que su uso podría tener interés en pacientes con ACG. No obstante, un estudio realizado por nuestro grupo, demostró que

el tratamiento con estatinas no disminuye el número de recidivas clínicas ni los requerimientos de prednisona. Cabe decir que este trabajo presentaba ciertas limitaciones. Además de ser un estudio retrospectivo que incluía una serie no muy amplia de pacientes, las dosis de estatinas utilizadas fueron pequeñas y no uniformes, por lo que no fue posible descartar que a dosis más elevadas, pudieran contribuir a una reducción más rápida de la dosis de prednisona (*García-Martínez et al, 2004*). Se adjunta el artículo completo al final de esta introducción.

4.1. El intento de bloquear el TNFα

La propuesta de administrar infliximab nació tras observar que tanto los niveles circulantes, como la expresión tisular de TNFα se correlacionaban con la intensidad de la RIS y los requerimientos terapéuticos (*Hernández-Rodríguez et al, 2002; Hernández-Rodríguez et al, 2003*). Estos resultados condujeron a la hipótesis de que el TNFα podía jugar un papel central en el desarrollo y mantenimiento de la respuesta inflamatoria en pacientes con ACG, por lo que cabía esperar que su bloqueo facilitase el control de la enfermedad.

Se realizó un estudio multicéntrico, randomizado, controlado, a doble ciego en el que se incluyeron 44 pacientes con ACG que habían alcanzado la remisión con corticoides, con el objetivo de determinar si el infliximab podía ser de utilidad en el mantenimiento de esta remisión. Los objetivos del estudio fueron: número de pacientes que presentaban

recidivas a la semana 22 y efectos secundarios derivados del tratamiento. Al final de la introducción se ha adjuntado el artículo completo. El estudio fue suspendido de forma precoz ya que al realizar un análisis intermedio a la semana 22, la administración de infliximab (5 mg/kg) no había demostrado superioridad con respecto a placebo (*Hoffman et al, 2007*). No obstante, estos resultados no permiten descartar el posible beneficio del infliximab administrado en fases más tardías, únicamente en aquellos pacientes que presentan una enfermedad refractaria.

La elevación de un mediador inflamatorio como el TNFα, a pesar de ser un indicador de mal pronóstico y persistencia de la enfermedad, no significa que participe en el mantenimiento de la misma. La respuesta inflamatoria está vehiculizada por un complejo entramado de proteínas con múltiples vías de señalización reguladas por mecanismos complejos. Es posible que el bloqueo de uno de estos mediadores desencadene la activación de citocinas redundantes que compensen su función. Sería interesante identificar las moléculas que intervienen al inicio de la cascada de señales, ya que un bloqueo selectivo precoz de las mismas podría modificar el curso de la enfermedad de forma más eficaz. Además, se hace necesario la búsqueda de modelos de estudio que permitan realizar análisis preclínicos de posibles nuevos fármacos (*Lozano et al, 2008; Brack et al, 1997*).

4.2. Descripción del sistema RANKL/RANK/OPG.

En los últimos años, con el conocimiento de la secuencia del genoma humano, se están identificando múltiples citocinas que comparten una gran semejanza estructural con el TNFα y sus receptores y que se han englobado dentro de la superfamilia del TNFα. Sus principales funciones están relacionadas con la regulación de la proliferación celular y la apoptosis, aunque se ha visto que algunos grupos también tienen propiedades proinflamatorias (*Locksley, Killen y Lenardo, 2001; Walsh y Choi, 2003*).

A esta familia pertenece el sistema constituido por el ligando del receptor activador del factor nuclear κB (RANKL) y sus dos receptores: la osteoprotegerina (OPG) (receptor soluble) y el receptor activador del factor nuclear κB (RANK) (receptor de membrana). Este sistema fue descrito a finales de los años 90 y se ha erigido como la principal vía efectora del metabolismo óseo. El RANKL es producido por osteoblastos y células del estroma óseo y puede expresarse de forma soluble o asociado a membrana. El RANK se expresa en células pre-osteoclásticas y osteoclastos maduros. La unión de RANKL-RANK estimula la formación, diferenciación, activación y supervivencia de los osteoclastos, favoreciendo la osteoclastogénesis. Los osteoblastos y células del estroma óseo también producen OPG, cuya unión al RANKL bloquea su interacción con el RANK e inhibe la actividad osteoclástica. La dimerización de la OPG aumenta su afinidad por RANKL (*Reid y Holen, 2009*). Además de RANKL, la OPG también es el receptor soluble del ligando inductor de la apoptosis

relacionado con el TNF (TRAIL), factor que favorece la apoptosis celular. Los miembros de la familia del TNFα y sus receptores tienen mecanismos comunes para la transmisión de señales, que suelen producirse a través de la unión del receptor de membrana con proteínas adaptadoras llamadas factores asociados al receptor del TNF (TRAFs). El TRAF6 parece jugar un papel importante en la resorción ósea. A través de los TRAFs se activan diversas vías de transducción de señales entre las que se encuentran: factor nuclear (NF)-kB, c-Fos, JNK, c-Src y la serina/treonina cinasa Akt/PkB (*Hofbauer y Heufelder, 2001; Kong, Boyle y Penninger, 2000; Bolon et al, 2002*). Se conocen múltiples factores con capacidad para regular el metabolismo óseo. La mayoría de ellos ejerce esta función a través de regular la expresión del sistema RANKL/OPG a nivel de osteoblastos y células del estroma óseo (Tabla 2).

Tabla 2. Efecto de diversos factores sobre la expresión de RANKL y OPG

	RANKL	OPG
PTH	↑	↓
$1,25(OH)_2D_3$	↑	↓
Estrógenos		↑
Corticoides	↑	↓
Bifosfonatos		↑
Raloxifeno		↑
TNFα, IL-1ß, IL-6	↑	↑
TGFß	↓	↑
BMP		↑
bFGF		↓
IGF-I		↓
PGE_2	↑	↓

PTH: hormona paratiroidea
TGFβ: factor de crecimiento transformador beta
BMP: proteína morfogenética del hueso
bFGF: factor de crecimiento fibroblástico
IGF: factor de crecimiento-I similar a la insulina
PGE2: prostaglandina E2

Estudios realizados con modelos animales pusieron de manifiesto que, además de la osteoclastogénesis, RANKL y OPG ejercían funciones reguladoras sobre otros sistemas. Los ratones OPG knock-out, además de osteoporosis severa, presentaban calcificaciones arteriales así como alteraciones en la maduración de las células B y de la respuesta mediada por anticuerpos (*Bucay et al, 1998; Yun et al, 1998*). De forma similar, los ratones RANKL o RANK knock-out, además de desarrollar osteopetrosis mostraban defectos en la maduración de las células T y B y en el desarrollo de los ganglios linfáticos (*Baud'huin et al, 2007; Papadopouli, Klonaris y Theocharis, 2008*). Estos hallazgos despertaron el interés por estudiar el papel de estas citocinas en la regulación del sistema inmune y la homeostasis vascular.

4.3. Efecto del sistema RANKL/RANK/OPG sobre el sistema inmune.

La función inmunorreguladora de RANKL y OPG ha sido estudiada principalmente en estudios in vitro. La activación antigénica de las células T aumenta la expresión de RANKL por estas células, tanto en su forma soluble como la asociada a membrana. El RANK se expresa de forma primaria en células de la línea macrofágica/monocítica, que además de células preosteoclásticas, incluye células T y B, fibroblastos, monocitos, macrófagos y células dendríticas maduras que son las principales presentadoras de antígenos. La interacción RANKL-RANK estimula el gen Bcl-xL que aumenta la supervivencia de estas células. Esta interacción también produce un aumento de la expresión de citocinas proinflamatorias

(IL-1, IL-6, IL-12 e IL-15), algunas de ellas fundamentales en la regulación de la respuesta inmune Th1 (*Bachmann et al, 1999; Wong, Josien y Choi, 1999; Josien et al, 1999; Walsh y Choi, 2003; Chino, Draves y Clark, 2009; Anderson et al, 1997*). La administración de una proteína de fusión soluble RANK.Fc, disminuye in vitro la secreción de IFNγ por las células T activadas tras su unión a las células presentadoras de antígenos (*Chen, Huang y Hsieh, 2001*). Esto podría tener interés en pacientes con ACG, ya que el IFNγ se considera una de las principales citocinas en la diferenciación de la respuesta inmune Th1. Por otra parte, RANKL estimula la migración celular. Estudios *in vitro* han demostrado que la exposición de monocitos a la acción de RANKL aumenta su capacidad migratoria (*Breuil et al, 2003; Mosheimer et al, 2004*). En definitiva, todos estos datos sugieren que la interacción RANKL-RANK podría potenciar la respuesta inflamatoria.

Efectos de RANKL sobre células del sistema inmune

RANKL
RANK
OPG

Linfocitos

- ↑ supervivencia
- Activación células T
- Secreción citocinas
- Estimula respuesta inmune
- Maduración células B

Células dendríticas

Monocitos/Macrófagos

- ↑ supervivencia
- Activación celular
- Secreción citocinas
- Estimula presentación Ag

- ↑ supervivencia
- Secreción citocinas
- Estimula respuesta inmune
- Quimiotaxis
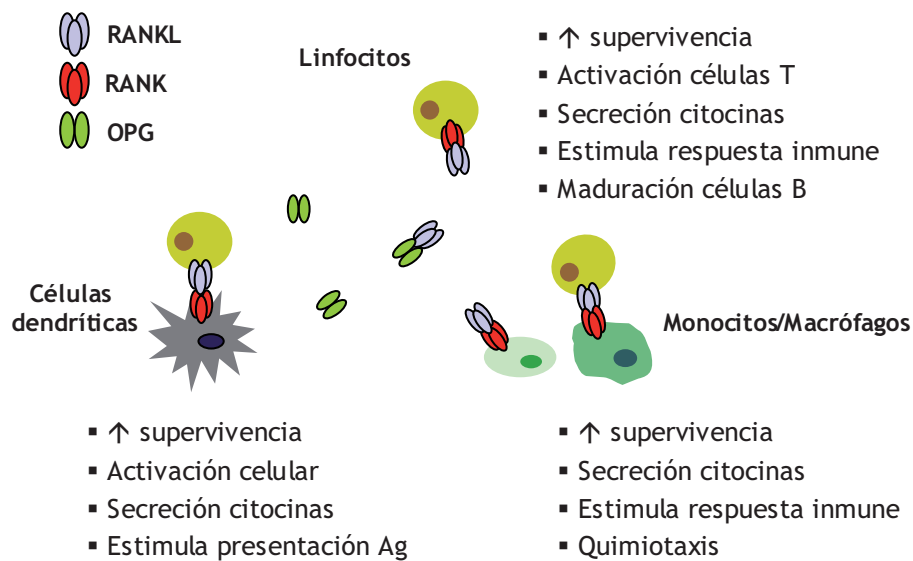
4.4. Función del sistema RANKL/RANK/OPG en biología vascular.

Por otra parte, es posible que el sistema RANKL/OPG intervenga en el mantenimiento de la homeostasis vascular. Mientras que la OPG se expresa en una gran variedad de tejidos, incluido el árbol arterial normal, RANKL y RANK no suelen detectarse en vasos no enfermos (*Collin-Osdoby, 2004*). A nivel vascular, tanto las células endoteliales como las células musculares lisas expresan OPG. RANKL es producido fundamentalmente por células T infiltrantes y células endoteliales activadas mientras que RANK se expresa en los monocitos precursores de osteoclastos y células dendríticas (*Van Campenhout y Golledge, 2009*). La expresión de RANKL y OPG a nivel vascular se ve modificada por la acción de múltiples factores y a su vez, ambas citocinas pueden desencadenar diversas respuestas a nivel vascular.

La expresión de RANKL aumenta en células endoteliales expuestas a la acción del factor de crecimiento transformador (TGF)-β (*Ishida et al, 2002*). La expresión de mRNA de RANKL y OPG en células endoteliales microvasculares humanas (HMVEC) también aumenta tras la acción del TNFα y la IL-1, así como la expresión proteica de RANKL en las HMVEC estimuladas con TNFα (*Collin-Osdoby et al, 2001*). Por otra parte, RANKL es capaz de aumentar la expresión de moléculas de adhesión (ICAM-1 y VCAM-1) en las células endoteliales, lo que favorecería la adhesión leucocitaria y la amplificación de la respuesta inflamatoria (*Min J, 2005*). También la OPG es capaz de aumentar la expresión de moléculas de
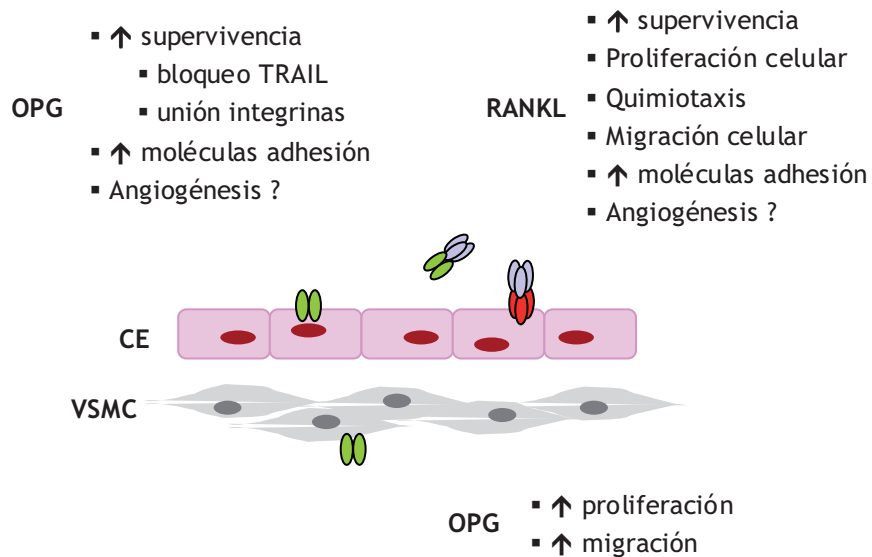
adhesión en células endoteliales estímuladas con TNFα. Este efecto tiene lugar a través de aumentar la expresión de angiopoietina-2, que sensibiliza la célula endotelial a la acción del TNFα que a su vez aumenta la transcripción génica de ICAM-1, VCAM-1 y E-selectina (*Mangan et al, 2007*). En este sentido, OPG podría jugar un papel fundamental como puente entre la inflamación y el daño vascular.

Una de las principales áreas de interés respecto al papel del sistema RANKL/OPG en biología vascular se centra en su posible relación con el desarrollo de enfermedad arteriosclerótica. Los ratones deficitarios para OPG desarrollan mayores calcificaciones a nivel de los vasos, lo que sugiere que la OPG pueda actuar como un factor de protección vascular (*Bucay et al, 1998*). No obstante, de forma repetida se ha observado que existe una correlación entre la severidad de enfermedad coronaria y los niveles séricos de OPG (*Van Campenhout et al, 2009*). Hasta la fecha, no ha sido posible concluir si la OPG interviene en el desarrollo de enfermedad arteriosclerótica o simplemente se libera en respuesta a la agresión. En lo que sí parecen coincidir los diversos autores, es en la potencial utilidad de la OPG como marcador de enfermedad vascular.

La agresión inflamatoria vascular desencadena una serie de respuestas que dan lugar a cambios en la estructura de los vasos. La OPG se almacena en los cuerpos de Weibel-Palade de las células endoteliales, donde se encuentra unida al factor von Willebrand. En respuesta a un estímulo inflamatorio ambas moléculas son rápidamente liberadas al

torrente circulatorio (*Zannettino et al, 2005*). En pacientes con ACG, el factor von Willebrand se mantiene elevado durante los primeros años tras el diagnóstico y se ha sugerido que podría ser un marcador de persistencia de actividad de la enfermedad (*Cid et al, 1996*). Desconocemos en el momento actual si la OPG pudiera seguir un comportamiento similar al factor von Willebrand.

Efectos de RANKL y OPG en el sistema vascular

OPG
- ↑ supervivencia
  - bloqueo TRAIL
  - unión integrinas
- ↑ moléculas adhesión
- Angiogénesis ?

RANKL
- ↑ supervivencia
- Proliferación celular
- Quimiotaxis
- Migración celular
- ↑ moléculas adhesión
- Angiogénesis ?

CE

VSMC

OPG
- ↑ proliferación
- ↑ migración

CE: célula endotelial, VSMC: célula muscular lisa vascular

En arterias normales, las células musculares lisas de la capa media presentan un fenotipo quiescente y contráctil. Tras la agresión inflamatoria, estas células adquieren capacidad secretora, además de potencial para proliferar y migrar hacia el interior de la pared, dando lugar a la

hiperplasia de la íntima responsable de los fenómenos isquémicos. El PDGF estimula la proliferación y migración de células musculares lisas procedentes de arteria temporal, así como la secreción de diversas citocinas y quimiocinas por esas células (*Lozano et al, 2008*). Diversos estudios realizados en otras áreas de investigación, sugieren que el sistema RANKL/OPG podría tener alguna función en este proceso de remodelado vascular. El PDGF aumenta la expresión de OPG en células musculares lisas de vena umbilical y de aorta humanas (*Malyankar et al, 2000; Zhang et al, 2002*). En arterias pulmonares, OPG fue capaz de inducir la proliferación y la migración de células musculares lisas, sugiriendo un posible papel de la OPG en los mecanismos patogenéticos de la hipertensión pulmonar (*Lawrie et al, 2008*). Por otra parte, el fenómeno de remodelado vascular requiere la secreción de enzimas proteolíticos. En arterias temporales de pacientes con ACG la secreción de metaloproteasas se ha relacionado con la destrucción de la lámina elástica, fenómeno imprescindible para la migración de los miofibroblastos y el desarrollo de hiperplasia intimal (*Segarra et al, 2007; Rodríguez-Pla et al, 2005*). El sistema RANKL/OPG ha demostrado capacidad para modificar la actividad proteolítica *in vitro* en distintos tipos celulares a través de regular la expresión, tanto de algunas metaloproteasas como de sus inhibidores naturales, los TIMPs (*Moran et al, 2005; Wittrant et al, 2002, 2003, 2004*).

Las lesiones inflamatorias de la ACG muestran gran formación de neovasos que podrían tener un papel protector frente a la isquemia. El sistema RANKL/OPG también ha demostrado tener capacidad

angiogénica, aunque los estudios publicados hasta la fecha aportan resultados contradictorios. La OPG aumenta la supervivencia de las células endoteliales a través de varios mecanismos. Por una parte, inhibe el TRAIL con capacidad apoptótica (*Ki et al, 2003*). Además, favorece la unión de las células endoteliales a integrinas de matriz extracelular ($\alpha v \beta 3$), lo que también promueve la supervivencia de esas células (*Malyankar et al, 2000*). En modelos de angiogénesis, OPG mostró actividad pro-angiogénica mientras que RANKL era capaz de inhibir la angiogénesis (*McGonigle, Giachelli y Scatena, 2009*). Sin embargo, otros estudios sugieren resultados opuestos. Son estudios realizados in vitro y con modelos animales, en los que RANKL fue capaz de estimular la supervivencia, proliferación y migración de las células endoteliales así como la formación de estructuras tubulares, respuestas todas ellas, pro-angiogénicas. El cultivo de HUVECs con VEGF aumentó la expresión de RANK en dichas células, haciéndolas más sensibles a la acción del RANKL y facilitando un efecto angiogénico (*Kim et al, 2002; Kim et al, 2003; Min et al, 2003*).

Nuestro interés por estudiar el sistema RANKL/RANK/OPG nació tras observar que el bloqueo del TNFα con infliximab no producía efectos significativos en la evolución de los pacientes con ACG. Los miembros de la superfamilia del TNFα, además de gran similitud estructural, comparten vías de señalización comunes con el TNFα. La ausencia de respuesta al infliximab nos hizo pensar que probablemente existan otras vías paralelas que se activen ante el bloqueo del TNFα y compensen su función, o bien que existan citocinas redundantes que continúen activando vías de

señalización comunes. El sistema RANKL/RANK/OPG podría ser una de estas vías debido a su similitud con el TNFα. Por otra parte, estudios previos sugerían que este sistema de citocinas podría jugar un papel en la regulación de la respuesta inflamatoria y la homeostasis vascular. Ambos puntos confluyen y son de gran interés en una enfermedad vascular inflamatoria como la ACG. Por ese motivo, pensamos que el estudio del sistema RANKL/RANK/OPG podría tener interés para comprender mejor los mecanismos que regulan la respuesta vascular a la inflamación en esta enfermedad. El último estudio de esta tesis tiene como principal objetivo conocer la expresión de estas citocinas en pacientes con ACG.

5. Trabajos publicados relacionados con esta tesis doctoral.

En las siguientes páginas se han incluido tres artículos que han sido mencionados a lo largo de la introducción. Los estudios han sido realizados por nuestro grupo de investigación y en cierto modo, constituyen el punto de partida o tienen relación con los trabajos desarrollados en esta tesis doctoral.

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A Strong Initial Systemic Inflammatory Response is Associated With Higher Corticosteroid Requirements and Longer Duration of Therapy in Patients With Giant-Cell Arteritis

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Objective. To assess whether the intensity of the initial systemic inflammatory response is able to predict response to therapy in patients with giant cell arteritis (GCA).

Methods. Retrospective review of 75 patients (49 women and 26 men) with biopsy-proven GCA who had regular followup and were treated according to uniform criteria. Four parameters were used to evaluate the baseline inflammatory response at diagnosis: fever, weight loss, erythrocyte sedimentation rate $\geq$ 85 mm/hour, and hemoglobin < 110 gm/liter. Patients were considered to have a weak inflammatory response if they had 2 or fewer inflammatory parameters (group 1) and a strong inflammatory response if 3 or 4 parameters were present (group 2). Time required to achieve a maintenance dose of less than 10 mg prednisone/day was recorded and analyzed by the Kaplan–Meier survival analysis method. Tumor necrosis factor α (TNFα) and interleukin 6 (IL-6) serum levels were also determined in 62 patients and 15 controls.

Results. Forty patients had a weak (group 1) and 35 had a strong (group 2) initial inflammatory response. Patients in group 2 had significantly higher levels of circulating TNFα (31.9 $\pm$ 16.8 versus 22.3 $\pm$ 9 pg/ml; P = 0.01) and IL-6 (28.2 $\pm$ 17.4 versus 16.6 $\pm$ 13 pg/ml; P = 0.004) than patients in group 1. In group 1, 50% of patients required a median of 40 weeks (95% CI 37–43) to reach a maintenance dose of <10 mg, whereas in group 2 a median of 62 weeks (95% CI 42–82) was necessary (P = 0.0062). Patients in group 2 experienced more flares than patients in group 1 (P = 0.01) and received higher cumulative steroid doses (8.974 $\pm$ 3.939 gm versus 6.893 $\pm$ 3.075 gm; P = 0.01).

Conclusion. GCA patients with a strong initial systemic inflammatory reaction have more elevated circulating levels of IL-6 and TNFα, have higher and more prolonged corticosteroid requirements, and experience more disease flares during corticosteroid therapy than patients with a weak systemic acute phase response.

KEY WORDS. Vasculitis; Inflammatory response; Giant-cell arteritis; Corticosteroids.

INTRODUCTION

Giant cell (temporal) arteritis (GCA) is a chronic granulomatous vasculitis preferentially targeting large- and medium-sized arteries (1). Most of the classic disease manifestations result from symptomatic involvement of the carotid artery branches. Typical symptoms include headache, jaw claudication, scalp tenderness, and a variety of aches in the craniofacial area (1,2). Ischemic complications derived from vessel occlusion include visual loss

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Submitted for publication March 23, 2001; accepted in revised form July 19, 2001.

mainly due to anterior ischemic optic neuritis and, less frequently, stroke or scalp necrosis (3).

In addition to the above-mentioned clinical manifestations, GCA is a disease characterized by a prominent systemic inflammatory reaction (1–3). The acute phase response to infection or injury is a complex and not completely understood phenomenon that, globally, is thought to be protective and meant to avoid excessive tissue destruction. It encompasses a series of reactions distant from the areas of inflammation in which many organs and systems participate. As a consequence, such clinical manifestations as fever, anorexia, weight loss, hematologic abnormalities (i.e., anemia and thrombocytosis), biochemical alterations (acute phase protein synthesis), and metabolic changes (i.e., increased lipolysis and muscle loss) characteristically occur. This systemic reaction to injury is driven by proinflammatory cytokines, mostly interleukin 1 (IL-1), tumor necrosis factor α (TNFα), and IL-6, which are produced mainly by macrophages at the sites of inflammation. Although most cytokines act in an autocrine/paracrine fashion, proinflammatory cytokines drive the acute-phase response in a distant, systemic way. They have pleiotropic effects on a variety of cells that, in turn, secrete a wide array of products. A complex network of stimulatory and inhibitory mediators determines, eventually, the intensity of the acute phase response (4,5).

Approximately 50% of GCA patients experience fever and weight loss. Most patients have remarkably elevated erythrocyte sedimentation rates (ESR) and chronic anemia (1–3). Acute phase proteins such as C-reactive protein (CRP), orosomucoid, and haptoglobin are elevated in a substantial proportion of patients (3,6,7). However, the intensity of the acute phase response is highly variable among patients. Patients with no constitutional symptoms (3,8) and normal or near-normal ESR have been reported repeatedly (3,9–12). The mechanisms underlying this variability have not been investigated.

Corticosteroids are the treatment of choice for patients with GCA; they rapidly relieve most symptoms in the majority of cases. However, the duration of corticosteroid therapy is highly variable (13,14). Some individuals achieve a sustained remission after a few months of treatment. Most patients require 1–2 years of therapy and some patients require long-term corticosteroid therapy. Some patients are able to maintain remission with less than 10 mg/day of prednisone, and other patients require at least 20 mg/day to remain asymptomatic (13–15). Corticosteroid-related morbidity is elevated in patients with GCA, and iatrogenic complications are heavily influenced by the intensity and duration of corticosteroid treatment (14,16). To date, no clinical or analytic parameters have been identified that can consistently predict the intensity and duration of corticosteroid therapy in a large and homogeneous cohort of people with GCA.

The goals of our study were to determine whether circulating levels of proinflammatory cytokines are related to the intensity of systemic inflammatory response in GCA and to assess whether the intensity of the systemic inflammatory response may be an indicator of the magnitude and duration of corticosteroid treatment.

SUBJECTS AND METHODS

The study group consisted of 75 patients, 49 women and 26 men, with biopsy-proven GCA diagnosed and treated at our Internal Medicine Department over a 14-year period. Patients were selected consecutively among those who had regular followup. Patients who were transferred to another institution or treating physician, had low compliance, or died early (within 3 months) in the course of the disease were excluded. Although this study is retrospective in design, patients included were evaluated and treated by the authors according to uniform criteria. The treatment schedule began with an initial prednisone dose of 1 mg/kg/day (up to 60 mg/day) for 1 month. Subsequently, prednisone was tapered by 5 mg/week. Reductions below 20 mg/day were slower and individualized. A rate of 2.5 mg every 3 months was attempted. A disease flare was considered when ESR rose above 50 mm/hour and disease-related manifestations (cranial symptoms, polymyalgia rheumatica, fever, or malaise) appeared or hemoglobin fell below 110 gm/liter. When clear and worsening symptoms occurred with a normal or slightly elevated ESR, a flare was also considered. When ESR rose with no clinical symptoms or anemia, the maintenance dose was held until it went back to normal or a flare could be defined. When a disease flare was suspected, prednisone was increased to 10 mg above the previous effective dose; to be fully considered a flare, symptoms had to remit after adjusting the prednisone dose.

Data recorded at entry included age, sex, number and type of cranial symptoms, transient or permanent ischemic complications, polymyalgia rheumatica, fever (>37°C), weight loss (>5 kg), duration of clinically symptomatic disease before diagnosis and time (weeks) of followup. Laboratory parameters included ESR, CRP, hemoglobin (Hgb), haptoglobin, γ-glutamyl transpeptidase, alkaline phosphatase, albumin, α2-globulin, and platelet count.

To evaluate the initial systemic inflammatory response, the following 4 parameters were considered: fever, weight loss, ESR $\geq$ 85 mm/hour, and Hgb < 110 gm/liter because they have been previously demonstrated to be useful in discriminating patients at high and low risk of developing ischemic events (3). Patients were considered to have a weak systemic inflammatory response when they had 2 or fewer inflammatory parameters (group 1) and a strong systemic inflammatory response when they had 3 or 4 parameters (group 2). The time (weeks) required to achieve a maintenance dose of <10 mg prednisone per day and the cumulative dose of prednisone received at that point were recorded. The number of flares and the number of patients out of treatment at the end of followup were also included.

Sera was obtained from 62 patients (36 from group 1 and 26 from group 2) with active disease and from 15 age- and sex-matched healthy individuals. Aliquots were frozen and stored at −80°C until use. IL-1β, TNFα, and IL-6 concentrations were determined by enzyme-linked immunosorbent assay (ELISA). Commercially available ELISA kits for TNFα were obtained from Medgenix (Fleurus, Belgium) and kits for IL-1β and IL-6 from Genzyme (Minneapolis, MN). The assays were performed according to the manufacturer's instructions.

Table 1. Clinical findings in patients with weak (group 1) and strong (group 2) systemic inflammatory reactions		
Clinical characteristics	**Group 1 (n = 40)**	**Group 2 (n = 35)**
General characteristics		
Age in years, mean (range)	76 (57–90)	73 (58–87)
Sex, male/female	16/24	9/26
Duration of symptoms in weeks, mean (range)	14 (1–80)	16 (2–104)
Followup time in months, mean (range)	31 (4–84)	40 (4–166)
Cranial symptoms (%)		
Headache	85	74
Jaw claudication	45	46
Scalp tenderness	52.5	43
Facial pain/edema	27.5	31
Abnormal temporal arteries*	83	79
Ocular pain	15	8.6
Tongue pain	7.5	3
Earache	22.5	31
Trismus	0	6
Carotidynia	5	3
Toothache	15	11
Odynophagia	7.5	23
Ischemics events (%)	30†	9
Amaurosis fugax	12.5	3
Established amaurosis	17.5	3
Transient diplopia	5	6
Permanent diplopia	5	0
Stroke	2.5	0
Symptomatic involvement of other vascular territories (%)	2.5	3
Systemic manifestations (%)		
Fever	22.5‡	77
Weight loss	32.5‡	86
Polymyalgia rheumatica	40	46

* Prominent and hard arteries; pulse asymmetric, weak or absent; or inflammatory signs.
† $P = 0.04$ versus patients with a high inflammatory status.
‡ $P < 0.0001$

Fisher's exact test was used for qualitative comparisons. For quantitative comparisons among groups of individuals, Student's unpaired t test was employed. The Pearson's correlation coefficient was used. The time required to achieve a maintenance prednisone dose of <10 mg/day was compared between group 1 and group 2 by the Kaplan–Meier survival analysis method.

RESULTS

According to the previously mentioned criteria, 40 patients had a weak (group 1) and 35 had a strong (group 2) systemic inflammatory response at first evaluation. The main clinical findings in both groups of patients are summarized in Table 1. In accordance with previously reported data (3,17,18), ischemic events were significantly more frequent in patients with a weak systemic inflammatory response. No differences were found in the distribution of other major clinical manifestations between both groups of patients except for the clinical criteria (fever and weight loss) used to define them. No differences existed in the duration of clinically apparent disease between the groups, suggesting that differences in the intensity of the systemic inflammatory response may be constitutive rather than reflect early or late time points in the course of the disease. No differences existed in the duration of followup.

In addition to Hgb and ESR values employed as criteria to define both groups of patients, parameters related to the acute phase response, such as CRP, haptoglobin, platelet count, and α2 globulins, were more elevated in patients with a strong systemic inflammatory reaction (Table 2). In contrast, albumin, a negatively-regulated protein during the acute phase response, was lower in patients with a strong systemic inflammatory reaction.

Circulating TNFα levels were moderately but significantly higher in GCA patients (26.4 ± 13.7 pg/ml) than in controls (16 ± 9.5 pg/ml; $P = 0.007$) (Figure 1). As was shown previously (19–21), IL-6 concentrations were more elevated in patients than in healthy controls (21.4 ± 16 pg/ml versus 5 ± 11 pg/ml; $P = 0.0004$) (Figure 2). In addition, remarkable differences in the concentrations of both cytokines were observed between patients with a strong systemic inflammatory response and patients with a weak inflammatory reaction. TNFα concentrations were 22.3 ± 9 pg/ml in group 1 and 31.9 ± 16.8 pg/ml in group 2 ($P = 0.01$) and IL-6 concentrations were 16.6 ± 13 pg/ml in group 1 and 28.2 ± 17.4 in group 2 ($P = 0.004$) (Figures

Table 2. Blood chemistry and hematologic values in patients with weak (group 1) and strong (group 2) systemic inflammatory reactions*

Parameter	Group 1 mean (range)	Group 2 mean (range)
ESR, mm/hour	80 (28–130)†	114 (65–147)
CRP, mg/dl	4.7 (0.5–25.5)‡	12 (1.9–33.3)
Haptoglobin, gm/liter	3.221 (0.079–6.770)§	4.877 (3.024–7.490)
Hemoglobin, gm/liter	120 (66–156)†	98 (75–119)
Platelets, $\times 10^9$/liter	315 (105–493)¶	378 (130–768)
Albumin, gm/liter	35 (24–42)#	32 (25–43)
α2-globulin, gm/liter	9 (5–18)¶	10.4 (4.4–20)
Alkaline phosphatase, units/liter	219 (139–450)	304 (98–1682)
γ-glutamyl transpeptidase, units/liter	32 (8–140)	63 (10–383)

* ESR = erythrocyte sedimentation rate; CRP = C-reactive protein.
† $P < 0.0001$ versus patients with a high inflammatory status.
‡ $P = 0.001$
§ $P = 0.004$
¶ $P = 0.03$
# $P = 0.02$

1 and 2), indicating that TNFα and IL-6 may participate in the development of the acute phase response in GCA. TNFα concentrations correlated positively with ESR values ($r = 0.364$, $P = 0.018$) and haptoglobin levels ($r = 0.448$, $P = 0.022$), and negatively with hemoglobin concentration ($r = -0.329$, $P = 0.033$). Similarly, IL-6 levels significantly correlated with CRP ($r = 0.378$, $P = 0.025$). In contrast, IL-1β was below the detection threshold in most patients and controls.

Corticosteroid requirements were significantly higher in patients with a strong systemic inflammatory response. While in group 1, 50% of patients required a median of 40 weeks (95% CI 37–43) to reach a maintenance dose of prednisone lower than 10 mg/day; in group 2, 50% of patients required a median of 62 weeks to reach maintenance dose (95% CI 42–82; $P = 0.0062$) (Figure 3). The cumulative dose of prednisone received during this period was 6.893 ± 3.075 gm in group 1 and 8.974 ± 3.939 gm in group 2 ($P = 0.01$). During the followup, 22 of 40 (55%) patients in group 1 and 27 of 35 (77%) in group 2 experienced at least 1 disease flare ($P = 0.054$). The main manifestation of a GCA recurrence was headache in 29 flares, polymyalgia rheumatica in 37, fever in 7, malaise in 12, and anemia in 5. Headache was more frequent in flares of patients in group 1 (17 versus 12; $P = 0.0046$) and malaise was slightly more frequent in patients in group 2 ($P = 0.0495$). No additional differences in the nature of flares were observed between groups. Nine (22.5%) patients in group 1 and 18 (51.4%) in group 2 had more than 1 disease flare ($P = 0.01$). At the end of followup, 17 (42.5%) patients in group 1 and 6 (17%) patients in group 2 were out of treatment (OR 3.6; CI 95% 1.2–10.5; $P = 0.02$). Six (15%) patients in group 1 and 7 (20%) patients in group 2 had died by the end of followup ($P = $ NS).
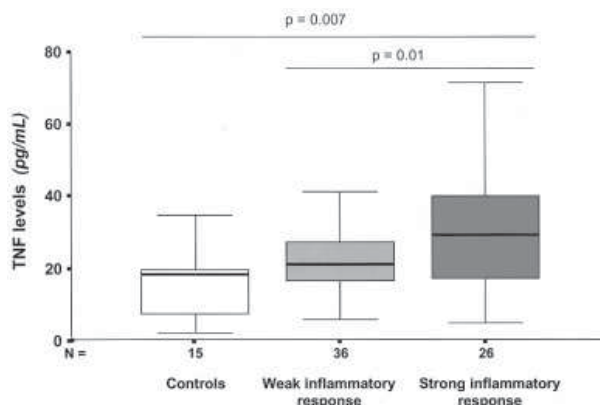

Figure 1. Box plots indicating range (error bars), 25–75% interval and median valve (horizontal line) of serum tumor necrosis factor (TNF)α levels in 62 patients with giant cell arteritis classified according to the intensity of their systemic inflammatory response as defined in the Subjects and Methods section, and in 15 age- and sex-matched healthy controls.

Figure 2. Box plots indicating range (error bars), 25–75% interval and median valve (horizontal line) of circulating concentrations of interleukin 6 (IL-6) in 62 patients with giant cell arteritis classified according to the intensity of their systemic inflammatory response as defined in the Subjects and Methods section, and in 15 age- and sex-matched healthy controls.
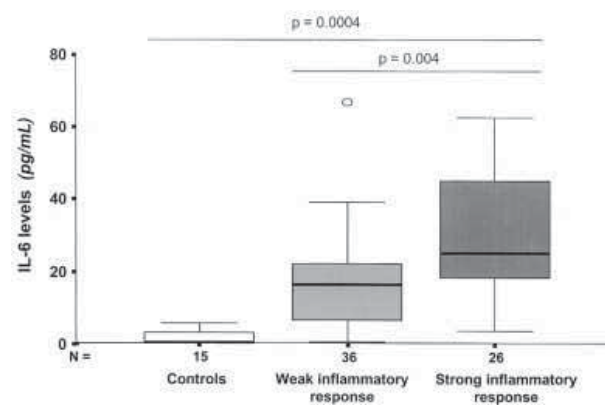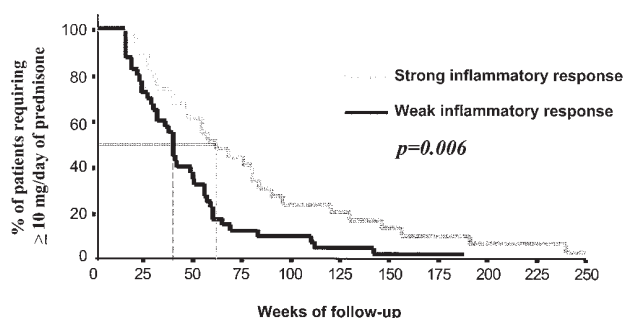
Figure 3. Percentage of patients requiring a maintenance dose of prednisone equal or greater than 10 mg/day over time.

DISCUSSION

GCA is a disease characterized by a strong acute phase response, and proinflammatory cytokine transcripts, namely IL-1β, TNFα, and IL-6 have been detected in temporal artery lesions by reverse transcriptase–polymerase chain reaction and in situ hybridization (22,23). However, when evaluating the influence of these cytokines in the development of the systemic inflammatory response, determining circulating levels may be more significant than their detection in tissue. First, most cytokines have instability sequences at their 3' untranslated region and are subjected to a strict posttranscriptional regulation, and the amount of mRNA at a given time point may not represent the actual resulting protein synthesis. Second, activated circulating monocytes may also contribute to the production of cytokines in the bloodstream (19,23); and third, circulating levels of cytokines may better reflect their overall systemic effects. With the exception of IL-6, previous attempts to determine circulating levels of cytokines have been performed mostly in patients with polymyalgia rheumatica, including just a few patients with GCA (24–28). In a homogeneous series of 19 and 20 patients with biopsy-proven GCA, Roche et al (19) and Roblot et al (20) found elevated levels of circulating IL-6 in patients with active disease. A trend towards elevated levels of TNFα in patients versus controls was also found, but the difference was not significant, probably due to the small number of patients included and to the fact that, according to our data, TNFα may only be elevated in a subset of patients. In an extended prospective followup study including 25 patients, Weyand et al (21) found IL-6 to be a sensitive marker of disease activity, but no correlation with clinical findings was investigated. In the present study of a large and homogeneous series of patients with biopsy-proven GCA, we found elevated levels of both TNFα and IL-6 in sera from active patients compared to controls. In addition, in active patients, IL-6 levels correlated with CRP concentrations; TNFα levels correlated positively with haptoglobin and ESR values and inversely with Hgb concentrations. Both TNFα and IL-6 levels were significantly higher in patients with strong overall systemic inflammatory reaction evaluated with previously established clinical and analytic parameters. Taken together, these data indicate an important role for circulating TNFα and IL-6 in the pathogenesis of the acute phase response in GCA. In contrast, although IL-1β mRNA can be detected in temporal artery lesions from patients with GCA (22,23), serum IL-1β levels were below the detection threshold in most patients. This result supports the concept that tissue cytokine mRNA may not correlate with circulating cytokine concentrations and suggests a less relevant participation of IL-1β in the generation of the systemic inflammatory response in GCA.

Corticosteroid requirements are highly variable among patients with GCA (13–15). Although some patients treated for a few months sustain remission, others require long-term therapy and higher-than-desirable maintenance prednisone doses with their ensuing iatrogenic complications (13–16). To date, no clinical or analytic findings able to predict the outcome of patients with GCA have been identified in large and homogeneous series of patients. Our results indicate that the intensity of the initial systemic inflammatory reaction is a major predictor of disease duration and corticosteroid requirements. Patients with a strong initial systemic inflammatory response evaluated with previously established clinical and analytic parameters have more disease flares and require a significantly longer duration of corticosteroid therapy. A similar trend has been observed by other investigators. In this regard, Weyand et al (29) found that elevated pretreatment ESR was associated with longer duration of corticosteroid treatment in a series of 27 patients with polymyalgia rheumatica.

We have previously published that patients with a strong inflammatory reaction have a lower risk of developing ischemic complications (3). The reason the intensity of the initial systemic inflammatory response is able to delineate patient subpopulations with different prognoses is unknown. As suggested by our results, an intense systemic inflammatory reaction may reflect higher proinflammatory cytokine production. Higher cytokine production may be constitutive in some patients, caused by more widespread inflammatory lesions; by a more sustained, self-perpetuating, inflammatory response; or due to a combination of these or other factors. The intensity of the acute phase response probably reflects upstream cytokine and growth factor production, which influences vessel permeability and remodeling and determines the fate of inflammatory lesions in GCA (30–35). In this regard, we have previously shown that patients with strong systemic inflammatory reactions have more striking inflammation-induced angiogenesis and expression of endothelial adhesion molecules for leukocytes in their lesions (36,37). Taken together, our data suggest that some patients would develop an obliterative, self-limiting disease with high risk of vessel occlusion and ischemic events, whereas other patients would develop a chronic self-perpetuating disease. In the latter, continuous release of unknown mediators would prevent vessel occlusion, and neovessels would, at distal sites, compensate for ischemia but, at the same time, would continue recruiting leukocytes through adhesion molecule expression. The intensity of acute phase response, although probably an epiphenomenon derived from more directly related upstream events, would be able to distinguish between these 2 disease patterns.

Our data suggest that different cytokine production

might, at least partially, account for these 2 different disease patterns. Both IL-6 and TNFα levels correlated with the intensity of the inflammatory response in our patients. TNFα is upstream of IL-6 production in many macrophage responses and it is one of its major inducers (4,5,38,39). Perhaps TNFα blockade, which appears to be promising in several chronic inflammatory disorders including rheumatoid arthritis and inflammatory bowel disease (40–43), and which is currently being tested in other vasculitides such as Wegener's granulomatosis (44), might also be of help for GCA patients, particularly those with strong systemic inflammatory response and high TNFα who, according to our results, have higher and longer corticosteroid requirements.

ACKNOWLEDGMENT

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CONTRIBUTION FROM THE FIELD

Treatment With Statins Does Not Exhibit a Clinically Relevant Corticosteroid-Sparing Effect in Patients With Giant Cell Arteritis

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Introduction

Giant cell arteritis (GCA) is a chronic granulomatous vasculitis preferentially targeting large- and medium-sized vessels in aged people. The inflammatory lesions eventually lead to vessel occlusion and ~15% of patients develop cranial ischemic complications, particularly visual loss (1,2).

The granulomatous nature of GCA lesions, with the frequent presence of multinucleated giant cells, has classically suggested a delayed-type hypersensitivity reaction but the potential triggering agents remain unknown (1–3). CD4 T cells infiltrating the vessel wall display a T helper type 1 functional differentiation with copious production of interferon γ (IFNγ), a major cytokine in macrophage activation (3). Activated macrophages produce angiogenic factors and proinflammatory cytokines, such as interleukin-1β, tumor necrosis factor α, and interleukin-6 (IL-6) (2,3). These mediators amplify the inflammatory response by inducing endothelial cell adhesion molecules for leukocytes and chemokines, and by generating new vessels through which additional leukocytes may subsequently invade the vessel wall (2,4–7). Macrophages also participate in tissue destruction by producing oxidative damage and secreting metalloproteases, and in tissue repair by secreting fibrogenic cytokines that eventually may lead to vessel occlusion with its ensuing ischemic complications (3).

Corticosteroids are, at present, the treatment of choice for patients with GCA (1–3). Although their ability to modify the course of the disease or to cure it is questionable, corticosteroids induce dramatic functional changes in GCA lesions both in humans and in human arteritis–severe combined immunodeficient mouse chimeras (4,8,9). These functional changes result in a rapid relief of symptoms and prevention of ischemic complications. However, therapeutic requirements are highly variable among patients. Some patients achieve persistent remission of the disease within a few months, whereas others present recurrent relapses and need maintenance doses of corticosteroids for long periods of time.

Sustained corticosteroid therapy has been associated with the development of dyslipidemia by inducing insulin resistance, increasing the hepatic synthesis of very low-density lipoproteins and triglyceride, enhancing the activity of hydroxymethylglutaryl-coenzyme A (HMG-CoA) reductase, and inhibiting lipoprotein lipase. Moreover, studies in vitro have demonstrated that corticosteroids inhibit the activity of the low-density lipoprotein (LDL) receptor leading to an increase in LDL levels in patients (10–13). For that reason, some patients diagnosed with GCA receiving corticosteroid therapy may require lipid-lowering agents during their followup.

HMG-CoA reductase inhibitors, statins, are widely and effectively used as hypolipidemic agents. They competitively inhibit HMG-CoA reductase, the rate-limiting enzyme in cholesterol biosynthesis. Treatment with statins results in a reduction of the cholesterol levels through a decrease in cholesterol synthesis and by increasing the expression of hepatic LDL receptors, which clear LDL and LDL precursors from the bloodstream (14). Numerous clinical trials have demonstrated that statins reduce coronary heart disease mortality and the incidence of cardiovascular events (14–16). Recently, it has been demonstrated that statins also have antiinflammatory properties, through various mechanisms (14). The effect of statins on the inflammatory component of atherosclerosis is considered to be an important mechanism through which statins reduce cardiovascular events and death (14,17,18). Furthermore, statins have demonstrated to be of therapeutic benefit in animal models of chronic inflammatory conditions (19–21) and have been shown to reduce graft rejection in heart

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and kidney transplantation in humans, even at low or moderate doses (22).

The objective of this study was to evaluate whether concomitant use of statins had any significant impact in the outcome of patients with GCA in terms of corticosteroid requirements, frequency of relapses, and disease activity markers.

Patients and Methods

The study group consisted of 54 biopsy-proven GCA patients (38 women and 16 men) aged 76 years (range 57–91 years) selected among those diagnosed during the past 7 years. Patients eligible were those who had not received statins at all (group 1) and those who had received statins for at least 12 months during GCA treatment, starting before the diagnosis of GCA or during the first year of corticosteroid therapy (group 2). Patients who did not fit into these categories were excluded. Although this study is retrospective in design, these patients were prospectively evaluated at baseline as part of other ongoing studies and they all were treated by the authors according to uniform criteria. Patients included were treated with corticosteroids at an initial prednisone dosage of 1 mg/kg/day (up to 60 mg/day) for 1 month. Subsequently, prednisone was tapered by 10 mg/week. Reduction below 20 mg/day was slower and individualized. A further reduction to a maintenance dose of 10 mg/day was attempted over a 2-month period. If tolerated, a reduction to 7.5 mg/day was tried after ~3 months. If the erythrocyte sedimentation rate (ESR) rose to >40, corticosteroid dose was held. If disease-related symptoms (cranial manifestations or polymyalgia rheumatica), persistent malaise, or anemia occurred, the prednisone dosage was increased by 10 mg/day above the previous effective dose. All patients had a sustained and regular followup for an average of 2.8 years (range 9 months to 6.7 years).

Variables recorded were time (in weeks) required to reach a prednisone maintenance dosage <10 mg/day, not followed by a relapse during at least 3 months, and cumulative dose of prednisone received at that point. ESR and serum C-reactive protein (CRP) were determined at baseline evaluation, before starting therapy, and serially during followup.

Student's *t*-test was used for statistical comparison between quantitative variables. Fisher's exact test was employed for contingency tables. Time required to achieve a maintenance prednisone dosage <10 mg/day was compared between group 1 and group 2 by the Kaplan-Meier survival analysis method.

Results

Thirty-seven patients did not receive statins at all during the followup period (group 1) and 17 received statins for 2.7 years (range 1–6.5 years; group 2). The type and dosages of statins received are displayed in Table 1. No evidence of secondary adverse effects attributable to statins was observed during the followup.

No differences in age, sex, clinical manifestations, or disease duration before diagnosis were observed between the groups (Table 2). They also had a similar intensity of

Table 1. Type and dose of statins used

Simvastatin, 10–40 mg: 7 patients
Lovastatin, 10 mg: 3 patients
Atorvastatin, 10 mg: 4 patients
Pravastatin, 10 mg: 3 patients

the initial systemic inflammatory response assessed by clinical or analytic parameters (Tables 2 and 3). This is important because patients with a weak acute-phase response may require less corticosteroid (23).

No differences in corticosteroid requirements were found between both groups: patients who had not received statins required a median of 27 weeks (95% confidence interval [95% CI] 22–32) to reach a maintenance prednisone dosage <10 mg/day, and patients who had received statins required a median of 40 weeks (95% CI 21–59, $P = 0.39$; Figure 1). The mean ± SD cumulative dose of prednisone received until the patients reach a maintenance dose of <10 mg/day was 5.7 ± 2.3 gm in group 1 and 5.81 ± 2.1 gm in group 2 ($P = 0.87$). Nineteen (51.3%) patients in group 1 relapsed during the first year compared

Table 2. Clinical findings in patients not receiving statins (group 1) or under therapy with statins (group 2)

Clinical characteristics	Group 1 (n = 37)	Group 2 (n = 17)
General characteristics:		
Age, mean (range) years	76 (58–91)	75 (57–86)
Sex, no. male/female	10/27	6/11
Duration of symptoms, mean (range) weeks	17 (1–96)	24 (2–104)
Followup time, mean (range) months	33 (9–81)	33 (12–78)
Cranial symptoms (%)		
Headache	86.4	88.2
Jaw claudication	45.9	47
Scalp tenderness	51.3	64.7
Facial pain	24.3	29.4
Ischemic events (%)		
Amaurosis fugax	10.8	5.8
Permanent visual loss	16.2	5.8
Diplopia	10.8	5.8
Systemic manifestations (%)		
Fever	32.4	35.2
Polymyalgia rheumatica	43.2	41.1
Weight loss	35.1	52.9

Table 3. Baseline laboratory parameters reflecting acute phase response in patients not receiving statins (group 1) and those under statin therapy (group 2)*

Parameter	Group 1 (n = 37) mean (range)	Group 2 (n = 17) mean (range)
ESR, mm/hour	89 (28–131)	87 (24–130)
CRP, mg/dl	7.4 (0.5–30.4)	7.9 (0.4–20.2)
Albumin, gm/liter	35 (24–44)	35 (28–41)
Hemoglobin, gm/dl	11.2 (8.3–14.5)	11.9 (9.6–14)
Haptoglobin, gm/liter	3.87 (1.72–7.49)	3.77 (1.87–6.77)

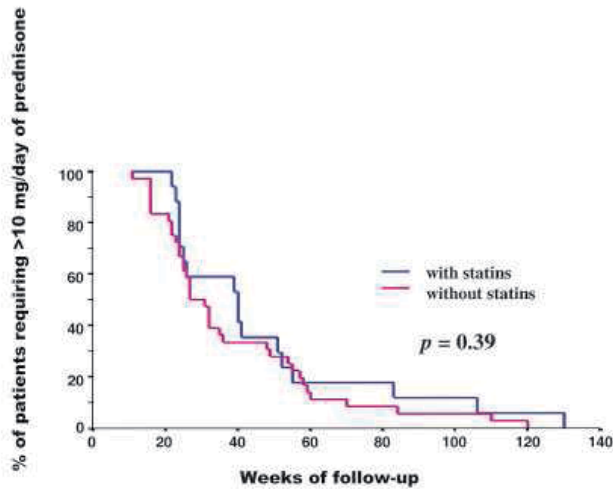* ESR = erythrocyte sedimentation rate; CRP = C-reactive protein.

Figure 1. Percentage of giant cell arteritis patients achieving a maintenance prednisone dose <10 mg/day over time.

with 5 (29.4%) in group 2 (odds ratio [OR] 2.4, 95% CI 0.71−8.15, *P* = 0.24). Six (16.2%) patients in group 1 and 3 (17.6%) in group 2 suffered more than 1 relapse during the first year (OR 0.88, 95% CI 0.19−4.01, *P* = 1). As shown in Figures 2 and 3, ESR and CRP values were equivalent in both groups, at baseline evaluation and at every time point during followup.

Discussion

Although corticosteroids induce a rapid remission of clinical manifestations in GCA, 30−50% of patients have disease exacerbations during corticosteroid tapering, especially during the first 2 years (1,2). Population-based analyses have shown that only 50% of patients are able to successfully discontinue therapy after 2 years (24−28). The numerous complications associated with corticosteroid therapy (24−28) have urged the pursuit of corticosteroid-sparing agents. The use of immunosuppressive agents has been assayed in isolated cases and uncontrolled small series (29−31), with the exception of methotrexate, which has been tried in 3 double-blind, placebo-controlled trials with variable results (32−34).
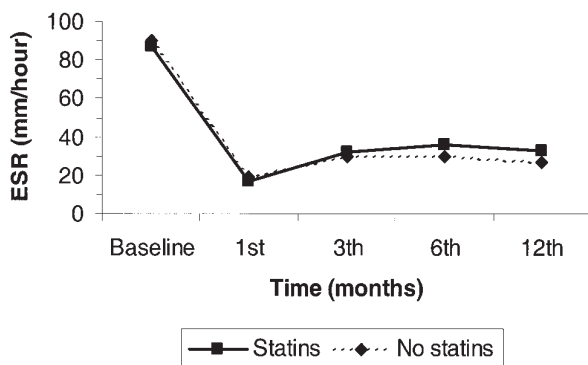

Figure 2. Serial determination of erythrocyte sedimentation rate (ESR) during followup in giant cell arteritis patients receiving or not receiving statins.
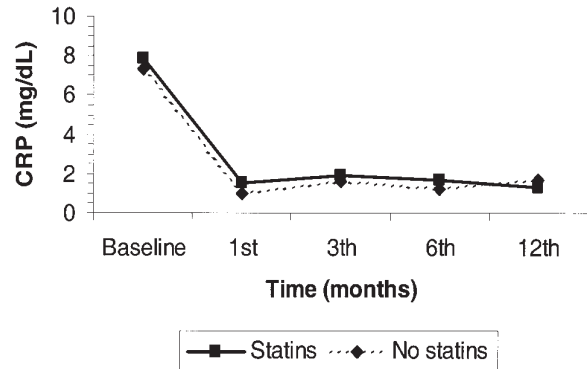

Figure 3. Serial measurement of serum C-reactive protein (CRP) in giant cell arteritis patients during followup with and without statins.

As mentioned, HMG-CoA reductase inhibitors, statins, have antiinflammatory and immunosuppressive properties (14,21,35). Statins decrease major histocompatibility complex class II antigen expression induced by IFNγ in a variety of cells and decrease T cell activation and proliferation (19,35). Statins are able to block interactions between lymphocyte integrin lymphocyte function–associated antigen 1 (LFA-1) and its counterreceptor intercellular adhesion molecule 1 by locking LFA-1 integrin in a nonadherent conformation status (36); they are also able to downregulate endothelial adhesion molecules (37). Given the important role of LFA-1 in contact-dependent T-cell activation and lymphocyte−endothelial cell interactions required for lymphocyte transmigration into tissues, this effect may have immunodepressive and antiinflammatory consequences. In addition to these experimental observations, treatment of humans with statins actually results in a decrease of circulating molecules that are known to participate in inflammatory reactions, such as soluble adhesion molecules (P-selectin) chemokines (IL-8 and monocyte chemotactic protein 1), and acute phase proteins (CRP) (38−40). In addition, statins also have effects on vessel wall components. Statins restore endothelial cell function assessed by nitric oxide production and decrease smooth muscle cell proliferation and its ensuing intimal hyperplasia (14,18). Consequently, statins influence a variety of immune pathways, inflammatory cascades, and vascular responses to inflammatory mediators that are known to play a significant role in the pathogenesis of GCA (2−4,6,7).

We did not observe a significant benefit derived from the use of statins on disease outcome in patients with GCA. However, the conclusions of our study cannot be considered definitive given that it has several limitations. Although all statins used had proven effects on inflammation in other experimental conditions and were administered in doses within the therapeutic range, the type and dose of statins, as well as the duration of treatment, were variable among patients. In addition, the doses used were in the low to moderate range. Furthermore, even though these patients were prospectively evaluated at baseline and prospectively followed as part of other research projects, this study was not randomized and was retrospective in de-

sign. Its retrospective nature, however, guarantees that therapeutic decisions in terms of corticosteroid tapering were not biased by the knowledge of whether or not patients were receiving statins. In spite of these limitations, our data do not suggest that concomitant use of statins during the first year of treatment has a clinically relevant impact on disease outcome in individuals with GCA receiving corticosteroids. However, we cannot exclude that at higher doses or with a more aggressive corticosteroid tapering schedule, statins may show some clinically apparent beneficial effects on disease activity and disease duration in these patients. Yet, a potential benefit in later stages of the disease (allowing an earlier corticosteroid withdrawal in patients in sustained remission with low corticosteroid doses) cannot be excluded from our data. These possibilities deserve further investigation with prospective, randomized trials.

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Infliximab for Maintenance of Glucocorticosteroid-Induced Remission of Giant Cell Arteritis
A Randomized Trial

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Background: Tumor necrosis factor-α is present in arteries in giant cell arteritis.

Objective: To evaluate the efficacy of infliximab, an anti–tumor necrosis factor-α agent, in giant cell arteritis.

Design: Randomized, controlled trial.

Setting: 22 sites in the United States, the United Kingdom, Belgium, Italy, and Spain.

Patients: 44 patients with newly diagnosed giant cell arteritis that was in glucocorticosteroid-induced remission.

Intervention: Participants were randomly assigned in a 2:1 ratio to receive infliximab (5 mg/kg of body weight) or placebo. Sixteen patients were assigned to glucocorticosteroid plus placebo, and 28 patients to glucocorticosteroid plus infliximab.

Measurements: End points were measured through week 22, when an interim analysis resulted in early stopping of the planned 54-week trial. Primary end points were the number of patients who remained free of relapse through week 22 and adverse events. Secondary end points were time to first relapse, biomarkers, cumulative glucocorticosteroid dose, and the number of patients who remained relapse-free while the glucocorticosteroid dosage was tapered to 10 mg/d.

Results: Infliximab therapy did not increase the proportion of patients without relapse at week 22 compared with placebo (43% vs. 50%, respectively; difference, −7 percentage points [95% CI, −38 to 23 percentage points; $P = 0.65$]), nor did it increase the proportion of patients whose glucocorticosteroid dosages were tapered to 10 mg/d without relapse (61% vs. 75%, respectively; difference, −14 percentage points [CI, −42 to 14 percentage points]; $P = 0.31$). The incidence of infection was 71% with infliximab and 56% with placebo (difference, 15 percentage points [CI, −14 to 45 percentage points]).

Limitations: The sample was too small to rule out modest effects of infliximab and included only patients with a new diagnosis. Only one dose of infliximab was evaluated, and the study was terminated early.

Conclusions: This trial is too small to draw definitive conclusions, but it provides evidence that using infliximab as maintenance therapy in patients in glucocorticoid-induced remission of newly diagnosed giant cell arteritis is of no benefit and may be harmful. If infliximab has benefit, it is unlikely to be great.

In northern Europe and North America, the estimated annual incidence of giant cell arteritis is 19 to 32 cases per 100 000 persons older than 50 years of age. In Mediterranean countries, the annual incidence appears to be lower: 6 to 10 cases per 100 000 persons (1–5). Treatment with glucocorticosteroids dramatically alters the symptoms and course of giant cell arteritis, reducing the likelihood that the patient will develop blindness (6, 7). However, relapses usually occur when glucocorticosteroid dosages are tapered, resulting in frequent re-treatment and glucocorticosteroid dependence and toxicity (8–10). Approximately 80% of patients with giant cell arteritis will eventually experience at least 1 adverse event attributable to glucocorticosteroids, and about 60% will have 2 or more adverse events. Compared with age- and sex-matched controls, patients with giant cell arteritis have an increased risk for fractures and corticosteroid-related cataracts (9).

Adjunctive treatments are needed that would effectively reduce the dose and duration of glucocorticosteroid therapy and provide more durable remissions of giant cell arteritis. Other investigators have evaluated the utility of cytotoxic and anti-inflammatory agents in giant cell arteritis. However, the reports have been anecdotal, of uncontrolled studies, or of controlled studies with conflicting results in terms of efficacy (11, 12).

Increased knowledge of cell types and mediators within vessels damaged by giant cell arteritis has led to speculation about the potential therapeutic role of several cytokine antagonists. Interleukin-1, interleukin-6, tumor necrosis factor (TNF)–α, and interferon-γ have been implicated in contributing to vascular injury in patients with giant cell arteritis (13–16). Published case studies reported

Context

Up to 80% of patients with giant cell arteritis (GCA) experience complications related to glucocorticoid therapy. Case reports suggest that patients with GCA who received infliximab achieved sustained disease remission and independence from glucocorticoids.

Contribution

Patients with glucocorticoid-induced GCA remission were randomly assigned to infusions of infliximab, 5 mg/kg, or placebo at 0, 2, and 6 weeks and every 8 weeks thereafter. The investigators found that infliximab did not reduce rates of relapse or any secondary end point.

Caution

The study was small and stopped early (after week 22 of the planned 54 weeks), so it could not definitively identify harms or small benefits.

Implication

Infliximab is unlikely to cause large reductions in rates of relapse of GCA.

—*The Editors*

that some patients with giant cell arteritis or polymyalgia rheumatica who received the anti–TNF-α agent infliximab had sustained remission and became glucocorticosteroid-independent (17, 18). However, the investigators cautioned that randomized, controlled studies were needed to validate these results.

We report the results of the first randomized, placebo-controlled, double-blind, multicenter trial of standardized treatment with glucocorticosteroids and adjunctive treatment with placebo or infliximab in patients with newly diagnosed giant cell arteritis.

METHODS

Design

We designed a multicenter, randomized, double-blind, placebo-controlled study to determine whether infliximab added to a standardized program of glucocorticosteroid therapy (equivalent daily doses of prednisone or prednisolone) in patients with newly diagnosed giant cell arteritis would decrease the frequency of relapse, cumulative glucocorticosteroid requirement, and glucocorticosteroid-associated toxicity. The study protocol was approved by the institutional review boards or ethics committees of the individual study sites. The study was conducted according to the current regulations of the U.S. Food and Drug Administration, the International Conference on Harmonization guidelines, and the principles of the Declaration of Helsinki. All patients provided written informed consent before participating in any protocol-specific procedures. An independent safety monitoring committee reviewed safety information during the trial. The first patient was

enrolled on 22 October 2003, and the last patient completed the study on 29 July 2005.

The primary objective was to obtain preliminary evidence on the safety and efficacy of infliximab therapy in patients with glucocorticoid-induced remission of newly diagnosed giant cell arteritis, as measured by the proportion of patients who were relapse-free through week 22 and the incidence of adverse events. The secondary objective was to further evaluate the preliminary evidence of the efficacy of infliximab therapy, as measured by the proportion of patients who remained relapse-free through week 54, time to first relapse, levels of biochemical markers of inflammation and disease activity, and cumulative dose of glucocorticosteroids.

Setting

The study was conducted at 22 sites in the United States, United Kingdom, Belgium, Italy, and Spain.

Participants

To be eligible for the study, patients must have had a diagnosis of giant cell arteritis within 4 weeks of enrollment, satisfied the American College of Rheumatology criteria for giant cell arteritis (19), had an erythrocyte sedimentation rate 40 mm or greater in the first hour at the time of diagnosis, and achieved clinical remission before randomization. For at least 1 week before randomization, patients were required to be receiving prednisone or prednisolone at a stable dosage of 40 to 60 mg/d, have a normal erythrocyte sedimentation rate ($<$40 mm in the first hour, as determined by using the Westergren method), and have no symptoms or signs of active giant cell arteritis.

Patients were excluded if they had received a diagnosis of giant cell arteritis or polymyalgia rheumatica more than 4 weeks before screening, did not respond to glucocorticosteroid therapy within 5 days of initiation of therapy, received intravenous glucocorticosteroid therapy with an equivalent dose of methylprednisolone ($>$1000 mg/d for $>$3 days), or received other forms of immunosuppressive therapy (such as methotrexate, azathioprine, or other cytotoxic agents) or any investigational or biological agents within the 3 months before screening. Patients with screening blood test results within the following ranges were also excluded: leukocyte count less than 3.5×10^9 cells/L, neutrophil count less than 1.5×10^9 cells/L, hemoglobin level less than 85 g/L, platelet count less than 100×10^9 cells/L, or hepatic aminotransferase or alkaline phosphatase levels greater than 3 times the upper limit of normal. We excluded patients with serious or chronic infections in the previous 3 months; opportunistic infections within the 6 months before screening; cancer within the 5 years before screening (with the exception of treated and cured squamous or basal cell carcinoma of the skin); a history of severe congestive heart failure or demyelinating disease; current signs or symptoms of severe, progressive, or uncontrolled renal, hepatic, hematologic, gastrointestinal, endocrine, pulmonary, cardiac, neurologic, or cerebral disease; a

transplanted organ (with the exception of corneal transplantation done more than 3 months before screening); or evidence of active or previous tuberculosis.

Randomization and Intervention

Patients were randomly assigned in a 2:1 ratio to receive infliximab, 5 mg/kg, or placebo by using adaptive treatment allocation (20, 21) stratified by baseline glucocorticosteroid dosage (40 to 50 mg/d or 51 to 60 mg/d prednisone equivalent). Patients received infusions at weeks 0, 2, and 6 and every 8 weeks thereafter. Allocation to treatment group was performed by using a central randomization procedure through an interactive voice response system. Patients, investigators, and study personnel were blinded to treatment assignments during the study; the site pharmacists, who prepared study medication, were not blinded to this information.

Infliximab and placebo were supplied as sterile, white, lyophilized powders that were reconstituted with sterile water for injection. The reconstituted placebo solution contained the same excipients as the infliximab solution but did not contain infliximab.

Glucocorticosteroid dosages were tapered according to a predefined schedule (**Table 1**). Each week, the daily dose of prednisone or prednisolone was decreased by 10 mg until the dosage reached 20 mg/d. It was then tapered by 2.5 mg until it reached 10 mg/d and then by 1 mg until the dosage was 0 mg/d. In the absence of a relapse, this schedule results in a glucocorticosteroid dosage of 10 mg/d after 4 months and no glucocorticosteroid use after 6 months. If a relapse occurred, the patient was to resume treatment with the previous higher dose of prednisone or prednisolone that provided disease remission, plus 10 mg/d. If the relapse resolved within 72 hours, the patient was to continue receiving that dosage for 2 weeks and then resume tapering according to the protocol. If relapse did not resolve within 72 hours, the patient was to receive another increase of 10 mg and resume treatment according to the protocol.

If relapse included visual symptoms, the patient was to receive at least 40 mg/d or the previous higher dosage of prednisone or prednisolone, plus 10 mg (whichever was higher). If the visual symptoms improved within 48 hours, the patient was to resume tapering according to the protocol above. If the visual symptoms did not resolve within 48 hours, the patient's vision was threatened, or there was concern about any other catastrophic event, the investigator was to take any measures necessary according to clinical judgment to treat the patient, including but not limited to increasing the glucocorticosteroid dosage to more than 60 mg/d. If a patient received more than 60 mg of oral prednisone or prednisolone daily or more than 1000 mg of intravenous glucocorticosteroid daily for more than 3 days, study infusions were discontinued, but the patient continued to return for study visits.

Table 1. **Schedule of Dosage Tapering for Glucocorticosteroid Therapy***

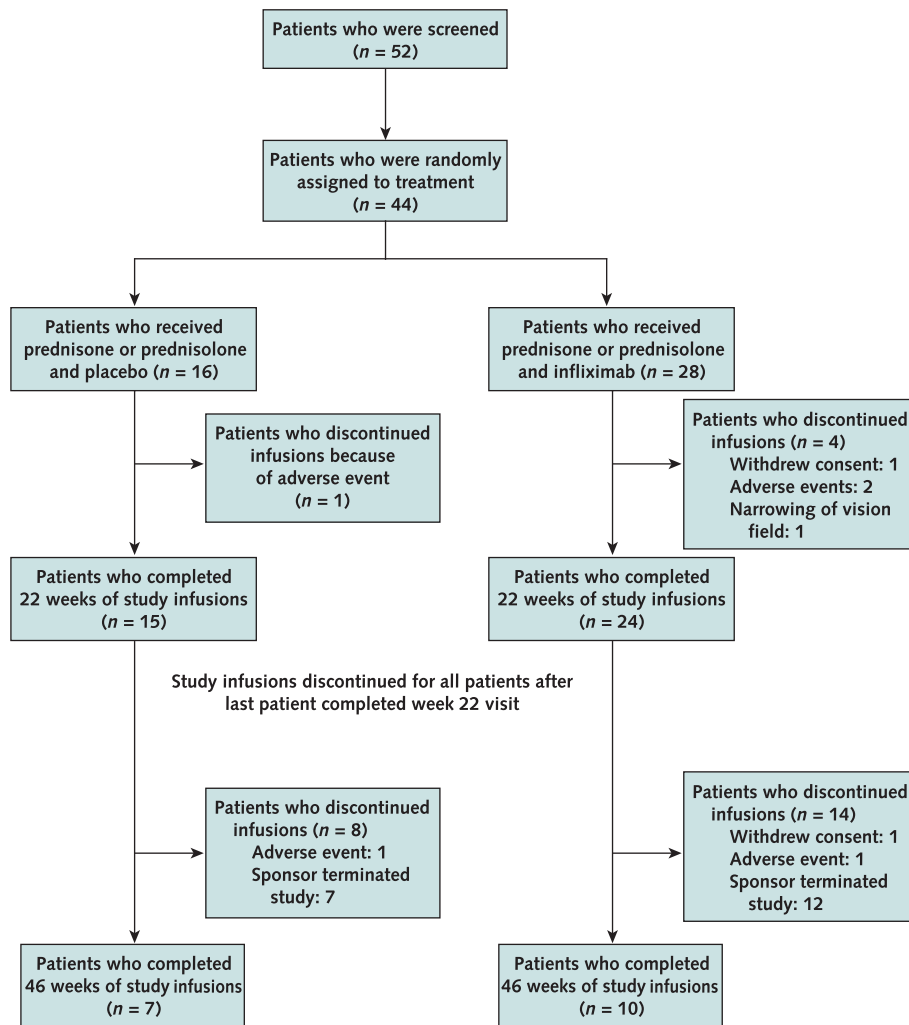| Week	Starting Glucocorticosteroid Dosage, *mg/d*		
	40	**41–50**	**51–60**
0	40	41–50	51–60
1	40	41–50	51–60
2	30	40	50
3	20	30	40
4	20	20	30
5	17.5	20	20
6	17.5	17.5	20
7	15	17.5	17.5
8	15	15	17.5
9	12.5	15	15
10	12.5	12.5	15
11	10	12.5	12.5
12	9	10	12.5
13	8	9	10
14	7	8	9
15	6	7	8
16	5	6	7
17	4	5	6
18	3	4	5
19	2	3	4
20	1	2	3
21	0	1	2
22		0	1
23	–	–	0
24	Discontinued before week 24		

* Prednisone or prednisolone.

Outcomes and Measurements

Disease relapse was defined as an increase in erythrocyte sedimentation rate from normal to 40 mm or greater in the first hour, plus at least 1 symptom or sign of giant cell arteritis: sustained fever (temperature > 100.4 °F [38 °C] for > 1 week) that was not attributable to a cause other than giant cell arteritis; new or recurrent headache or pain or tenderness of the scalp; new, recurrent, or worsening ischemic retinopathy, optic neuropathy, or visual loss not attributable to other causes; new or recurrent pain or claudication of the tongue or jaw; new or recurrent claudication of the extremities; new, recurrent, or worsening thickness, tenderness, or ulcers or nodules over the temporal or occipital arteries; new, recurrent, or worsening angiographic abnormalities compatible with vasculitis of the aorta or its primary branches; new, recurrent, or worsening transient cerebral ischemia or stroke not attributable to cardiac arrhythmias or atherosclerotic disease; or new, recurrent, or worsening classic polymyalgia rheumatica–like symptoms, including malaise and fatigue that were unexplained by processes other than giant cell arteritis. In addition, patients with symptoms or signs of giant cell arteritis other than those listed above that could not be attributed to any cause other than giant cell arteritis and that were accompanied by an increase in the dose of glucocorticosteroids used to treat giant cell arteritis were considered to have had relapse.

Clinical remission was defined as an erythrocyte sedi-

Figure 1. Study flow diagram.

Patients who were screened
(*n* = 52)

Patients who were randomly
assigned to treatment
(*n* = 44)

Patients who received
prednisone or prednisolone
and placebo (*n* = 16)

Patients who received
prednisone or prednisolone
and infliximab (*n* = 28)

Patients who discontinued
infusions because
of adverse event
(*n* = 1)

Patients who discontinued
infusions (*n* = 4)
Withdrew consent: 1
Adverse events: 2
Narrowing of vision
field: 1

Patients who completed
22 weeks of study infusions
(*n* = 15)

Patients who completed
22 weeks of study infusions
(*n* = 24)

Study infusions discontinued for all patients after
last patient completed week 22 visit

Patients who discontinued
infusions (*n* = 8)
Adverse event: 1
Sponsor terminated
study: 7

Patients who discontinued
infusions (*n* = 14)
Withdrew consent: 1
Adverse event: 1
Sponsor terminated
study: 12

Patients who completed
46 weeks of study infusions
(*n* = 7)

Patients who completed
46 weeks of study infusions
(*n* = 10)

mentation rate less than 40 mm in the first hour and lack of the symptoms or signs of giant cell arteritis. Complete clinical remission was defined as maintenance of clinical remission for 12 weeks after discontinuation of glucocorticosteroid therapy. C-reactive protein was evaluated by using the Tinaquant assay (Roche, Indianapolis, Indiana) (normal range, 0 to 6 mg/L). Interleukin-6 was evaluated by using assays from R&D Systems (Minneapolis, Minnesota) (normal range, 0.45 to 9.96 ng/mL). All laboratory tests were done in a single batch by a central laboratory. Antibodies to infliximab, antinuclear antibodies, and antibodies to double-stranded DNA were evaluated at baseline, week 22, and 20 weeks after the last dose of study medication, by using a method described elsewhere (22). Antibodies to infliximab were assessed by measuring the optical density of antibodies in serum samples, using a double-antigen enzyme immunoassay in which infliximab served as the detection and capture reagent (23). Because the presence of infliximab in serum samples can interfere with de-

tection of antibodies to infliximab, samples were classified as inconclusive when infliximab levels in patient sera were greater than 0.1 μg/mL.

At each site, one clinician-investigator provided comprehensive care for an individual patient. A second independent physician-assessor (who did not have access to any other clinical information on the patient) evaluated the patient at each study visit and indicated on standardized forms whether symptoms or signs of giant cell arteritis were present or absent. Both physicians were blinded to treatment group assignment. Patients who discontinued study infusions were to be followed, according to the study schedule, for clinical and safety assessments.

Statistical Analysis

Primary study end points were the proportion of patients who remained relapse-free through week 22 and the incidence of adverse events. Secondary end points included the proportion of patients who remained relapse-free

through week 54, time to first relapse, levels of biochemical markers of inflammation and disease activity (erythrocyte sedimentation rate, C-reactive protein level, and interleukin-6 level), cumulative dose of glucocorticosteroid, the proportion of patients who remained relapse-free during tapering of the glucocorticosteroid dosage to 10 mg/d, and the duration of complete clinical remissions beyond week 22.

The Cochran–Mantel–Haenszel 2-sided chi-square test at a 5% level of significance with stratification by baseline prednisone or prednisolone dosage (40 to 50 mg/d or 51 to 60 mg/d) was used for the efficacy analysis. An intention-to-treat analysis was performed. Patients who discontinued treatment before week 22 because of lack of efficacy were considered to have had relapse. Patients who did not return for an evaluation or for whom data were insufficient to assess whether they were relapse-free before week 18 were considered to have had relapse. Patients who were relapse-free through evaluations at week 18 but who did not have sufficient evaluations at week 22 were considered to have achieved the primary end point (the week 18 value was carried forward). Patients whose glucocorticosteroid dose was increased to treat giant cell arteritis were considered to have had relapse because they did not follow

the glucocorticosteroid dose-tapering schedule; this was called an "analytical relapse." A Kaplan–Meier analysis was used to estimate the proportion of patients who remained relapse-free through week 22.

The study was designed with a planned sample size of 42 patients (14 in the placebo group and 28 in the infliximab group). The power calculations were based on a chi-square test with no stratification and a type I error rate of 5%. The power of the study was expected to be greater than 80% if the relapse-free response rate was approximately 80% in the infliximab group and approximately 30% in the placebo group (11, 12).

A prespecified interim safety and efficacy analysis was performed by 1 of the authors after the last enrolled patient completed the week 22 study visit. The objective of the interim analysis was to aid in directing the clinical development program. The primary and major secondary end points were examined, although the specific end points to be examined were not prespecified. No formal stopping rules were prespecified for the interim analysis because the results were not expected to affect the conduct of the study. The independent safety monitoring committee was not involved in the interim analysis.

Table 2. Demographic and Clinical Characteristics of Patients at Randomization*

Characteristic	Placebo Group (n = 16)	Infliximab Group (n = 28)
Women, n (%)	11 (69)	24 (86)
White persons, n (%)	16 (100)	28 (100)
Median age (IQR), y	69.5 (65.0–77.0)	71.5 (66.0–75.5)
Median body weight (IQR), kg	67.8 (59.6–72.1)	66.9 (57.3–73.6)
Symptoms and signs of relapse of giant cell arteritis, n (%)		
Fever	8 (50)	5 (18)
Headache, or pain or tenderness of the scalp or temporal artery	14 (88)	21 (75)
Visual impairment	5 (31)	2 (7)
Pain or claudication of the tongue or jaw	7 (44)	12 (43)
Polymyalgia rheumatica–like symptoms	6 (38)	8 (29)
Extremity claudication	0 (0)	2 (7)
Angiographic abnormalities	1 (7)	1 (4)
Transient cerebral ischemia or stroke	1 (6)	0 (0)
Positive temporal artery biopsy	10 (67)†	24 (92)‡
Median serum creatinine concentration (IQR)		
μmol/L	79.5 (61.9–88.4)	70.7 (61.9–79.5)
mg/dL	0.9 (0.7–1.0)	0.8 (0.7–0.9)
Median hematocrit (IQR)	0.38 (0.35–0.41)	0.39 (0.37–0.41)
Median erythrocyte sedimentation rate (IQR), mm/h		
At diagnosis of giant cell arteritis	76.0 (51.0–130.0)	79.0 (52.0–102.0)
At screening	41.5 (26.5–74.5)	49.5 (27.0–67.0)
At remission (week 0)	30 (22.0–44.5)	36 (18.0–50.0)
Median C-reactive protein level (IQR), mg/L§		
At screening	5 (4–18)	4 (4–10)
At remission (week 0)	6 (4–17)	5 (4–8)
Median interleukin-6 level (IQR), ng/L‖		
At screening	3.0 (1.3–11.8)	3.8 (1.6–5.0)
At remission (week 0)	4.1 (1.6–7.3)	3.2 (2.1–7.4)
Starting glucocorticosteroid dosage, n (%)		
40–50 mg/d	9 (56)	17 (61)
51–60 mg/d	7 (44)	11 (39)

* Values are those obtained at screening, unless otherwise specified. IQR = interquartile range.
† Percentage is based on 10 of 15 patients.
‡ Percentage is based on 24 of 26 patients.
§ Normal range, 0.0 to 0.6 mg/dL.
‖ Normal range, 0.45 to 9.96 ng/L.

Table 3. **Efficacy Outcomes at 22 Weeks**

Outcome	Placebo Group (*n* = 16)	Infliximab Group (*n* = 28)
Patients who remained relapse-free*		
Total, *n (%)*	8 (50)	12 (43)
Difference (95% CI), *percentage points*		−7 (−38 to 23)
P value		0.65†
Patients whose glucocorticosteroid dosage was tapered to 10 mg/d		
Total, *n (%)*	12 (75)	17 (61)
Difference (95% CI), *percentage points*		−14 (−42 to 14)
P value		0.31†
Cumulative glucocorticosteroid dose at week 22		
Mean (SD), *mg*	3049.56 (769.54)	3154.10 (968.50)
Median (interquartile range), *mg*	2909.3 (2502.5–3143.0)	2982.0 (2461.0–3630.0)
P value		0.95‡
Glucocorticosteroid dosage at first relapse		
Mean (SD), *mg/d*	11.8 (16.9)	13.4 (17.5)
Median (interquartile range), *mg/d*	6.5 (0.5–17.5)	10 (1.0–20.0)
P value		0.59‡
Signs and symptoms of relapse through week 22, *n (%)*§		
Sustained fever	0 (0)	0 (0)
New or recurrent headache or pain or tenderness of the scalp or temporal artery	7 (44)	8 (29)
New, recurrent, or worsening visual symptoms specific to giant cell arteritis	1 (6)	6 (21)
New or recurrent pain or claudication of the tongue or jaw	3 (19)	4 (14)
New or recurrent claudication other than of the tongue or jaw	1 (6)	2 (7)
New, recurrent, or worsening temporal artery signs and symptoms	3 (19)	4 (14)
New, recurrent, or worsening angiographic abnormalities	0 (0)	0 (0)
New, recurrent, or worsening transient cerebral ischemia	0 (0)	1 (4)
New, recurrent, or worsening classic polymyalgia rheumatica–like symptoms	3 (19)	4 (14)
Other symptoms specified by the individual assessor	1 (6.3)	5 (18)
Other related symptoms specified by the investigator	1 (6.3)	3 (10.7)

* One patient in the infliximab group who withdrew consent at week 14 and did not return for the week 22 visit was considered to have had relapse.
† Cochran–Mantel–Haenszel 2-sided chi-square test stratified by baseline glucocorticosteroid dosage (40 to 50 mg/d or 51 to 60 mg/d).
‡ Analysis of variance on the van der Waerden normal scores.
§ Statistical testing was not done for this post hoc analysis because of the small number of patients and the error rates of statistical inferences caused by multiple comparisons.

All statistical analyses were done by using SAS software, version 8.2 (SAS Institute, Inc., Cary, North Carolina).

Role of the Funding Source

This study was funded by Centocor Research and Development, Inc. The study was led by a steering committee (Drs. Hoffman, Cid, Rendt-Zagar, Weyand, Stone, and Rahman), which was primarily responsible for the design of the study, interpretation of the results, and preparation of the manuscript. Dr. Xu (Centocor Research and Development, Inc.) conducted the statistical analysis. Employees of Centocor Research and Development, Inc., were also involved in the design of the study, interpretation of the results, and preparation of the manuscript. Dr. Hoffman wrote the first draft of the manuscript. All authors reviewed, contributed revisions to, and approved the manuscript before submission.

RESULTS

Patient Characteristics

Forty-four patients were enrolled, 16 in the placebo group and 28 in the infliximab group (**Figure 1**). Thirty-four patients (83%) had findings on baseline temporal artery biopsy that were consistent with giant cell arteritis. Baseline demographic and disease characteristics of the treatment groups were similar, except that fever was more frequent in the placebo group than the infliximab group (50% vs. 18%; *P* = 0.040, 2-sided Fisher exact test) (**Table 2**). The difference between the placebo and infliximab groups in the frequency of temporal artery biopsies demonstrating giant cell arteritis was not statistically significant (67% vs. 92%; *P* = 0.079, 2-sided Fisher exact test).

Five patients discontinued treatment before the week 22 visit (4 in the infliximab group and 1 in the placebo group) (**Figure 1**). Four of these patients returned for the assessment visit at week 22; 1 patient in the infliximab group withdrew consent at week 14 and did not return for assessment.

Efficacy

The proportion of patients who were relapse-free through week 22 was similar between the placebo and infliximab groups (50% vs. 43%, respectively; *P* = 0.65) (**Table 3**). The groups did not differ in time to first relapse (**Figure 2**) or in interleukin-6 and C-reactive protein levels and erythrocyte sedimentation rates at first relapse (**Figure 3**).

Of the 24 patients who had relapse by week 22, 16 met the primary definition of relapse (an increase in the erythrocyte sedimentation rate ≥40 mm in the first hour and at least 1 of the signs or symptoms of giant cell arteritis

listed in the Methods section). Eight patients (4 placebo recipients and 4 infliximab recipients) had an analytical relapse, in that they did not meet the primary definition of relapse but their glucocorticosteroid dose was increased to treat giant cell arteritis. Of these 8 patients, 5 had signs and symptoms of giant cell arteritis but did not meet the erythrocyte sedimentation rate criterion in the primary definition, 2 met the erythrocyte sedimentation rate criterion but did not have a sign or symptom of giant cell arteritis, and 1 had neither a sign nor symptom nor met the erythrocyte sedimentation rate criterion. If patients with analytical relapse were considered to be relapse-free and only patients who met the primary definition were considered to have relapsed, 12 of 16 patients (75%) in the placebo group and 16 of 28 patients (57%) in the infliximab group would have been relapse-free through week 22.

The groups did not differ in the cumulative dose of prednisone or prednisolone at week 22 or the mean glucocorticosteroid dose at relapse (**Table 3**). Only 3 patients (25%) in the placebo group and 4 patients (17%) in the infliximab group were not receiving glucocorticosteroids at the time of relapse ($P = 0.34$).

The week 22 results were analyzed during the preplanned interim analysis. The study steering committee and sponsor reviewed the results of the interim analysis and determined that although infliximab was generally well tolerated and had no unexpected safety issues, it appeared to provide no therapeutic benefit. Therefore, the steering committee and the sponsor decided to discontinue study infusions for all patients. Each patient had a safety follow-up visit 4 weeks after infusions were stopped. Patients also had a visit 20 weeks after their last dose of infliximab to evaluate antibodies to infliximab and disease activity. One patient in the infliximab group withdrew consent at week 26; all other patients who completed the week 22 visit returned for all follow-up visits.

Figure 2 shows the time to first relapse. The results after week 22 should be interpreted with caution because some patients completed the 54-week study, whereas only limited data were available for others because the study was terminated prematurely.

The mean number of relapses per patient during the study was 1.7 (SD, 1.45) in the placebo group and 1.8 (SD, 1.66) in the infliximab group. In the placebo group, 4 patients (25%) had no relapse, 5 patients (31%) had 1 relapse, 1 patient (6%) had 2 relapses, and 6 patients had 3 or more relapses (38%). In the infliximab group, 5 patients (18%) had no relapse, 10 patients (36%) had 1 relapse, 8 patients (29%) had 2 relapses, and 5 patients (18%) had 3 or more relapses ($P = 0.23$). **Table 3** summarizes signs and symptoms of relapse.

Seven patients (44%) in the placebo group and 11 patients (39%) in the infliximab group achieved complete clinical remission (no sign of active giant cell arteritis for at least 12 weeks after discontinuation of prednisone or prednisolone therapy) ($P = 1.00$). The median duration of complete clinical remission was 20.3 weeks (interquartile range, 18.0 to 25.0 weeks) in the placebo group and 21.0 weeks (interquartile range, 18.6 to 32.3 weeks) in the infliximab group. Of the patients who achieved complete clinical remission, 6 (86%) in the placebo group and 8 (73%) in the infliximab group later had relapse.

Pharmacokinetics

The median trough serum infliximab concentration in the infliximab group at week 22 was 1.7 μg/mL (interquartile range, 0.0 to 5.1 μg/mL; range, 0.0 to 113.6 μg/mL).

Adverse Events

Patients in each group received an average of 7 study infusions, and the median total dose of infliximab was 35.0 mg/kg. The groups did not differ clinically or statistically in the frequency of adverse events or serious adverse events (**Table 4**). Although the incidence of infections was numerically higher among infliximab recipients than placebo recipients, the difference was not statistically significant (71% vs. 56%; difference, 15 percentage points [95% CI, −14 to 45 percentage points]). The incidence of infections requiring oral or parenteral antimicrobial treatment for each treatment group did not differ (57% of infliximab recipients vs. 50% of placebo recipients; difference, 7 percentage points [CI, −23 to 38 percentage points]). Four patients reported serious infections (3 [11%] in the infliximab group vs. 1 [6%] in placebo group; difference, 5 percentage points [CI, −12 to 21 percentage points]). The patient in the placebo group had ischemic colitis 13 days after the week 22 infusion. In the infliximab group, 1 patient had herpes keratitis in the right eye 35 days after the week 30 infusion, 1 had bronchitis 7 days after the week 2 infusion, and 1 had a *Staphylococcus aureus*–infected hema-
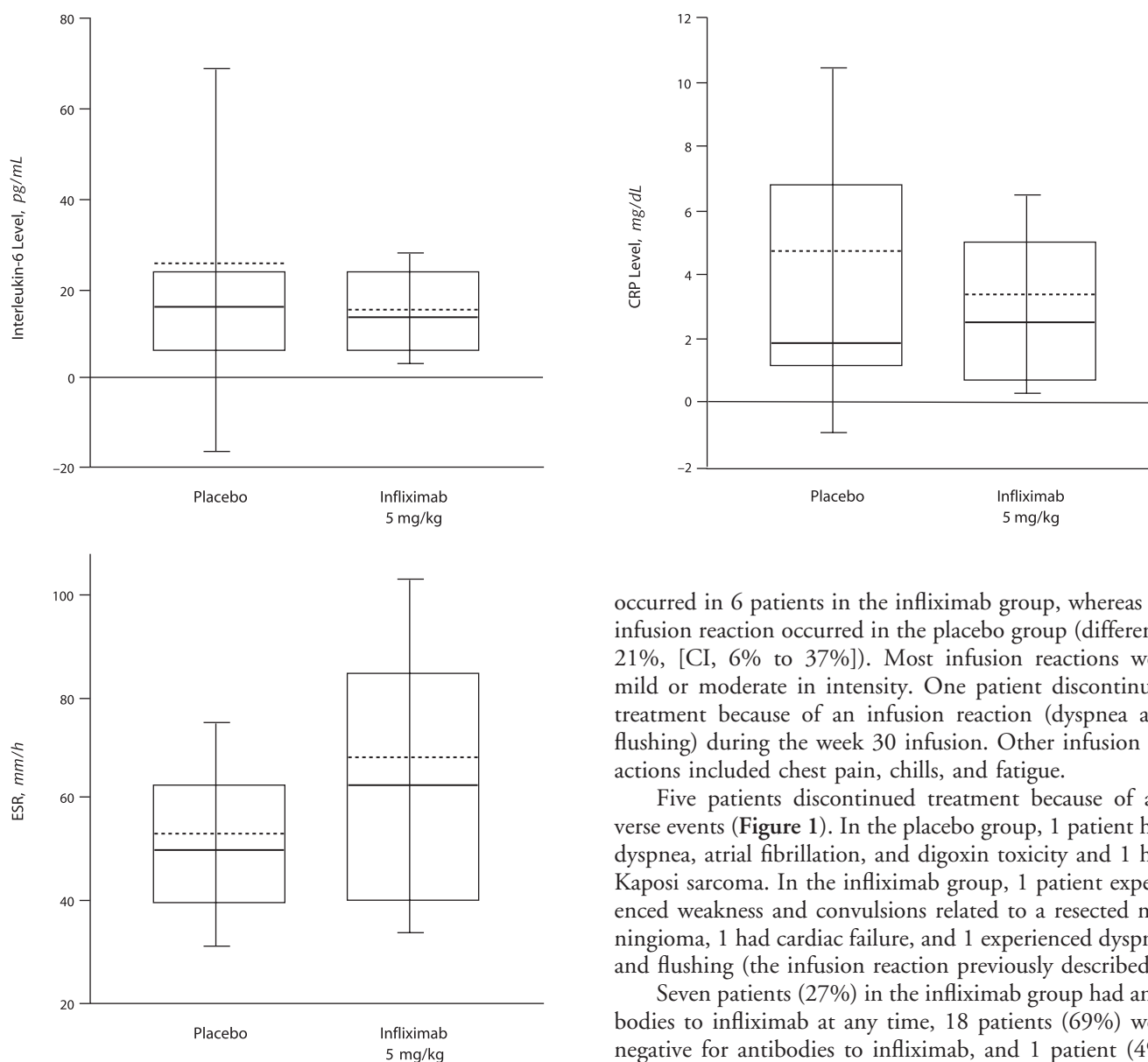
Figure 2. **Kaplan–Meier estimate of the proportion of patients who remained relapse-free through the end of the study.**
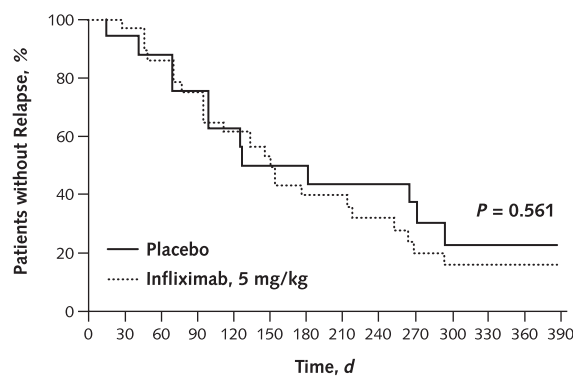
The groups did not differ significantly in the time to first relapse, according to a log-rank test.

Figure 3. Box plots showing interleukin-6 level, C-reactive protein (*CRP*) level, and erythrocyte sedimentation rate (*ESR*) at the time of first relapse.

Solid horizontal lines represent medians, boxes represent interquartile ranges, dashed horizontal lines represent means, and error bars represent SDs. Values were available for 13 placebo recipients and 21 infliximab recipients.

toma 23 days after the week 2 infusion and pleuropneumonia 89 days after the week 46 infusion. There were no cases of tuberculosis or sepsis. One patient in the placebo group developed Kaposi sarcoma. No cases of cancer were observed in patients receiving infliximab.

An infusion reaction was predefined as any adverse event that occurred during an infusion or within 1 hour after completion of an infusion. Ten infusion reactions occurred in 6 patients in the infliximab group, whereas no infusion reaction occurred in the placebo group (difference 21%, [CI, 6% to 37%]). Most infusion reactions were mild or moderate in intensity. One patient discontinued treatment because of an infusion reaction (dyspnea and flushing) during the week 30 infusion. Other infusion reactions included chest pain, chills, and fatigue.

Five patients discontinued treatment because of adverse events (**Figure 1**). In the placebo group, 1 patient had dyspnea, atrial fibrillation, and digoxin toxicity and 1 had Kaposi sarcoma. In the infliximab group, 1 patient experienced weakness and convulsions related to a resected meningioma, 1 had cardiac failure, and 1 experienced dyspnea and flushing (the infusion reaction previously described).

Seven patients (27%) in the infliximab group had antibodies to infliximab at any time, 18 patients (69%) were negative for antibodies to infliximab, and 1 patient (4%) had an inconclusive antibody status 20 weeks after the last dose of infliximab. Five patients (33%) in the placebo group and 13 patients (52%) in the infliximab group developed antinuclear antibodies during the study. Antibodies to double-stranded DNA were not found in the placebo group and developed in 16% of patients in the infliximab group. However, no clinical syndromes associated with antinuclear antibodies or antibodies to double-stranded DNA were observed.

DISCUSSION

To address the unmet need for a treatment that would allow patients with giant cell arteritis to reduce their dependence on glucocorticosteroids, we conducted the first

Table 4. Rates of Adverse Events and Development of Autoantibodies

Event	Placebo Group (*n* = 16)	Infliximab Group (*n* = 28)
Patients with adverse events, *n* (%)		
≥1 adverse event	15 (94)	26 (93)
≥1 serious adverse event	4 (25)	8 (29)
Discontinuation due to an adverse event	2 (13)	3 (11)
Infection		
All infections, *n*	23	47
Patients with ≥1 infection, *n* (%)	9 (56)	20 (71)
Patients with ≥1 infection requiring oral or parenteral antimicrobial treatment, *n* (%)	8 (50)	16 (57)
Patients with ≥1 serious infections, *n* (%)	1 (6)	3 (11)
Infusion reactions*		
Infusions, *n*	110	182
Infusion reactions, *n*	0 (0)	10 (5)
Patients with ≥1 infusion reactions, *n* (%)	0 (0)	6 (21)
Development of autoantibodies, *n* (%)		
Antinuclear antibodies (newly positive)	5 (33)	13 (52)
Antibodies to double-stranded DNA (newly positive)	0 (0)	4 (16)

* Defined as any adverse event reported during the infusion or within 1 hour after the infusion.

double-blind, randomized, placebo-controlled trial in which a biological agent was used as an adjunct to glucocorticosteroid therapy for giant cell arteritis. Our results failed to demonstrate that infliximab improved the duration of remissions or decreased the glucocorticosteroid requirement in patients with newly diagnosed giant cell arteritis. These conclusions were based on a duration of follow-up of at least 22 weeks for all patients and a median of 7 infliximab infusions per patient. The results are consistent with a difference in the proportion of relapse-free patients ranging from a 38% advantage for placebo to a 23% advantage for infliximab. The results are also consistent with a difference in the proportion of relapse-free patients whose glucocorticosteroid dosages were tapered to 10 mg/d, ranging from a 42% advantage for placebo to a 14% advantage for infliximab. Thus, our study provides evidence that infliximab therapy is unlikely to greatly reduce the proportion of patients with relapse of giant cell arteritis.

The study was not powered to detect modest effects of adding infliximab to glucocorticosteroid therapy for newly diagnosed giant cell arteritis. However, the expense and risk of the intervention would not likely be justified in routine practice if studies in a much larger sample demonstrated only a small benefit of infliximab.

Several matters regarding the study drug and trial design merit discussion. One might question whether a higher dose of infliximab might be efficacious for giant cell arteritis. The infliximab dose of 5 mg/kg, rather than the usual initial dose of 3 mg/kg used to treat for inflammatory arthritis, was chosen to reduce the possibility of failure merely because of an inadequate dose. The 5-mg/kg induction and maintenance regimen has been shown to be effective and well tolerated in patients with psoriasis (24), spon-

dyloarthropathy (25), and Crohn disease (26). Infliximab was not administered with concomitant methotrexate in these studies. We did not address whether efficacy may have been achieved by using even higher doses of infliximab, greater frequency of administration, or concurrent administration of methotrexate.

We found that approximately one quarter of patients who received infliximab developed antibodies to infliximab 20 weeks after the last dose was administered. Thus, for most patients, the lack of efficacy could not be attributed to antibodies to infliximab. In EXPRESS (European Infliximab for Psoriasis [Remicade] Efficacy and Safety Study), which studied infliximab therapy in patients with psoriasis (24), the rate of antibody formation to infliximab (27%) 20 weeks after the last dose was similar to that in our study. However, contrary to our results, the EXPRESS investigators found that infliximab was much more effective than placebo at reducing the signs and symptoms of psoriasis.

Salvarani and colleagues' study of polymyalgia rheumatica (27), a forme fruste of giant cell arteritis, further supports our findings. The researchers used a trial design that was similar to that of our study and found no statistically significant therapeutic benefit of infliximab. Thus, these 2 studies indicate that the addition of infliximab to glucocorticosteroids does not markedly decrease relapse rates or cumulative glucocorticosteroid requirements of patients with newly diagnosed giant cell arteritis or polymyalgia rheumatica. However, the role of anti–TNF-α therapy in patients with glucocorticosteroid-refractory giant cell arteritis or polymyalgia rheumatica has not been systematically studied.

Although TNF is found in abundance in biopsy samples with vascular damage from giant cell arteritis, the exact role of TNF in the pathogenesis of giant cell arteritis remains to be elucidated. It is possible that other pathways and mediators play more important or pivotal roles in the pathogenesis of giant cell arteritis.

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APPENDIX: THE INFLIXIMAB-GCA STUDY GROUP

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II. HIPÓTESIS DE TRABAJO

Las manifestaciones craneales constituyen el síntoma guía de la ACG. Los síntomas locales suelen acompañarse de una intensa respuesta de fase aguda, con producción de proteínas y mediadores de la respuesta inflamatoria, que son liberados a la circulación y dan lugar a los síntomas sistémicos de la enfermedad. El tratamiento con corticoides produce una rápida mejoría de los síntomas y disminuye la síntesis de mediadores inflamatorios. Sin embargo, un porcentaje elevado de pacientes presenta recidivas clínicas al reducir la dosis de tratamiento. La intensidad de la RIS es muy variable entre pacientes, y esta variabilidad permite diferenciar entre subgrupos de pacientes con características clínicas y pronóstico distinto. De esta manera, se ha observado que los pacientes con una RIS intensa en el momento del diagnóstico presentan más recidivas clínicas y precisan pautas de tratamiento más largas.

En los últimos años se ha sugerido que la persistencia de actividad inflamatoria crónica tiene efectos deletéreos sobre el paciente, principalmente relacionados con el desarrollo de enfermedad cardiovascular. En pacientes con ACG se ha observado que los marcadores de inflamación pueden persistir moderadamente elevados meses después del diagnóstico, incluso en pacientes que han alcanzado la remisión y no precisan tratamiento. Se desconoce, tanto el origen de esta actividad inflamatoria subclínica, como si ésta tiene consecuencias negativas sobre el paciente. La secreción de citocinas y factores de crecimiento se produce como consecuencia de la persistencia de lesiones inflamatorias arteriales, aunque también durante el proceso de remodelado vascular que tiene lugar

tras la agresión inflamatoria. Ambas situaciones podrían favorecer la aparición de alteraciones estructurales vasculares durante el seguimiento.

Según los estudios publicados hasta la fecha, la prevalencia de aneurismas o disección aórticos en pacientes con ACG oscila entre el 9.5% y el 18%. Es posible que la cifra sea superior, ya que estos datos provienen de estudios retrospectivos que incluyen pacientes diagnosticados hace muchos años, cuando la disponibilidad de técnicas de imagen era menor y por tanto era más difícil realizar un diagnóstico precoz. En la actualidad no se conoce si la intensidad o persistencia a lo largo del tiempo de la respuesta inflamatoria pueda favorecer el desarrollo de estas complicaciones. Los dos primeros trabajos de esta tesis tienen como objetivo, por un lado, determinar el estado de la RIS en pacientes con un periodo de seguimiento prolongado que están en remisión clínica, y por otro, conocer si dicha RIS se asocia a un mayor desarrollo de complicaciones clínicas significativas, centrándonos principalmente en el desarrollo de alteraciones estructurales aórticas.

Por otra parte, es evidente que las pautas de tratamiento utilizadas en la actualidad son insuficientes en aquellos pacientes que presentan múltiples recidivas clínicas. Uno de los principales objetivos de los últimos años ha sido la búsqueda de nuevos fármacos que permitan reducir la exposición a los corticoides y minimizar las complicaciones derivadas del tratamiento. El tercer trabajo de esta tesis nació en un momento en el que se intentó, sin éxito, bloquear el TNFα en pacientes con ACG. En los últimos

años se han identificado diversos sistemas de proteínas que tienen gran similitud estructural con el TNFα y han sido englobados dentro de la superfamilia del TNFα. Muchas de estas citocinas tienen acciones similares y vías de señalización comunes, por lo que se pensó que el bloqueo del TNFα podría potenciar la expresión de citocinas redundantes que compensasen su función. Uno de estos sistemas de proteínas es el constituido por el RANKL y sus receptores (OPG y RANK) cuya principal función conocida es la regulación del metabolismo óseo. Además, múltiples estudios han sugerido que estas citocinas intervienen en la regulación de la respuesta inmune y en biología vascular. Estos dos puntos convergen y tienen máximo interés en una enfermedad vascular inflamatoria como la ACG. Sería importante conocer si el sistema RANKL/RANK/OPG participa en la regulación, tanto de la respuesta inflamatoria, como del remodelado vascular que tiene lugar posteriormente en estos pacientes. En la actualidad disponemos de fármacos con capacidad para modular la función de esta familia de citocinas, por lo que puede ser interesante conocer qué papel juegan en esta enfermedad. Hasta la fecha, nunca se había estudiado la función del sistema RANKL/RANK/OPG en pacientes con ACG.

III. OBJETIVOS
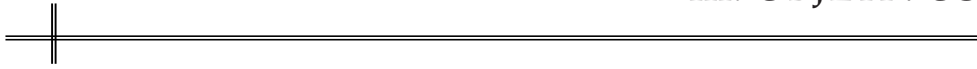
✓ Evaluar el estado de la respuesta inflamatoria sistémica biológica en pacientes con ACG que están en remisión clínica y tienen un periodo de seguimiento mínimo de cuatro años. Se realizará una valoración clínica, se determinarán los niveles de citocinas proinflamatorias (IL-6 y TNFα) así como los niveles de proteínas secretadas durante la respuesta de fase aguda (VSG, PCR, haptoglobina).

✓ Determinar si la persistencia de actividad inflamatoria subclínica se relaciona con el desarrollo de complicaciones después del diagnóstico de la vasculitis. Se valorarán las complicaciones relacionadas con la propia enfermedad (pérdida de visión por neuritis óptica y dilatación aórtica) así como otros eventos vasculares relacionados o no con la enfermedad (angina o infarto de miocardio, accidente isquémico transitorio o accidente vascular cerebral, claudicación intermitente o isquemia de extremidades). También se analizará si existe relación entre la intensidad de esta respuesta inflamatoria y la persistencia de actividad de la enfermedad valorada por el número de recidivas y los requerimientos terapéuticos. Por último, se analizará si la intensidad de la respuesta inflamatoria se correlaciona con el desarrollo de eventos adversos relacionados con una mayor exposición al tratamiento corticoideo.

✓ Estudiar la prevalencia de alteraciones estructurales aórticas (aneurisma o dilatación) en el mismo grupo de pacientes mediante un protocolo diagnóstico que incluye la realización de una ecografía

abdominal, una radiografía de tórax y una TC torácica en los casos en los que la radiografía no es concluyente o es sugestiva de dilatación aórtica.

✓ Determinar posibles factores que puedan favorecer el desarrollo de alteraciones estructurales aórticas. En concreto se analizará si estas complicaciones tienen relación con la intensidad de la respuesta inflamatoria sistémica o con la persistencia de actividad inflamatoria durante el seguimiento.

✓ Estudiar la expresión del sistema RANKL/RANK/OPG en pacientes con ACG. Valorar si la expresión de estas proteínas se relaciona con la expresión clínica de la enfermedad o con factores pronósticos (recidivas durante el seguimiento o requerimientos de corticoides).

IV. RESULTADOS
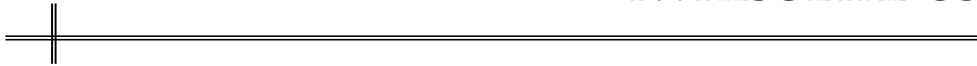
En las siguientes páginas se incluyen los artículos que han dado lugar a esta tesis doctoral.

1) **Clinical relevance of persistently elevated circulating cytokines (TNFα and IL-6) in the long-term follow-up of patients with giant-cell arteritis. Arthritis Rheum 2010;62:835-841. (IF: 6.787)**

2) **Development of aortic aneurysm/dilatation during the follow-up of patients with giant-cell arteritis: a cross-sectional screening of fifty-four prospectively followed patients. Arthritis Rheum 2008;59:422-430. (IF: 6.787)**

3) **Expression and clinical relevance of Receptor activator of NFκB (RANK) ligand (RANKL), RANK and Osteoprotegerin (OPG) in patients with giant-cell arteritis. (Remitido a publicación)**

ORIGINAL ARTICLE

Clinical Relevance of Persistently Elevated Circulating Cytokines (Tumor Necrosis Factor α and Interleukin-6) in the Long-Term Followup of Patients With Giant Cell Arteritis

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Objective. To assess the clinical relevance of increased circulating cytokines in patients with giant cell arteritis (GCA) after long-term followup.

Methods. We performed a cross-sectional evaluation of 54 patients with biopsy-proven GCA prospectively followed for a median of 5.4 years (range 4–10.5 years). GCA-related complications, vascular events, relapses, current prednisone dose, time required to achieve a maintenance prednisone dosage <10 mg/day, cumulated prednisone at that point, and adverse effects during followup were recorded. Serum interleukin-6 (IL-6) and tumor necrosis factor α (TNFα) were determined by immunoassay.

Results. All patients were in clinical remission. Both cytokines were significantly higher in patients than in controls (mean ± SD 21 ± 35 versus 5 ± 11 pg/ml; $P < 0.001$ for IL-6 and mean ± SD 32 ± 14 versus 16 ± 9 pg/ml; $P < 0.001$ for TNFα). No differences were found in patients with or without GCA-related complications or vascular events during followup. Circulating cytokines were significantly higher in patients who had experienced relapses (mean ± SD 25 ± 39 versus 10 ± 11 pg/ml; $P = 0.04$ for IL-6 and mean ± SD 34 ± 15 versus 25 ± 11 pg/ml; $P = 0.042$ for TNFα). IL-6 was significantly higher in patients still requiring prednisone (mean ± SD 29 ± 45 versus 13 ± 17 pg/ml; $P = 0.008$), and TNFα correlated with cumulated prednisone dose ($r = 0.292$, $P = 0.04$). No significant relationship was found between elevated cytokines and prednisone adverse effects or patients' quality of life.

Conclusion. Circulating TNFα and IL-6 may persist elevated in GCA patients after long-term followup and remain higher in patients who have experienced more relapsing disease. However, in this patient cohort, elevated circulating cytokines were not associated with increased frequency of GCA complications, vascular events, or treatment-related side effects.

INTRODUCTION

Giant cell arteritis (GCA) is the most common systemic vasculitis among people age >50 years. GCA inflammatory lesions preferentially target large and medium-sized vessels. Typical symptoms of the disease (headache, jaw claudication, scalp tenderness, facial aches, and visual loss) derive from inflammatory involvement of the carotid artery branches. Involvement of other arteries such as the aorta and its major tributaries remains asymptomatic unless complications such as dilatation or stenosis occur (1–4).

Vascular inflammatory infiltrates are mainly composed

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Submitted for publication August 20, 2009; accepted in revised form January 22, 2010.

of T lymphocytes and macrophages, which are the source of a variety of inflammatory mediators, including proinflammatory cytokines interleukin-1β (IL-1β), tumor necrosis factor α (TNFα), and IL-6 (5–9). These cytokines are mainly involved in local autocrine/paracrine responses, but TNFα and IL-6 may be released into the bloodstream and trigger systemic effects, including fever, malaise, weight loss, anemia of chronic disease type, and elevation of acute-phase proteins, all common in patients with GCA. Accordingly, local production of proinflammatory cytokines TNFα, IL-1β, and IL-6 in involved temporal arteries and circulating TNFα and IL-6 correlate with the intensity of the acute-phase response at diagnosis (8,10). Interestingly, a strong systemic inflammatory response and markedly increased expression of IL-6 are negatively associated with the development of disease-related cranial ischemic events at diagnosis (11,12).

Patients with GCA experience a rapid relief of their symptoms with high-dose corticosteroids. However, disease activity may not be completely abrogated, and 40–60% of patients relapse when corticosteroids are tapered (13,14). In addition, corticosteroid-treated patients may develop GCA-related vascular complications during followup: approximately 10–15% of patients with visual symptoms continue to have deteriorating vision during the first weeks of treatment (13,15), 22.2% of patients develop significant aortic dilatation (3), and 5–15% develop extremity artery stenosis (2,16). It is not clear at present whether these vascular complications arise from subclinical vascular inflammation or result from maladaptive remodeling driven by the initial inflammatory injury.

High-dose corticosteroid treatment results in sharp decreases in erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) level (17–19). However, when corticosteroids are tapered, acute-phase proteins may remain slightly or moderately elevated above the normal levels in some patients, even maintaining clinical remission (17–19). In keeping with this observation, it has been shown that circulating IL-6 persists elevated for several months, but it is not known whether IL-6 persists elevated after long-term followup (20). Elevated IL-6 in asymptomatic patients with GCA has been considered to reflect remaining subclinical vascular inflammation. Persistent vascular inflammation raises concerns about its long-term clinical consequences, including late GCA-related complications, accelerated atherosclerotic disease, or IL-6–induced osteopenia (20).

The aim of our study was to determine whether circulating proinflammatory cytokines persist elevated in patients with GCA after long-term followup, and whether persistent increase in circulating cytokines is associated with a higher frequency of GCA-related complications or other vascular events, clinically apparent disease activity, corticosteroid requirements, and corticosteroid-derived side effects.

PATIENTS AND METHODS

The study group consisted of 54 patients (14 men and 40 women) with biopsy-proven GCA and a median age of 79

years (range 63–91 years) who were prospectively treated and followed by the authors for a median of 5.4 years (range 4–10.5 years). This patient cohort was subjected to a cross-sectional screening for aortic dilatation in a previous study, and details regarding patient selection have been previously published (3). Briefly, patients were consecutively selected among those who had regular followup visits every 4–6 months for at least 4 years. All of the patients were treated according to a defined protocol (3). A relapse was defined as reappearance of disease-related symptoms (cranial, polymyalgic, systemic symptoms, or anemia of chronic disease type not attributable to other causes) that resolved with an increase in prednisone dose 10 mg above the previous dose able to maintain remission.

At the time of the screening for aortic aneurysm, patients were subjected to a clinical evaluation in search of disease-related symptoms. Patients' quality of life was self-estimated with a visual analog scale (VAS; 0–100 mm) for pain or other physical limitations, psychological well-being, independence for self-care, and the ability to perform work, social, or recreational activities. The average of these 4 assessments was considered.

Followup data were categorized into disease-related complications, other vascular events, disease activity, corticosteroid requirements, and corticosteroid-related adverse events. Disease-related complications included aortic dilatation and visual deterioration due to anterior ischemic optic neuritis during followup (confirmed by an ophthalmologist). Other vascular events included clinically symptomatic cardiovascular (angina or myocardial infarction), cerebrovascular (transient ischemic attack or stroke), or lower extremity arteriopathy (intermittent claudication or ischemia). Disease activity data comprised the number of relapses and the corticosteroid requirement assessed as time (in weeks) necessary to achieve a maintenance prednisone dosage <10 mg of prednisone/day, cumulated prednisone dose at that point, and prednisone treatment (any dose) at the time of evaluation. Corticosteroid-related side effects included new or worsening hypertension, diabetes mellitus and hypercholesterolemia, symptomatic fractures, gastrointestinal bleeding, mild or serious (requiring hospitalization) infection, and symptomatic cataracts requiring intervention.

At the time of the evaluation, general laboratory analysis, including hemoglobin and acute-phase reactants ESR, CRP, and haptoglobin, was performed. Circulating levels of IL-6 and TNFα were measured by immunoassay using Quantikine kits from R&D Systems, according to the manufacturer's instructions. Other cytokines thought to be relevant in the pathogenesis of GCA such as interferon-γ and IL-1β were not determined because concentrations of these cytokines in human serum are usually around the detection threshold. IL-6 and TNFα were also measured in 15 healthy donors with similar age and sex distribution. Mann-Whitney test or Student's *t*-test, when applicable, was employed for quantitative variables, and Spearman's or Pearson's test was employed for correlations.

The study was approved by the ethics committee of our institution, and all of the patients signed informed consent.
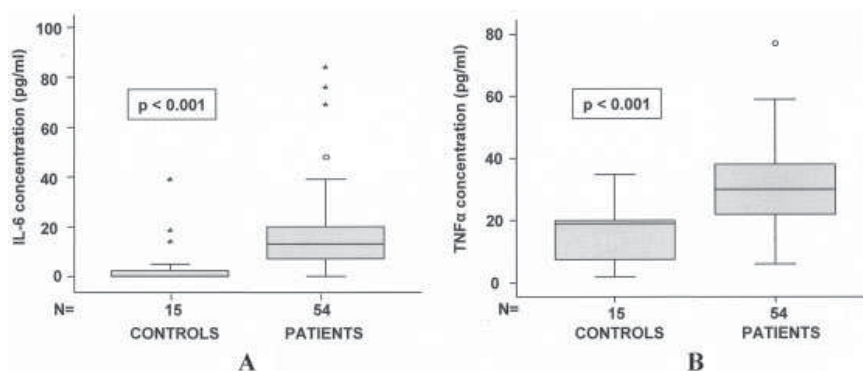
Figure 1. A, Interleukin-6 (IL-6) and **B,** tumor necrosis factor α (TNFα) serum concentrations in patients with giant cell arteritis and controls. * = extreme cases; ○ = outliers.

RESULTS

IL-6 and TNFα concentrations in sera from patients with GCA after long-term followup. The median IL-6 concentration in the patient cohort was 13 pg/ml (range 0–237) and the median TNFα concentration was 30 pg/ml (range 6–77). Seventy-eight percent of patients with GCA had IL-6 levels and 79% had TNFα concentrations above the reference values for the general population (0–5 pg/ml for IL-6 and 0–20 pg/ml for TNFα). As shown in Figure 1, circulating levels of IL-6 and TNFα were significantly higher in GCA patients than in healthy controls with similar age and sex distribution.

At the time of the evaluation, the median ESR was 32 mm/hour (range 8–66), the median CRP level was 0.7 mg/dl (range 0.2–5.5), the median haptoglobin level was 1.63 gm/liter (range 0.08–2.86), and the median hemoglobin level was 129 gm/liter (range 108–167). Circulating IL-6 significantly correlated with TNFα concentrations ($r = 0.378$, $P = 0.005$) and with CRP plasma levels ($r = 0.296$, $P = 0.03$). No significant correlations were found between IL-6 or TNFα and the rest of the laboratory parameters determined (Table 1).

IL-6 and TNFα concentrations and the development of GCA-related complications during followup. As previously published, 12 (22.2%) of the 54 patients developed aortic aneurysm or dilatation during the followup period (3). Two patients (3.7%) experienced GCA-related worsen-

ing of vision after the initiation of corticosteroid treatment. As shown in Table 2, no differences in cytokine concentrations were found between patients who had or had not developed disease-related vascular complications during followup.

IL-6 and TNFα concentrations and the development of vascular events. Seven patients (13%) experienced symptomatic vascular complications in other territories. The relative contribution of GCA versus traditional vascular risk factors in the development of vascular disease could not be fully ascertained. Four patients presented lower extremity ischemia that required percutaneous revascularization and stenting (1 patient), bypass surgery (1 patient), and extremity amputation (1 patient). Three patients developed transient cerebral ischemic attacks and a Doppler sonography disclosed significant carotid stenosis. One patient experienced a stroke 1 month after the diagnosis of GCA, and the magnetic resonance angiography exhibited thrombosis of the right carotid artery. Finally, 1 patient developed myocardial infarction that required percutaneous angioplasty and stenting. Overall, 2 of the 7 patients exhibited symptomatic vascular involvement in more than one territory (extremity and cerebrovascular ischemia). As shown in Table 2, there were no significant differences in circulating levels of proinflammatory cytokines between patients with or without symptomatic vascular events during followup.

Correlation between IL-6 and TNFα concentrations and GCA activity and corticosteroid requirements. At the time of the evaluation, all of the patients were in stable clinical remission with no evidence of relapse, infection, or other chronic inflammatory diseases within the previous 4 months. Four patients had an ESR ≥50 mm/hour and 5 had a CRP concentration ≥2 mg/dl (normal value <1). These patients had persistent mild elevation of acute-phase reactants with no development of disease-related symptoms during the following 6 months. Thirteen patients (24.1%) had not presented disease flares during the entire followup, 15 (27.8%) had experienced one relapse, and 26 (48.1%) had presented more than one. At the time of the evaluation, 27 patients had successfully discontinued prednisone, whereas the remaining 27 patients were

Table 1. Correlation between circulating cytokines and acute-phase reactants at the time of the evaluation*

	IL-6, pg/ml		TNFα, pg/ml	
	r	P	r	P
ESR, mm/hour	0.078	ns	0.248	ns
CRP level, mg/dl	0.296	0.03†	0.19	ns
Haptoglobin, gm/liter	0.034	ns	0.089	ns
Hemoglobin, gm/liter	−0.176	ns	−0.136	ns

* IL-6 = interleukin-6; TNFα = tumor necrosis factor α; ESR = erythrocyte sedimentation rate; ns = not significant; CRP = C-reactive protein.
† Spearman's rho test.

Table 2. Circulating cytokines and vascular complications during the followup of patients with giant cell arteritis*

	IL-6, pg/ml			TNFα, pg/ml		
	Present, mean ± SD	Absent, mean ± SD	P	Present, mean ± SD	Absent, mean ± SD	P
Aortic aneurysm/dilatation (n = 12)†	13 ± 8	24 ± 39	ns	30 ± 19	32 ± 13	ns
Worsening of vision (n = 2)	15 ± 3	22 ± 36	ns	17 ± 6	32 ± 14	ns
Other vascular events (n = 7)	19 ± 29	22 ± 35	ns	32 ± 13	31 ± 15	ns

* IL-6 = interleukin-6; TNFα = tumor necrosis factor α; ns = not significant.
† Data concerning aortic aneurysm/dilatation have been previously published (3).

still receiving low-dose prednisone treatment (median 3.75 mg/day, range 1.25–12.5).

Patients who had experienced at least one relapse during followup had higher levels of TNFα and IL-6 than patients with no relapsing disease (Figures 2A and B). IL-6 concentrations were significantly higher in patients who still required corticosteroid treatment at the time of the evaluation (Figure 3A). No significant differences were found in TNFα levels between patients still receiving prednisone compared with those who had successfully discontinued corticosteroid treatment (Figure 3B). Circulating levels of both IL-6 and TNFα remained significantly higher in patients who had been able to discontinue therapy than in healthy controls (mean ± SD 13 ± 17 versus 5 ± 11 pg/ml; $P < 0.001$ for IL-6 and mean ± SD 32 ± 12 versus 16 ± 9 pg/ml; $P = 0.005$ for TNFα). TNFα concentrations tended to correlate with the time required to reach a maintenance daily prednisone dosage <10 mg (r = 0.235, $P = 0.09$) and significantly correlated with the cumulative prednisone dose at that point (r = 0.292, $P = 0.04$) (Figure 3C). As shown in Table 3, the longer duration of treatment observed in patients with elevated TNFα or IL-6 levels did not result in more corticosteroid-related side effects.

The median VAS score in the entire series was 90 mm (range 37–100). No significant correlation was found between IL-6 or TNFα levels and VAS scores (r = −0.228, not significant for IL-6 and r = −0.048, not significant for TNFα).

DISCUSSION

Previous studies have shown that IL-6 may persist elevated for several months after the beginning of corticosteroid treatment in patients in remission, but longer followup studies have not been performed (18,20). To our knowledge, the present study is the first attempt to evaluate circulating cytokine levels in patients in remission after long-term followup and indicates that circulating proinflammatory cytokines IL-6 and TNFα remain elevated in a substantial proportion of patients with GCA.

The source of elevated cytokines in patients in remission is not clear. Surgical or necropsy specimens from long-term treated patients with GCA have disclosed extensive vascular remodeling with persistent, small foci of inflammatory cells (3,21,22). However, it is important to remark that inflammatory cells may not be the only source of cytokines. We have previously shown that cultured myointimal cells derived from temporal arteries are able to produce substantial amounts of IL-6 (23,24). Therefore, both remaining inflammatory cells and regenerating smooth muscle cells may contribute to proinflammatory cytokine production, particularly IL-6. Increased circulating cytokines in patients with GCA may then represent long-lasting persistence of low-grade inflammatory activity and/or continuous vascular remodeling. Differences observed among patients may reflect the extent of persistent subclinical vascular inflammation/remodeling or may
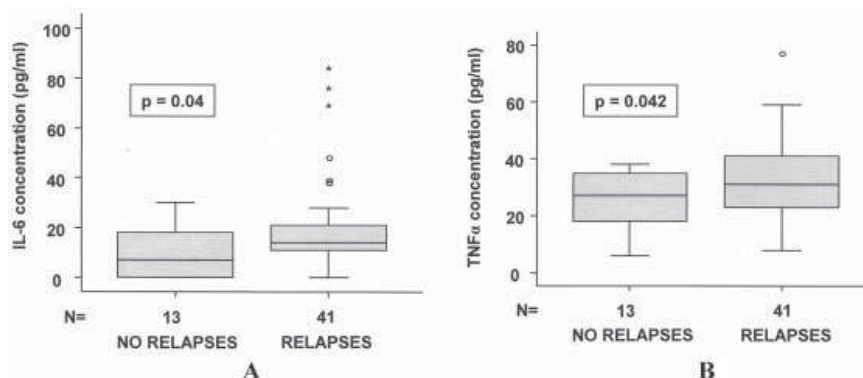

Figure 2. A, Interleukin-6 (IL-6) and **B,** tumor necrosis factor α (TNFα) levels in patients with giant cell arteritis according to the occurrence of relapses. * = extreme cases; ○ = outliers.
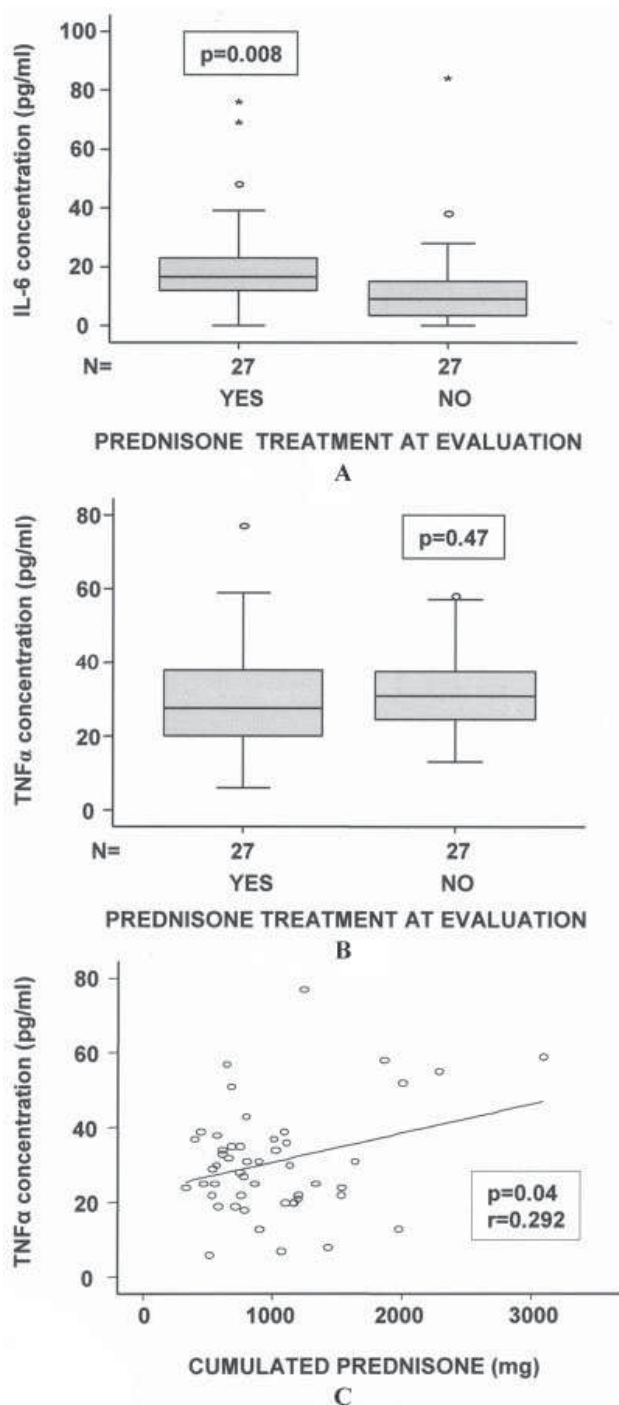
Figure 3. Serum cytokine concentrations and corticosteroid requirements. **A,** Interleukin-6 (IL-6) and **B,** tumor necrosis factor α (TNFα) levels in patients with giant cell arteritis still receiving prednisone treatment compared with those with successful treatment discontinuation at the time of evaluation. **C,** Correlation between TNFα concentration and cumulated prednisone dose when reaching a maintenance dosage of <10 mg/day. * = extreme cases; ○ = outliers.

be due to functional polymorphisms in cytokine genes. Polymorphisms in the TNFα or IL-6 gene promoters result-

ing in higher cytokine production have been identified (25,26).

Several studies have shown that chronic inflammatory diseases such as rheumatoid arthritis or systemic lupus erythematosus are associated with accelerated atherosclerosis and a higher risk of cardiovascular disease, which has been attributed to persisting low-grade inflammatory activity (27–30). Moreover, moderately increased serum levels of IL-6 and CRP in the general population are associated with a higher risk of cardiovascular events (31,32). In our patients, persistent increase in circulating cytokines was not associated with higher frequency of vascular complications, either GCA or atherosclerosis related, during followup. However, these results must be interpreted with caution, due to the relatively small number of patients included and the relatively low frequency of disease- or atherosclerosis-related complications in GCA patients in remission (15,33). We cannot exclude that a much larger series or a longer followup could evidence an association between elevated circulating cytokines and a higher frequency of vascular complications. However, the physiologically limited lifespan of patients with GCA reduces the significance of studies with a much longer followup and restricts its relevance to the youngest subset of patients.

Previous studies have shown that increased TNFα expression in lesions at diagnosis is associated with persistent disease activity. This observation is in accordance with results generated by several groups showing an association between a strong acute-phase response at diagnosis and more relapsing disease, both in patients with GCA and in patients with polymyalgia rheumatica (34,35). The present study shows that patients who have experienced more relapses or have required more corticosteroid doses still maintain significantly increased circulating TNFα and IL-6 levels after long-term followup. Although increased serum TNFα and IL-6 concentrations are associated with more refractory disease, TNFα blockade failed to reduce relapses and spare corticosteroids, indicating that elevated TNFα, even being a marker of disease persistence, may not be crucial in maintaining disease activity or may be compensated by redundant cytokines (36). Blocking IL-6 has not been attempted in GCA and may or may not face similar limitations. Taken together, these findings suggest caution in attributing functional roles to these or other elevated biomarkers and underline the need for functional studies before they can be considered candidate therapeutic targets (37).

Of interest, although patients with increased TNFα or IL-6 levels had higher corticosteroid requirements, this was not associated with an increase in corticosteroid-related adverse events. This observation may again be limited by the relatively small size of the patient cohort. Moreover, regarding corticosteroid-induced osteoporosis, only symptomatic fractures were taken into account and the asymptomatic collapse of dorsal vertebrae was not systematically assessed. In addition, the development of corticosteroid-related side effects does not only depend on the cumulated doses because some patients are particularly prone to develop these complications. Patients with osteopenia at diagnosis or patients with underlying metabolic syndrome are especially susceptible to developing

Table 3. Cytokine levels and corticosteroid-related side effects during followup*

	IL-6, pg/ml			TNFα, pg/ml		
	Present, mean ± SD	Absent, mean ± SD	*P*	Present, mean ± SD	Absent, mean ± SD	*P*
Hypertension (n = 25)	12 ± 10	30 ± 45	0.041	31 ± 16	32 ± 13	ns
Diabetes mellitus (n = 7)	17 ± 11	22 ± 37	ns	30 ± 23	32 ± 13	ns
Hypercholesterolemia (n = 20)	16 ± 17	25 ± 42	ns	31 ± 17	32 ± 13	ns
Osteoporotic fractures (n = 7)	22 ± 28	21 ± 36	ns	27 ± 10	32 ± 15	ns
Mild infection (n = 6)	56 ± 90	17 ± 18	ns	34 ± 14	31 ± 14	ns
Serious infection (n = 6)	24 ± 26	21 ± 36	ns	31 ± 14	32 ± 14	ns
Cataracts (n = 10)	36 ± 72	18 ± 19	ns	31 ± 9	32 ± 15	ns

* Only 1 patient experienced gastrointestinal bleeding due to erosive gastritis. IL-6 = interleukin-6; TNFα = tumor necrosis factor α; ns = not significant.

related complications when receiving corticosteroid therapy (38,39).

Although not associated with major clinical consequences, subclinical inflammation may potentially produce malaise, fatigue, or reduction in well-being, impairing quality of life. Self-estimated quality of life was surprisingly high in our elderly patient cohort. This may not be representative of the overall GCA population since patients included had been able to maintain a regular long-term followup. Although increased cytokines were associated with more relapsing disease, no correlation was found between cytokine levels and patients' quality of life scores at the time of the evaluation. A limitation of this conclusion may be that quality of life was evaluated with a VAS and not with a validated instrument. An instrument to specifically measure quality of life in GCA patients is in development but is still awaiting validation (40). Illiteracy, sight problems, and lack of training in answering questionnaires were the main reasons for choosing a simple VAS in this particular patient cohort. It may be possible that a more sensitive instrument could have detected differences between patients with or without persistent subclinical inflammatory activity.

In summary, our study shows long-term persistence of elevated circulating cytokines in patients with GCA. Although patients with higher cytokine levels had experienced a more relapsing disease, persistent elevation of circulating cytokines was not associated with clinically relevant complications related to GCA, atherosclerotic disease, or corticosteroid treatment.

AUTHOR CONTRIBUTIONS

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be submitted for publication. Dr. Cid had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.
Study conception and design. García-Martínez, Cid.
Acquisition of data. García-Martínez, Hernández-Rodríguez, Espígol-Frigolé, Prieto-González, Butjosa, Segarra, Lozano, Cid.
Analysis and interpretation of data. García-Martínez, Cid.

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ORIGINAL ARTICLE

Development of Aortic Aneurysm/Dilatation During the Followup of Patients With Giant Cell Arteritis: A Cross-Sectional Screening of Fifty-Four Prospectively Followed Patients

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Objective. Giant cell arteritis (GCA) may involve the aorta. Retrospective studies have demonstrated a higher prevalence of aortic aneurysm among patients with GCA compared with the general population. We investigated the prevalence of aortic aneurysm in a cohort of patients with biopsy-proven GCA using a defined protocol and assessed whether persisting low-grade disease activity is associated with higher risk of developing aortic aneurysm.

Methods. Fifty-four patients with GCA (14 men and 40 women) were cross-sectionally evaluated after a median followup of 5.4 years (range 4.0–10.5 years). The screening protocol included a chest radiograph, abdominal ultrasonography scan, and computed tomography scan when aortic aneurysm was suspected or changes with respect to the baseline chest radiograph were observed. Clinical and laboratory data, corticosteroid requirements, and relapses were prospectively recorded.

Results. Twelve patients (22.2%) had significant aortic structural damage (aneurysm/dilatation), 5 of them candidates for surgical repair. Aortic aneurysm/dilatation was more frequent among men (50%) than women (12.5%; relative risk 3.5, 95% confidence interval 1.53–8.01, $P = 0.007$). At the time of screening, patients with aneurysm/dilatation had lower serum acute-phase reactants, lower relapse rate, and needed shorter periods to withdraw prednisone than patients without aortic structural damage.

Conclusion. There is a substantial risk of developing aortic aneurysm/dilatation among patients with GCA. Our data do not support that aneurysm formation mainly results from persistent detectable disease activity. Additional factors including characteristics of the initial injury or the target tissue may also determine susceptibility to aortic aneurysm/dilatation.

INTRODUCTION

Giant cell arteritis (GCA) is a granulomatous vasculitis affecting large and medium-sized vessels. The most common vascular symptoms of the disease (headache, jaw claudication, scalp tenderness) derive from inflammatory involvement of the craniofacial arteries, but other vascular territories may also be affected (1,2).

Aortic inflammation in patients with GCA was first described in the late 1930s/early 1940s (3) and sporadically reported thereafter (4,5). The prevalence of aortitis in GCA is unknown but appears to be remarkable. Systematic necropsy studies performed by Ostberg in 1972 disclosed aortic inflammation in 12 (92%) of 13 patients with GCA (4). Due to the lack of appropriate imaging techniques able

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Submitted for publication October 27, 2006; accepted in revised form September 10, 2007.

to detect aortic inflammation in living individuals, the clinical relevance of aortic involvement has been neglected for years. Currently, computerized tomography or magnetic resonance imaging can detect thickening, increased mural contrast enhancement, and, possibly, edema in the aortic wall (6–8), but available data are still limited. ^{18}F-fluorodeoxyglucose (FDG) uptake measured by positron emission tomography (PET) scan is emerging as a useful method to assess inflammatory activity in large vessels. In recent studies, increased aortic FDG uptake has been detected in approximately 50–60% of untreated patients, decreasing after 3–6 months of corticosteroid treatment (9–11).

Aortic inflammation appears to be frequent in GCA but remains asymptomatic unless structural damage leads to aneurysm, dissection, or aortic valve dysfunction. All of these events may have relevant clinical consequences and increase mortality in patients with GCA (5,12). They may appear early in the course of the disease or, more frequently, as delayed complications.

The prevalence of aortic structural damage related to GCA is unknown given that the occurrence of aortic complications has only been evaluated in retrospective, chart-review studies encompassing long periods. Reported prevalences range from 9.5% to 18% (13–15). These studies include patients diagnosed over very extended periods (20–50 years) including times when awareness of GCA was lower, treatment delay was longer, recommended corticosteroid doses were lower, duration of corticosteroid regimens were more brief, and life expectancy was much shorter (3,4). These factors may all influence both the intensity of aortic inflammation and the detection of clinically apparent complications.

Corticosteroid treatment usually elicits satisfactory relief of symptoms as well as normalization of acute-phase reactants in patients with GCA. However, when corticosteroids are tapered, relapses are frequent and persistent mild to moderate elevation of inflammatory markers can be observed in a substantial proportion of patients in clinical remission, suggesting subclinical activity (16–18). Corticosteroid tapering and withdrawal are currently guided by assessment of clinical activity and acute-phase reactants, mainly erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP). Based on the reported finding of inflammatory lesions in surgical or necropsy specimens from patients with aneurysm or dissection, there is some concern regarding whether or not persistent subclinical activity may eventually lead to the development of these complications (4,19,20). To date, it is not known whether or not persistent disease activity or persistent elevation of inflammatory markers is associated with a higher risk of developing delayed complications such as aortic aneurysm or dissection.

The goal of our study was 1) to investigate the prevalence and distribution of aortic aneurysm/dilatation detected with a defined screening protocol in a series of 54 patients with biopsy-proven GCA who were prospectively evaluated and treated and 2) to investigate factors associated with the development of this complication, particularly whether persistent subclinical inflammatory activity or a smoldering/relapsing course is associated with higher incidence of aneurysm formation.

PATIENTS AND METHODS

Patient selection. Between September 1995 and July 2001, 125 patients were diagnosed with biopsy-proven GCA at our department (Internal Medicine, Hospital Clínic, Barcelona, Spain). Seven were subsequently treated and followed at other departments/institutions, 16 died during followup, 5 were transferred to nursing homes for advanced dementia, and 38 were lost or had incomplete followup for a variety of reasons, including moving to other regions or not returning for periodic visits by study physicians after successful corticosteroid withdrawal. During the planned study period (2000–2005), 59 patients had already completed or would complete a prospective followup of at least 4 years and were considered eligible for aneurysm screening. This period was arbitrarily selected on the basis that aneurysm is considered to be a delayed complication. Five of the 59 patients declined participation due to advanced age or comorbidities and the remaining 54 agreed to participate and were included.

All patients were prospectively treated and followed by the investigators according to a defined protocol. All patients received an initial prednisone dosage of 1 mg/kg/day (up to 60 mg/day) for 1 month. Subsequently, prednisone was tapered 10 mg/week. Reduction below 20 mg/day was slower and individualized. A further reduction to a maintenance dosage of 10 mg/day was attempted over a 2-month period. If tolerated, reduction to 7.5 mg/day was attempted after 3 months and maintained for 3 additional months. A maintenance dosage of 5 mg/day was attempted for 6 months. If patients were asymptomatic with normal acute-phase proteins and ESR <40 mm/hour, tapering at an approximate rate of 1 mg per 3 months was attempted until discontinuation. If patients responded well but elevation of acute-phase proteins persisted, the maintenance dosage of 5 mg/day was maintained for 1 year before attempting withdrawal. If the ESR increased to >40 mm/hour, the corticosteroid dose was held for 2 months and if no clinical symptoms appeared, tapering was attempted again. Relapse was defined as reappearance of disease-related symptoms. Persistent malaise and anemia with elevation of acute-phase reactants were also considered relapses if they were not attributable to other causes after detailed evaluation and if they resolved after increasing steroids. When a relapse occurred, prednisone dosage was increased by 10 mg/day above the previous effective dose. Clinical findings and laboratory values at the time of diagnosis were prospectively recorded. These included ESR, CRP level, haptoglobin, α_2-globulin, blood cell counts, and liver function tests by usual automatized systems.

Screening protocol. Patients were screened once between 2000 and 2005 at their regular followup visits. Patients underwent a medical interview, complete physical examination, routine blood tests, and detection of serum concentration of proinflammatory cytokines (interleukin-6 [IL-6], tumor necrosis factor α, and IL-18). These were

determined by immunoassay (R&D Systems, Minneapolis, MN) according to the instructions of the manufacturer. Chest radiography was performed in all patients and carefully compared with that performed at the time of diagnosis. When aortic dilatation or changes with respect to the baseline radiograph were suspected, a contrast-enhanced spiral chest computed tomography (CT) scan was performed. The diameter of the aorta was measured at 3 different levels (ascending aorta, aortic arch, and descending aorta). Significant aortic structural damage was considered when an aortic aneurysm was found (defined as focal dilatation of the aortic wall) or when the aortic wall was diffusely dilated with a diameter >4 cm in the ascending aorta or at least 4 cm in the aortic arch and descending aorta. The aortic diameter at the same levels was measured in 28 consecutively selected age- and sex-matched individuals who underwent a chest CT scan for melanoma or gastric cancer as routine followup. The abdominal aorta was evaluated by ultrasonography. Prednisone requirements and relapse rate were prospectively recorded in all patients.

FDG uptake assessment by PET scan. To assess whether aneurysm development could be related to detectable subclinical inflammation, FDG uptake was evaluated by PET scan in 11 patients with GCA (7 with and 4 without aortic aneurysm or dilatation, all confirmed by CT scan), in 4 age- and sex-matched controls randomly selected among patients undergoing evaluation for cancer staging, and in 3 patients with noninflammatory thoracic aortic aneurysm who were scheduled for surgery. In these latter patients, the noninflammatory nature of the aortic aneurysm was confirmed by histopathologic examination after surgical repair. Two of the patients had aortic aneurysm secondary to myxoid degeneration of the aortic valve and 1 had severe atherosclerosis. Funding limitations precluded extension of PET scan study to the entire series.

After a fasting period of 6 hours and after verifying a blood glucose concentration <120 mg/ml, 370 MBq of FDG was injected intravenously and PET/CT was performed with a Biograph (Siemens Medical Solutions, Enlargen, Germany). Whole-body images from the base of the skull to mid-femur were acquired 50 minutes after the radiotracer injection. CT parameters were 50 mA, 130 kV, and 8-mm sections. Iterative reconstruction was performed and attenuation correction was based on CT. Attenuation-corrected and nonattenuation-corrected images were evaluated by 2 independent investigators. The maximum standard uptake value (SUV) and the median SUV were obtained from a zone of interest drawn on sagittal slices over the thoracic aorta. The study was approved by our local ethics committee and all patients gave informed consent.

Immunohistochemistry and gelatin zymography. Serial $4-6$-μm cryostat sections from a surgically removed aortic segment from a patient with GCA were air dried and fixed with cold acetone. Sections were incubated with a polyclonal rabbit anti-human matrix metalloprotease 2 (MMP-2; Chemicon, Temecula, CA) at 1:500 dilution or a

Table 1. Description of aortic characteristics in patients with aortic structural damage	
Patient	**Aortic characteristics**
1	Diffuse dilatation of aortic root and ascending aorta, maximum diameter of 5.7 cm. Moderate aortic insufficiency secondary to dilatation. Surgical repair declined because of age and concomitant diseases.
2	Diffuse dilatation of thoracic aorta with maximum diameter of 6 cm in ascending aorta. The aneurysm was surgically repaired. The histology showed moderate inflammation in adventitia and scattered inflammatory foci in the media layer.
3	Aneurysm of ascending aorta and aortic root with maximum diameter of 7.3 cm and severe aortic insufficiency. Surgical repair refused because of age and concomitant diseases.
4	Dilatation of ascending aorta with maximum diameter of 5 cm and important dilatation of aortic arch. Moderate aortic insufficiency. The patient refused surgical repair.
5	Aneurysm of ascending aorta with maximum diameter of 5.8 cm. Moderate aortic insufficiency secondary to dilatation. The aneurysm was surgically repaired. The histology showed moderate atherosclerosis with moderate chronic inflammation in the intima and adventitia.
6	Aneurysm of ascending aorta with maximum diameter of 5 cm.
7	Aneurysm of ascending aorta with maximum diameter of 4.8 cm.
8	Aneurysm of abdominal aorta (5.1 × 3.1 × 2.9 cm).
9	Dilatation of the ascending aorta (4.5 cm).
10	Dilatation of the ascending aorta (4.2 cm).
11	Dilatation of the aortic arch (4 cm).
12	Dilatation of the aortic arch and the descending aorta (4 cm).

mouse monoclonal anti-human MMP-9 (clone GE-213; Chemicon) at 1:1,000 dilution. Immunoglobulins obtained from the same species as the primary antibodies were used as negative controls at the same concentrations. Immunodetection was carried out with an HRP-labeled polymer conjugated to secondary antibodies (EnVision kit from Dako, Carpinteria, CA).

Elastic fibers were stained with 1% Shikata's orcein (Scharlau Chemie, Barcelona, Spain) in 70% ethanol. Gelatin zymography of tissue extracts from a normal temporal artery, a temporal artery with active GCA lesions, and a surgically excised GCA-related thoracic aortic aneurysm was performed as described (21).

Statistical analysis. Mann-Whitney U test and Student's *t*-test, when applicable, were applied to quantitative data. Kruskal-Wallis test was used for multiple comparisons. Fisher's exact test was used for contingency tables.

Table 2. Clinical data at baseline of patients with and without aortic abnormalities*			
	Altered aorta (n = 12)	Normal aorta (n = 42)	*P*
Sex, male/female	7/5	7/35	0.007
Age, median (range) years	76 (70–89)	79 (63–91)	NS
Followup, median (range) years	5.4 (4–8.5)	5.5 (4–10.5)	NS
Duration of symptoms, median (range) weeks	10 (2–52)	16 (1–104)	NS
Cranial symptoms			
Headache	75	83	NS
Jaw claudication	25	50	NS
Scalp tenderness	25	57	NS
Ischemic events	0	19	NS
Systemic symptoms			
Polymyalgia rheumatica	42	52	NS
Fever	25	43	NS
Weight loss	58	52	NS
Vascular risk factors			
Smoking	8	5	NS
Hypertension	75	79	NS
Diabetes	17	12	NS
Hypercholesterolemia	17	57	0.021

* Values are the percentage unless otherwise indicated. Vascular risk factors have been determined at baseline or during proper followup. NS = not significant.

The time required to achieve a stable maintenance prednisone dosage <10 mg/day and the time until definitive corticosteroid withdrawal were analyzed by the Kaplan-Meier survival analysis and compared by the log rank test.

RESULTS

Prevalence and characteristics of aortic structural damage in patients with GCA. Changes in the screening chest radiograph led to the performance of a chest CT scan in 28 (52%) patients. Significant structural abnormalities in the thoracic aorta were confirmed in 11 patients. In the remaining 17, suspected changes observed in the radiograph were positional or due to aortic elongation or hiatal hernia. No thoracic aortic aneurysm was found among controls and only 2 had an ascending aorta diameter >4 cm. Aortic diameters among individuals considered not to have aortic dilatation tended to be higher in patients with GCA at the level of the descending aorta when compared with controls (median 2.5 cm, range 2.1–3.6 versus median 2.3 cm, range 2–2.8; *P* = 0.018). No significant differences were found in the other segments. This finding indicates that a low degree of structural damage leading to slight diffuse dilatation is common in patients with GCA.

Ultrasonography revealed abdominal aortic aneurysm in only 1 patient. Overall, 12 (22.2%) patients developed significant structural aortic damage (aneurysm or dilatation) during a median followup of 5.4 years (range 4–10.5 years).

A brief description of the abnormalities detected is shown in Table 1. In 5 patients surgery was recommended because of the size of the aneurysm or resulting aortic valve insufficiency. Two of these patients underwent successful surgical repair of the aneurysm. One patient refused intervention. In the remaining 2 patients, surgery was eventually declined because of advanced age and comorbidities. Incidentally, the screening protocol led to the discovery of a thoracic hydatid cyst in 1 patient, lung cancer in 1 patient, hypernephroma in 1 patient, and ovarian mucinous cystadenoma in 1 patient.

Clinical findings associated with the development of significant aortic structural damage. No significant differences in age, duration of followup, or initial clinical manifestations were found between patients with and without aortic structural damage. The prevalence of traditional cardiovascular risk factors did not differ among patients with or without aortic structural damage except for hypercholesterolemia, which, surprisingly, was more frequent among patients who did not develop aortic structural damage (relative risk [RR] 0.29, 95% confidence interval [95% CI] 0.081–1.062, *P* = 0.021). In our series, significant aortic structural damage was detected in 50% of men but only 12.5% of women (RR 3.5, 95% CI 1.529−8.014, *P* = 0.007) (Table 2). Interestingly, patients who later developed aortic aneurysm/dilatation tended to have lower concentrations of acute-phase reactants at the time of diagnosis compared with patients who did not develop significant aortic damage (Table 3). When the overall intensity of the acute-phase response was evaluated combining clinical and analytical abnormalities as reported (22), aneurysm/dilatation was significantly more frequent among patients with a weak systemic inflammatory reaction (RR 1.7, 95% CI 1.166−2.626, *P* = 0.046) (Figure 1A). This was unexpected given that patients with strong acute-phase response usually have more resistant disease (22).

At the time of screening, all patients were in clinical remission. Twenty-seven were in stable remission without therapy and 27 still required low doses of corticosteroids (median dosage 3.75 mg/day, range 1.25–12.5). No significant differences in clinical outcome during followup were observed between patients with and without aortic abnor-

Table 3. Laboratory parameters at baseline and at the time of evaluation*			
	Altered aorta	**Normal aorta**	***P***
At diagnosis			
ESR, mm/hour	87 ± 24	95 ± 28	NS
CRP, mg/dl	6.5 ± 5.7	10 ± 9.3	NS
Haptoglobin, gm/liter	3.2 ± 1.27	4 ± 1.73	NS
Hemoglobin, gm/dl	117 ± 18	110 ± 15	NS
Alkaline phosphatase, units/liter	227 ± 80	283 ± 212	NS
GGT, units/liter	66 ± 107	48 ± 51	NS
Proteins, gm/liter	68 ± 8	68 ± 7	NS
Albumin, gm/liter	34 ± 5	35 ± 5	NS
α_2-globulin, gm/liter	8.4 ± 2.5	10.2 ± 3.6	NS
Platelet count, $\times 10^9$/liter	292 ± 52	341 ± 110	NS
At screening			
ESR, mm/hour	18 ± 9	34 ± 14	0.001
CRP, mg/dl	0.9 ± 1.4	1.1 ± 0.8	NS
Haptoglobin, gm/liter	1.38 ± 0.62	1.73 ± 0.51	NS
Hemoglobin, gm/dl	141 ± 17	128 ± 12	0.005
IL-6, pg/ml	13 ± 8	24 ± 39	NS
TNFα, pg/ml	30 ± 19	32 ± 13	NS
IL-18, pg/ml	290 ± 132	288 ± 159	NS

* Values are the mean ± SD unless otherwise indicated. ESR = erythrocyte sedimentation rate; NS = not significant; CRP = C-reactive protein; GGT = gamma glutamyl transpeptidase; IL-6 = interleukin-6; TNFα = tumor necrosis factor α; IL-18 = interleukin-18.

malities. Contrary to what was expected, patients with aneurysm/dilatation did not show a smoldering or relapsing course that might indicate stronger persisting inflammatory activity. In fact, as shown in Figure 1B, aneurysm/dilatation tended to be more frequent among patients who did not have recurrences compared with those who had a relapsing course (RR 2.9, 95% CI 1.214−7.965, P = 0.05). No significant differences in cumulated prednisone dosages during the first year (mean ± SD 6.3 ± 1.3 gm versus 6.2 ± 1.8; P = 0.86) or in the time required to reach a maintenance daily prednisone dosage <10 mg were observed between patients with or without significant aortic structural damage. However, patients with aortic structural damage needed shorter periods to withdraw prednisone therapy than patients without aortic structural damage (Figures 1C and 1D). At the time of screening, no significant differences in proinflammatory cytokine concentrations were observed between patients with or without aortic structural damage. Nevertheless, patients with aortic structural damage had significantly lower ESR (P = 0.001) and higher concentrations of hemoglobin (P = 0.005) than patients without significant aortic structural damage (Table 3). Although these data should be confirmed in larger series, they suggest that persistent subclinical inflammatory activity is not the major determinant of aneurysm formation in patients with GCA and that other factors may be involved.

FDG positron emission tomography. None of the patients examined showed remarkable FDG uptake by aortic tissue, as has been reported in active disease (9−11). Accurate measurement of maximal and median SUV did not show significant differences between patients with GCA-related aneurysm and patients with GCA with no aneurysm, controls, or patients with noninflammatory aneu-

rysm (Figure 2). The intensity of uptake was much lower than that reported in active patients and similar to that found in patients in remission or patients with atherosclerotic lesions. Although the number of patients examined was small, these findings suggest that, in appropriately treated patients, the development of aneurysm is not mainly related to major differences in persistent, detectable, local inflammatory activity.

Histopathologic examination of aortic specimens. Surgically removed specimens showed inflammatory infiltrates in the adventitial layer in both 2 patients with GCA and 3 patients with noninflammatory aneurysm. Scattered inflammatory foci were seen in the media only in patients with GCA and in the patient with severe atherosclerosis. No dense granulomatous lesions or giant cells were observed. Remaining foci of inflammatory cells immunostained positive for MMP-9 and MMP-2 (Figure 3A). MMP-2 expression by vascular smooth muscle cells was also observed. Elastic fibers were markedly disrupted in areas with remaining inflammatory cells, but also in many additional areas devoid of inflammatory infiltrates (Figure 3B). Gelatin zymography of tissue extracts revealed MMP-9 gelatinolytic signal in the temporal artery with active inflammatory lesions, whereas in a normal temporal artery and in a GCA-related aneurysm MMP-9 gelatinolytic signal was faintly detectable. MMP-2 gelatinolytic signal was observed both in active GCA lesions and in GCA-related aneurysm (Figure 3C).

DISCUSSION

Systematic screening of a cohort of 54 patients with biopsy-proven GCA demonstrated that 12 (22.2%) patients had
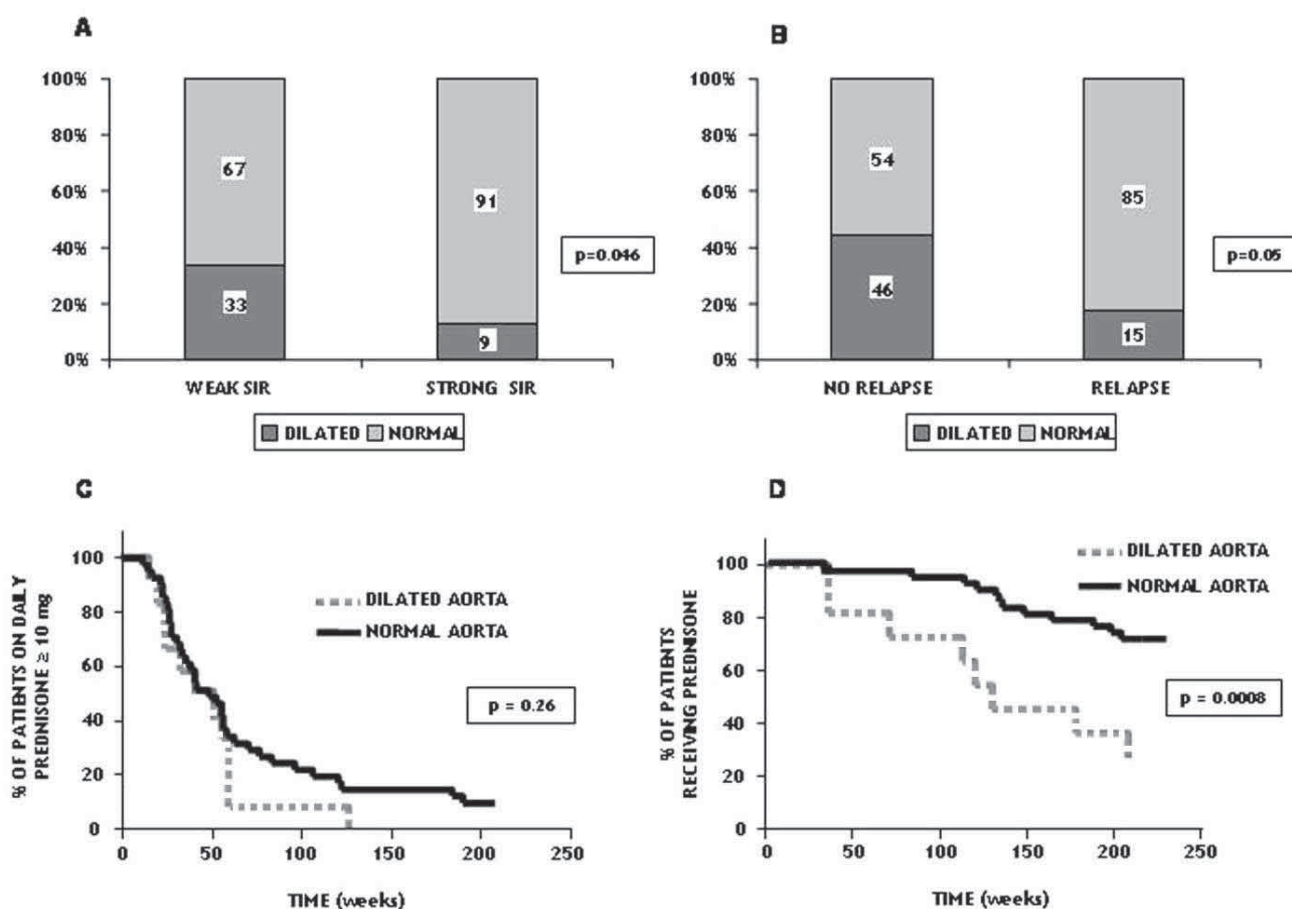
Figure 1. Systemic inflammatory response and clinical outcome in patients with giant cell arteritis with or without aortic structural damage. **A,** The proportion of patients with aortic structural damage was higher among patients with weak systemic inflammatory response (SIR; relative risk 1.7, 95% confidence interval 1.17–2.63, $P = 0.046$). Weak SIR was defined as the presence of $\leq$2 of the following: erythrocyte sedimentation rate $\geq$85 mm/hour, hemoglobin <110 gm/liter, fever >37°C, and weight loss >3 kg. Strong systemic inflammatory response was defined as the presence of 3–4 of the above items (20). **B,** Aortic structural damage did not preferentially occur in relapsing individuals. In contrast, aortic structural abnormalities tended to be more frequent among patients in sustained remission. **C,** Percentage of patients requiring $\geq$10 mg of daily prednisone over time. **D,** Percentage of patients requiring prednisone treatment over time.

significant aortic structural abnormalities (aneurysm or diffuse dilatation) after a median followup of 5.4 years. Thoracic aneurysms were much more frequent than abdominal aneurysms, as reported in retrospective studies.

Our screening method, chosen on the basis of its reasonable cost:benefit ratio and feasibility in general clinical practice, relied on a careful examination of a chest radiograph and an abdominal ultrasound, which may have reduced sensitivity. It is possible that performing echocardiography in patients with aortic murmurs, as recently suggested by Bongartz and Matteson (7), would increase sensitivity. Systematic screening with more sensitive imaging techniques such as CT scan would have probably revealed a higher prevalence of subtle aortic structural abnormalities, although perhaps not always clinically relevant. Despite the potential limitations of the screening method applied, the prevalence of aortic structural damage observed is higher than that reported in retrospective studies over a much more extended period. Based on the size of the aneurysm or the resulting aortic valve insufficiency, 5 patients (9.2% of the global series and 42% of

those with aneurysm or dilatation) were considered candidates for surgery. The development of aortic structural damage is, therefore, a major health threat in the outcome of patients with GCA, with a potentially increasing impact given the growing life expectancy of elderly persons in developed countries.

A relevant question arising from the recognition of aortic aneurysm/dilatation as a major and frequent complication of GCA is whether aortic structural damage appears as a consequence of the initial injury or develops progressively due to persisting, low-grade inflammatory activity. Concerns about the potential development of aortic damage as a consequence of persisting low-grade inflammatory activity despite an apparently appropriate response to steroids arise from the repeatedly reported finding of inflammatory infiltrates in surgical or necropsy aortic specimens (4,19,20). However, a critical analysis of the reported cases reveals that the characteristics and extent of inflammatory infiltrates as well as the dose and duration of the corticosteroid treatment received until surgery or necropsy are not described in detail in most reports. Therefore it is not
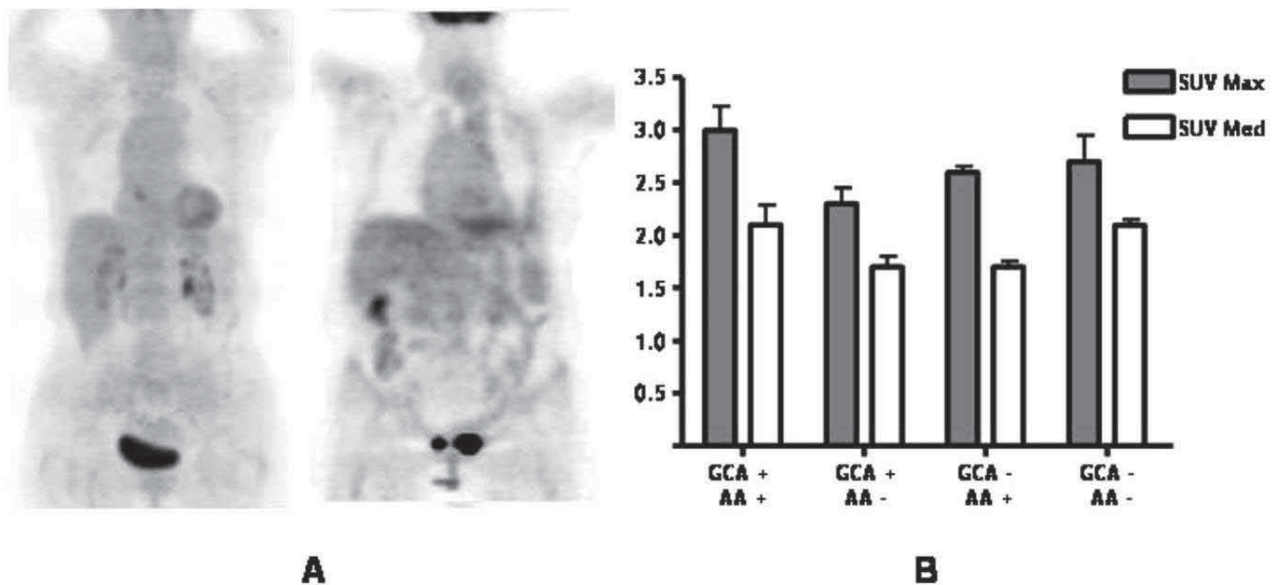
Figure 2. ^{18}F-fluorodeoxyglucose aortic uptake in patients with giant cell arteritis (GCA) according to the presence or absence of aortic aneurysm/dilatation. **A,** Positron emission tomography scan of a patient with GCA-related aneurysm (**left**) compared with a patient with noninflammatory aneurysm (**right**). **B,** Measurement of standard uptake value (SUV) maximal (Max) and median (Med) in patients with GCA and aortic aneurysm (GCA+, AA+), patients with GCA with a normal aorta (GCA+, AA−), patients with noninflammatory aneurysm (GCA−, AA+), and control individuals (GCA−, AA−).

clear whether active or residual inflammation is observed and whether specimens are obtained during active disease or in patients in remission under the current treatment strategy in terms of dose and duration of corticosteroids (13,20,23). Dense granulomatous lesions are usually described in specimens obtained from patients dying from aortic complications during active disease or in patients treated with low corticosteroid doses or treated for short periods (4,13). This important point was addressed by Lie who examined 35 aortic specimens from patients with GCA (19). Lie remarked that active granulomatous lesions were found in patients in whom the samples were obtained shortly after diagnosis, whereas the intensity and extent of inflammatory infiltrates were lower in treated patients. Our data, obtained from a cohort of prospectively treated patients according to the current standard of care, do not support that patients with smoldering or relapsing disease or patients with persistently elevated acute-phase reactants or proinflammatory cytokines are more prone to develop aneurysm/dilatation. The aortic specimens obtained during elective surgery from 2 of our patients showed scattered inflammatory foci in the media. Infiltrating leukocytes immunostained positive for MMP-2 and MMP-9. MMPs, particularly MMP-9, have been considered to be involved in elastin degradation and generation of aortic aneurysm in several models (24–27). However, gelatinolytic signal of MMP-9, which is mainly produced by activated inflammatory cells, was faint in aortic aneurysm compared with active GCA lesions in a temporal artery obtained at diagnosis. In contrast, active MMP-2, which can also be expressed by vascular smooth muscle cells and is involved in vascular reparative mechanisms (28,29), was detected equally in both active GCA lesions and aortic aneurysm. We cannot exclude that remaining infiltrates or

MMP-2 produced during vascular remodeling increases vessel wall damage over the years. However, persisting inflammatory infiltrates were very scarce, whereas elastic lamellae disruption, which is an early finding in experimental aneurysm formation (24), was extensive, possibly as a consequence of the initial inflammatory injury.

Characteristics of the target tissue may play a significant role in the extent of the initial injury. Some patients may have unique substrate characteristics in their aortic tissue, making it more susceptible to aortic inflammation, whereas in others the aorta may remain relatively spared. Once inflammation and injury are established, characteristics of vascular remodeling may vary in different aortic segments. Necropsy studies have indeed demonstrated that inflammatory lesions in GCA equally target the thoracic and the abdominal aorta (4). This is in accordance with recent studies showing a similar proportion of thoracic and abdominal FDG uptake in individuals with active disease (11). However, in all series, thoracic aneurysms are much more frequent than abdominal aneurysms in patients with GCA (12–15). Thoracic and abdominal aortas differ greatly in lumen diameter, wall thickness, vasa vasorum density, content of elastic and collagen fibers, propensity to atherosclerosis, and susceptibility to infection-induced vasculitis (30,31). Thoracic and abdominal aortas may then respond differently to inflammatory injury. In addition, the thoracic aorta is subjected to a higher pressure, which might favor progressive dilatation of a weakened wall. Sex may also influence the development of aortic damage. In our series, aortic structural abnormalities were more frequently observed in men. Male predominance in susceptibility to experimental aortic aneurysms has also been demonstrated in experimental settings (32,33).
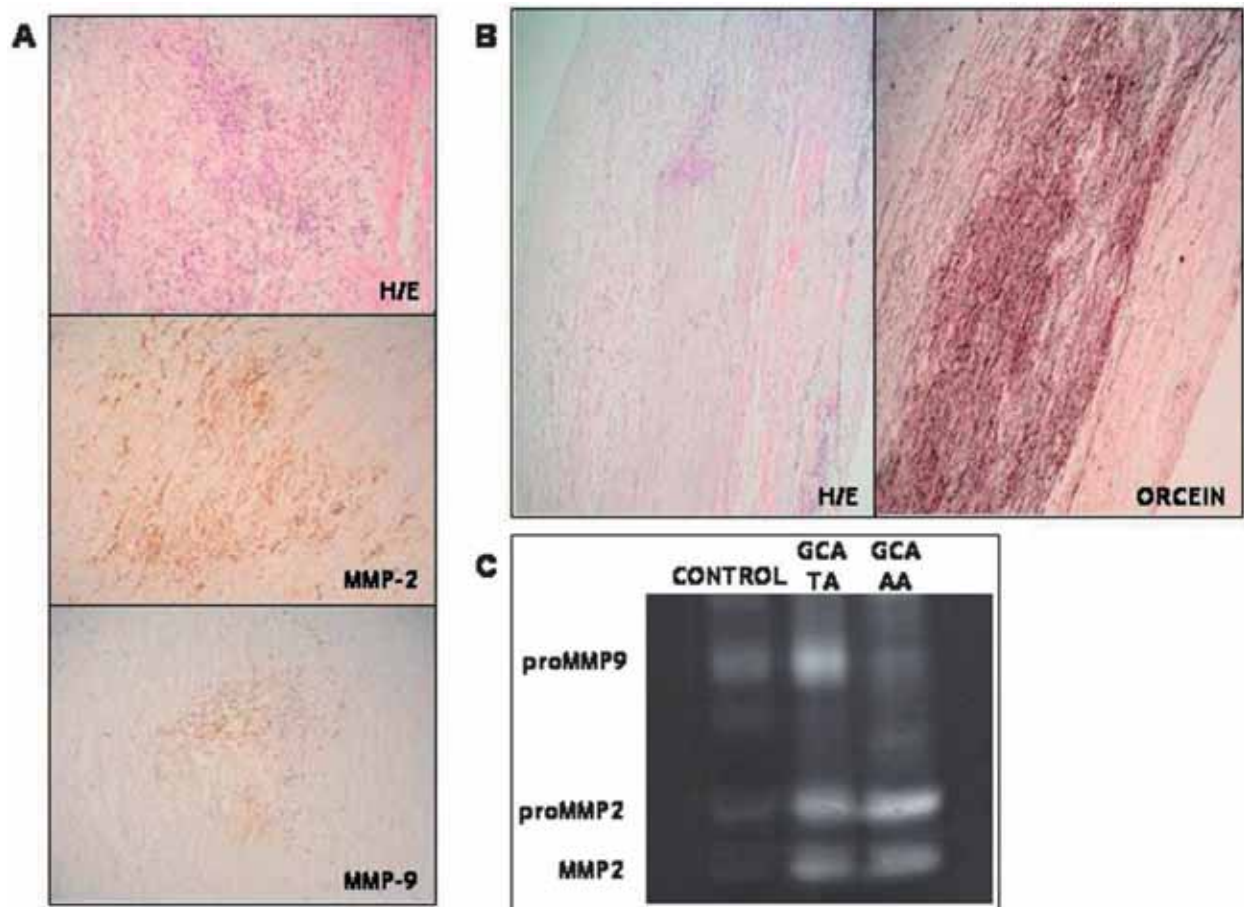
Figure 3. Histopathologic examination of the aortic wall in a patient with giant cell arteritis (GCA)–related aneurysm. **A,** Hematoxylin/eosin (H/E) staining showing scattered chronic inflammatory foci in the media and serial sections showing matrix metalloprotease 2 (MMP-2) and MMP-9 expression by inflammatory cells (magnification × 200). **B,** Lower magnification (magnification × 40) covering a wider area and showing a paucity of inflammatory infiltrates; orcein staining of elastic lamellae in a serial section displaying multiple foci of disruption in areas devoid of inflammatory infiltrates. **C,** Gelatin zymography of tissue extracts (80 μg/lane) from a normal temporal artery (TA); a TA with active, treatment-naive GCA lesions; and a GCA-related thoracic aortic aneurysm (AA). MMP-9 is only detected in active GCA lesions whereas active MMP-2 can be detected both in active lesions and in the GCA-related aneurysm. ProMMP = Promatrix metalloprotease.

Contrary to data gathered from retrospective studies (14,15), we did not observe a higher prevalence of aortic structural damage in patients with traditional cardiovascular risk factors. This may be due to the prospective nature of this study, in which tight control of vascular risk factors was part of the therapeutic approach. The higher prevalence of hypercholesterolemia, and consequently statin therapy, among patients with a preserved aortic wall raises the hypothesis of statins as protective agents against aortic wall structural damage.

In summary, prospective screening shows that a remarkable proportion of patients with GCA develop aneurysm/dilatation, in some instances severe enough to warrant surgical repair. The life-threatening nature of the potential complications derived from aortic structural damage indicates that patients with GCA should be subjected to a continuous surveillance by clinical examination and imaging. Our data do not support that in patients treated according to the current standard of care, aortic aneurysm formation results mainly from persistent activity; our data suggest interplay of heterogeneous factors. Investigating mechanisms involved in the development of aortic structural damage and its progression is of major relevance for patients with GCA.

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EXPRESSION AND CLINICAL RELEVANCE OF RECEPTOR ACTIVATOR OF NFκB (RANK) LIGAND (RANKL), RANK AND OSTEOPROTEGERIN (OPG) IN PATIENTS WITH GIANT-CELL ARTERITIS (GCA).

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Key words: giant-cell arteritis, TNFα superfamily, receptor activator of NFκB, osteoprotegerin.

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ABSTRACT

Objectives. Receptor activator of nuclear factor-κB (RANK) ligand (RANKL) and their receptors, RANK and osteoprotegerin (OPG) are members of the TNFα superfamily with regulatory functions over immune and vascular responses. We tried to investigate the expression and clinical relevance of these cytokines in patients with giant-cell arteritis (GCA).

Methods. Plasma levels of soluble RANKL (sRANKL) and OPG were determined by enzyme-linked immunosorbent assay in 50 patients and 14 controls. Immunohistochemical expression of RANKL, RANK and OPG and mRNA quantification of RANKL and OPG was evaluated in 29 GCA-temporal arteries and compared with control arteries. Clinical manifestations, intensity of the systemic inflammatory response (SIR), number of relapses and corticosteroid requirements were recorded. RANKL and OPG gene expression was also compared with the expression of other inflammatory mediators.

Results. Circulating levels of sRANKL and OPG did not show differences between patients and controls. However, immunohistochemical expression of RANKL and RANK was not detected in control arteries but was remarkable in GCA-temporal arteries, mainly in areas of granulomatous inflammation within the media. OPG was strongly expressed by endothelial cells in both GCA- and control temporal arteries and in a less intense way by the inflammatory infiltrate and smooth muscle cells. mRNA RANKL quantification was higher in patients than in controls. Although, mRNA RANKL was higher in patients with a strong SIR it was not correlated with clinical features or patients outcome. OPG mRNA levels correlated with the expression of other inflammatory mediators.

Conclusions. RANKL/OPG system is highly expressed in GCA-temporal arteries although its expression does not appear to be associated with disease phenotype or clinical outcome. However, we cannot rule out a possible role of these cytokines in vascular remodelling responses.

INTRODUCTION

Giant cell arteritis (GCA) is a granulomatous large- and medium-sized vessel vasculitis. Antigen exposure triggers de activation of resident immature dendritic cells in the adventitia. Once activated, dendritic cells become antigen presenting cells, secret chemokines and recruit CD4+ T cells into the artery wall providing necesary costimulatory signals to trigger T-cell activation and secretion of interferon γ (IFNγ), a pivotal cytokine in the Th1 immune response. Infiltrating macrophages and structural vascular cells secret pro-inflammatory cytokines, reactive oxigen intermediates and growth and angiogenic factors leading to vascular damage and abnormal vascular remodelling that may eventually have clinical consequences for patients, such as ischemic symptoms due to intimal hyperplasia and luminal narrowing or, development of aortic aneurysms. (1-3)

TNFα had been considered a pivotal cytokine in the maintenance of the inflammatory activity in patients with GCA since TNFα production was correlated with number of relapses and corticosteroid requirements. (4-5) However, the blockade of TNFα with infliximab failed to show any corticosteroid-sparing effect suggesting the involvement of other mediators in the maintenance of disease activity. (6)

In the late 1990s, novel members of the TNF/TNF receptor superfamily were identified. Receptor activator of NF-κB (RANK) ligand (RANKL), its membrane receptor, RANK and, its decoy receptor, osteoprotegerin (OPG) emerged as the final effectors of osteoclast activity and bone resorption. (7) Surprisingly, studies in animal models revealed regulatory functions of these cytokines over immune and vascular responses. (8-10)

In *in vitro* studies, RANKL increases the survival of dendritic cells, T cells and monocytes-macrophages and up-regulates the expression of proinflammatory cytokines, chemokines, co-stimulatory molecules, and cell adhesion molecules. (11-19) In animal models of sepsis, RANKL blockade suppressed the secretion of IFN-γ by activated T cells protecting mice from death. (20-21) IFN-γ is thought to be a key cytokine conducting the granulomatous inflammatory response in GCA affected vessels. These results suggest interesting inmunomodulatory functions of RANKL in GCA.

The RANKL/OPG system has also been involved in vascular homeostasis. OPG is a constitutive molecule of vascular cells (endothelial and vascular smooth muscle cells). After an inflammatory stimulus, OPG is released from the Weibel-Palade bodies in endothelial cells triggering cellular responses in the vascular bed such as up-regulation of cell adhesion molecules by endothelial cells or smooth muscle cell proliferation and migration. (22-24) This might be of interest in GCA arteries since smooth muscle cells evolve from their quiescent and contractile phenotype to proliferating and secreting cells that migrate into the intima leading to intimal hyperplasia and ischemic manifestations. Neovascularization is also a remarkable finding in GCA lesions. RANKL and OPG have also shown angiogenic properties in studies in vitro and in vivo models with contradictory results (25-28). OPG increases endothelial cell survival through integrin union and by blocking the tumor necrosis factor-related apoptosis inducing ligand (TRAIL), another member of the TNFα superfamily with apoptotic properties (29-31).

In summary, the RANKL/RANK/OPG axis is expressed by inflammatory and constitutive vascular cells. The expression of these cytokines may be regulated by cytokines and growth factors secreted in GCA lesions (32-34). In

addition, RANKL and OPG might induce immune and vascular cellular responses. Under these circumstances, we suggest that these cytokines might play a role in the pathogenesis of a primary vascular inflammatory disease such as GCA. This is the first attempt to study the role of these cytokines in GCA.

PATIENTS AND METHODS

Patients

Between 1996 and 2003, 110 patients were diagnosed with biopsy-proven GCA in our institution (Hospital Clinic, Barcelona). Clinical data were collected at the time of diagnosis and recorded in a database. Plasma-EDTA was obtained during active disease, before starting therapy or after a single prednisone dose and stored at -80º. Plasma levels of soluble RANKL (sRANKL) and OPG were determined by enzyme-linked immunosorbent assay in 50 patients and compared with 14 healthy controls with similar age and gender distribution. Temporal arteries were also obtained before starting therapy or after a single prednisone dose, included in OCT and stored at -80º. Immunohistochemical expression of RANKL, RANK and OPG was studied in 29 patients and compared with control arteries. Gene expression of RANKL and OPG was also determined in temporal artery tissue from 29 patients and 10 controls. All GCA-temporal arteries exhibited a panarteritic inflammatory infiltrate. Control temporal arteries were obtained from patients in whom GCA was initially considered but subsequently excluded. These patients were finally diagnosed with isolated polymyalgia (two patients), non vasculitic palsy of the VI cranial nerve (one patient), leukaemia (one patient),

rheumatoid arthritis (one patient), non specific headache (two patients) and chronic secondary anemia (three patients).

Clinical findings of the study group were similar to previously published series and are summarized in table 1. Patients were treated according to uniform criteria. The initial prednisone dose was 1 mg/kg per day for 1 month and was subsequently tapered 10 mg/week. Reductions below 20 mg/day were slower and individualized. A further reduction to a maintenance dosage of 10 mg/day was attempted over a 2-month period. The intensity of the systemic inflammatory response was evaluated according to the following parameters at diagnosis: fever, weight loss > 3 kg, ESR ≥ 85 mm/hour and hemoglobin < 110 g/l. (4) It was considered weak if patients had 0-2 inflammatory parameters and strong when they had more than 2 parameters. Follow-up data included number of relapses and glucocorticosteroid requirements assessed as time (weeks) necessary to achieve a maintenance prednisone dose < 10 mg/day, cumulated prednisone dose at that point, cummulated prednisone dose during the first year of follow-up and time to stop therapy.

The study was approved by the Ethics Committee of our institution (Hospital Clínic). Patients signed informed consent for the collection and storage of biologic material.

Measurement of circulating sRANKL and OPG

Circulating sRANKL and OPG were measured in the cohort of patients and controls by using Biomedica Immunoassays according to the manufacture's instructions (sRANKL: cat nº BI-20422 H, OPG: cat nº BI-20402). The detection limit of these kits were 0.08 pM/l for sRANKL and 0.14 pM/l for OPG.

Immunohistochemical study

We obtained 4-6 µm cryostat sections from frozen temporal arteries. Temporal artery sections were stained with the following antibodies: monoclonal mouse antihuman-OPG, polyclonal goat antihuman-RANK and monoclonal mouse antihuman-RANKL (from R&D Systems). Preliminary studies were carried out to determine the optimal concentration of the antibodies providing the strongest specific staining with the lowest background. Anti-OPG was used at a 1/125 dilution, anti-RANK at a 1/25 dilution and anti-RANKL at a 1/150 dilution. Temporal artery sections were air-dried and fixed with cold acetone. Then sections were incubated with the secondary antibodies (from the EnVision-Dako) according to the manufacture's protocol. The extension and distribution of cytokine immunostaining was evaluated at the media and the intima layer using a semiquantitative score from 1 to 4 according to the portion of the artery circumference stained (1: ≤ 25%, 2: 26-50%, 3: 51-75%, 4: 76-100%). Artery sections were independently evaluated by two investigators (MCC and AGM) and when the score was not coincident (< 10%), the average was considered.

Cytokine mRNA quantification

Total RNA was obtained from 120 serial sections of 5 µ thick per temporal artery sample using Trizol following the manufacture's instructions. One µg of total RNA was reverse transcribed to cDNA using the Archive kit (Applied Biosystems, Foster City, CA) employing random hexamers as the priming method. Samples were stored at -20º until use. RANKL and OPG expression was measured by real-time PCR using specific Assay-on-Demand Taqman Gene expression probes from

Applied Biosystems. Target probes and internal control probe (GUSb) were covalently linked to a reporter dye (FAM). PCR reaction was performed with 2 µl of cDNA together with Taqman PCR Universal Master Mix (Applied Biosystems) and the corresponding primers and probe. Each sample was tested twice. PCR reaction conditions followed the standard procedure established by the manufacturer. PCR was monitorized by measuring the fluorescence signal after each cycle with ABI Prism 7900 sequence detection system (Applied Biosystems). C_T Values (cycle number where fluorescence overpassed a fixed threshold) were obtained for each target probe and normalized with the corresponding C_T values for the internal control (GUSb). mRNA quantity was expressed as relative units.

mRNA RANKL and OPG quantification was compared with the expression of other inflammatory mediators: IL-6 (18 patients), IL-1 (18 patients), TNFα (24 patients), IFNγ (26 patients), TGFβ (27 patients), MMP-2, MMP-9, MMP-12, MMP-14, TIMP-1 and TIMP-2 (29 patients). (5, 35-36)

Statistical analysis

U-Mann Whitney test was used for comparing quantitative variables, Spearman test for correlations, and Fisher test for contingency tables.

RESULTS

Circulating levels of sRANKL and OPG

Plasma concentrations of OPG were higher in patients than in controls although the difference did not reach statistical significance (mean 7,731 ± 2,822 vs 6,279 ± 2,384 pM/L, p=0.083). Plasma levels of sRANKL hardly exceeded the

detection limit and did not show differences between patients and controls (mean 0,525 ± 0,161 vs 0,471 ± 0,138 pM/L). We found a positive correlation between OPG levels and age of patients (r=0,428, p<0.001).

Immunohistochemical expression of RANKL, RANK and OPG in temporal arteries

RANKL and RANK were not detected by inmunohystochemistry in control temporal arteries but were highly expressed in temporal arteries from patients with GCA. (Figure 1) Areas of inflammatory infiltrate exhibited the most intense immunostaining, specially macrophages and giant cells contributing to the granulomatous inflammation within the media. The expression of these cytokines was mostly concentrated in cell membranes. (Figure 2) Smooth muscle cells showed a diffuse but less intense expression of both cytokines. Diffuse RANKL staining but not RANK was observed around the luminal edge beneath the endothelial cell layer speacially in arteries with important intimal hyperplasia.

OPG was also expressed by inflammatory infiltrates and smooth muscle cells in GCA temporal arteries but in a less intense way than RANKL and RANK. OPG was also intensively expressed in giant cell membranes. Similarly to RANKL, some arteries exhibited OPG expression at the intima, beneath the endothelial cell layer. Opposite to RANKL, OPG expression at the intima was more frequent in arteries with low degrees of intimal hyperplasia. However, the most intense immunostaining of OPG was observed in endothelial cells from both control and GCA-temporal arteries. Vasa vasorum and neovessels within the media also exhibited intense OPG expression. (Figure 1)

Sixty-nine percent of patients exhibited a strong RANKL expression (score 3 or 4) and 31 % a low RANKL expression (score 1 or 2) by immunohistochemistry. For OPG we considered a strong expression when the immunostaining score was equal or higher than 2, as OPG was less intensively expressed than RANKL. Thus, 41 % of patients exhibited a strong OPG expression whereas 59 % had a weak OPG expression. The score of RANKL and OPG expression in GCA temporal arteries did not correlate with clinical manifestations, intensity of the systemic inflammatory response, disease relapses during follow-up or corticosteroid requirements.

Gene expression of RANKL and OPG in temporal arteries

RANKL mRNA levels were significantly higher in patients than in controls (mean 5,61 ± 3,34 vs 1,88 ± 1,24 relative units, $p<0.0001$) and among patients, they were higher in those with a strong systemic inflammatory response at diagnosis (mean 8,26 ± 2,84 vs 4,43 ± 2,89 relative units, $p=0.004$). (Figure 3A and 3C) However, mRNA RANKL levels were not correlated with number of relapses during follow-up or corticosteroid requirements.

OPG mRNA levels did not show differences between patients and controls (mean 1,32 ± 1,28 vs 1,12 ± 0,75 relative units, $p=0.64$) indicating constitutive expression in normal arteries. There were not differences either between patients with a weak and strong systemic inflammatory response. (Figure 3B and 3D) Opposite to what was observed in circulating levels, mRNA OPG quantification in temporal arteries was not correlated with age.

No significant correlation was found between RANKL and OPG mRNA levels ($r=-0.308$, $p=0.081$).

Correlation with other inflammatory mediators

mRNA RANKL and OPG levels were compared with mRNA levels of other inflammatory mediators involved in the pathogenesis of GCA and evaluated in previous studies. (5,35-36) Gene expression of OPG in GCA-patients correlated positively with mRNA IL-6 (n=18, r=0.577, p=0.012) and mRNA TIMP-1 (n=29, r=0.706, p<0.001) but was negatively correlated with mRNA MMP-9 expression (n=29, r= -0.45, p=0.014). (Figure 4) However, mRNA RANKL levels did not correlate with any of the genes evaluated.

DISCUSSION

The role of cytokines in the pathogenesis of GCA has been broadly demonstrated. They drive both autocrine-paracrine and systemic responses and some of them have been associated with clinical manifestations or disease outcome (4-5,37). In the last years, novel cytokine families have been described, making this network of mediators and signals more and more complex. Some of these cytokines activate common signaling pathways that ultimately activate transcription factors, such as the NFκB which plays a central role in the development of inflammation. NFκB is also activated by the RANKL/RANK/OPG system. The role of these cytokines has been extensively studied in metabolic bone disorders and denosumab, a human monoclonal antibody against RANKL, has shown promising results in the treatment of osteoporosis and bone metastasis. (38-40) However, biological activity of these cytokines over the immune and vascular systems has been scarcely studied in humans and remains unknown.

Our study demonstrates up-regulation of this set of cytokines in patients with GCA. The expression of RANKL and its receptors, RANK and OPG is up-regulated in GCA temporal arteries if compared with control arteries. However, the increase in tissue expression was not followed by an increase in circulating cytokine levels since we could not find significant differences in serum concentrations between patients and controls. Moreover, in spite of RANKL gene expression was significantly increased in patients than in controls, specially in patients with an intense systemic inflammatory response, it was not correlated with number of relapses or corticosteroid requirements. One possible explanation is that RANKL could act mainly locally in the artery wall, in an autocrine-paracrine manner. Contrary to other cytokines, such as TNFα or IL-6, RANKL would be scarcely secreted into the bloodstream and it would not exhibit activity over peripheral tissues in GCA. OPG gene expression and OPG circulating levels did not differ between patients and controls in spite of greater immunohistochemistry expression in GCA temporal arteries. The difference between vascular and peripheral cytokine levels might suggest that other tissues are contributing to circulating cytokine concentrations. Accordingly, the correlation found between OPG plasma levels and age might reflect an attempt to compensate the pro-resorptive bone status that eventually takes place in older patients by up-regulating OPG production. Other authors have also found a positive correlation between circulating OPG levels and age in general population or in patients with other conditions such as chronic kidney disease or systemic sclerosis (41-43). In addition, it might also indicate a more extense atherosclerotic vascular disease in older patients which correlates with higher levels of OPG in serum.

It should be remarked that, whereas circulating OPG levels were similar to those observed in previous studies, plasma concentrations of sRANKL hardly exceeded the detection limit. It is not possible to rule out that sRANKL concentration might be understimated in our study due to the long time of sample storage which might justify certain cytokine degradation (44). Moreover, the detection system could only measure the soluble fraction of RANKL but not membrane-bound RANKL which also have effector functions.

More than relevant systemic effects, the RANKL and OPG axis might conduct autocrine-paracrine responses in GCA. The greatest expression of RANKL and RANK was seen within the media, in areas of granulomatous inflammation where the macrophages and giant cells are the main cell type. It is not unreasonable to think that these cytokines might regulate macrophage function and giant cell formation. In bone physiology, RANKL/RANK interaction is necessary to produce mature osteoclasts which are also multinucleated cells derived from hematopoietic cells of the monocyte-macrophage lineage (45-47). In addition, RANKL and OPG might also modulate the expression of proteolytic systems in GCA arteries. In cultured osteoclasts RANKL increased the expression of MMP-9 and inhibited the expression of TIMP (1 and 2) favouring pro-resorptive responses in bone. A different study found that OPG increased the expression of MMP-9 but also increased the expression of TIMP-1 resulting in a reduction of the ratio MMP-9/TIMP-1 and inhibition of the proteolytic activity (48-49).

GCA temporal arteries exhibit higher proteolytic status driven by an increase in MMPs expression and in the ratio MMPs/TIMPs thus favouring vessel-wall disruption which is necessary for the development of intimal hyperplasia (36,50) In our study, mRNA OPG levels were negatively correlated with mRNA

MMP-9 and positively correlated with mRNA TIMP-1, the natural inhibitor of MMP-9. Thus, OPG gene expression is apparently associated with a lower ratio MMP9/TIMP1 therefore reducing the proteolytic gene status in GCA-temporal arteries.

It is likely that the RANKL/OPG system plays a role in the development of calcified lesions in atherosclerotic disease (51-53). Atherosclerosis does not appear to be a major clinical problem in GCA patients in whom major vascular complications occur as a result of abnormal vascular remodelling in response to inflammation. Smooth muscle cells expressed RANKL, RANK and, in a less intense way, OPG. Proliferation, migration and secretory properties of smooth muscle cells might be regulated by these cytokines therefore determining structural changes in the artery wall. RANKL staining was also observed at the intima, beneath the endothelial cell layer. It is likely that this reactivity might be provided by myofibroblasts that eventually migrate from the media since it is specially observed in arteries exhibiting major degrees of intimal hyperplasia. We suggest that OPG released after inflammatory injury might inhibit gelatinase activity which is essential for rupture of the elastic lamina and vascular remodelling. Maladaptative vascular remodelling accounts for the major vascular complications of large-vessel vasculitis. Smooth muscle cell degradation and rupture of elastic fibers by gelatinases are thought to be involved in aortic aneurysm development in GCA patients. Otherwise, ischemic manifestations in GCA have been attributed to the development of intimal hyperplasia which is also associated with an increase in proteolytic activity that leads to elastic membrane rupture and allows the migration of miofibroblasts towards the intima. Regulation of proteolytic systems might be of interest in the prevention of large-vessel complications.

Several inflammatory mediators stimulate up-regulation of RANKL and OPG by vascular cells (32-34). We found a positive correlation between gene expression of OPG and mRNA IL-6 levels. In previous studies, IL-6 exhibited angiogenic properties and its expression was associated with higher angiogenic scores and lower prevalence of ischemic events in patients with GCA (37). Accordingly, OPG expression which mostly relied on endothelial cells was correlated with mRNA IL-6 levels. OPG might also have pro-angiogenic properties as it increases endothelial cell survival, proliferation and migration through integrin attachment and by blocking TRAIL, a pro-apoptotic mediator of the TNF-superfamily (29-31,54) However, in our cohort of patients, tissue expression of OPG did not differ between patients with or without ischemic manifestations.

In summary, this is the first attempt to describe RANKL, RANK and OPG function in patients with GCA. At first glance, they do not appear to have correlation with clinical findings despite they were intensively expressed in GCA temporal arteries and correlated with other mediators secreted during the inflammatory response. However, the role of the RANKL/RANK/OPG triad in vascular homeostasis is poorly understood, and at this point it is not possible to rule out a possible role of these cytokines in the remodelling vascular phenomena that takes place after the inflammatory injury. We have described the expression and distribution of these cytokines in GCA lesions although it might be interesting to perform functional studies in order to determine specific roles of these cytokines in GCA. Confirmation of OPG as anti-proteolytic and vascular protective molecule might be interesting in order to prevent clinical manifestations due to vessel structural disease, such as ischaemic events or aortic aneurysms. Finally, we have not studied the effects of these cytokines over bone physiology in GCA patients,

that are prone to develop osteoporosis as a consequence of corticosteroid therapy and chronic inflammation. The demostration of a relevant clinical effect over bone resorption might offer new therapeutic targets to treat corticosteroid-related osteoporosis.

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Table 1. Clinical findings in the study cohort of patients with GCA

Clinical manifestations	Circulating cytokines (n=50)	mRNA quantification (n=29)
General characteristics		
Age, median (range)	77 (58-91)	76 (63-91)
Sex, no. female/male	35 / 15	19 / 7
Cranial symptoms, N (%)		
Headache	35 (70)	22 (84,6)
Jaw claudication	21 (42)	14 (53,8)
Scalp tenderness	20 (40)	17 (65,4)
Other cranial aches*	24 (48)	19 (73,1)
Abnormal temporal arteries[†]	45 (90)	25 (96,2)
Cranial ischemic events[‡]	16 (32)	5 (19,2)
Systemic symptoms, N (%)		
Fever	11 (22)	7 (26,9)
Weight loss	23 (46)	10 (38,5)
Polymyalgia rheumatica	25 (50)	10 (38,5)

* Other cranial aches (facial pain, ocular pain, tongue pain, earache, carotidynia, toothache, odynophagia).
[†] Abnormal temporal arteries at physical examination (painful, swollen, indurated and/or with decreased or absent pulsation).
[‡] Permanent visual loss, amaurosis fugax, transient diplopia, stroke, transient ischemic attack, tongue ischemia.

Figure legends

Figure 1. Immunohistochemical detection of RANKL, RANK and OPG in temporal arteries from GCA-patients and controls.

Figure 2. Immunohistochemical detection of RANKL, RANK and OPG in the granulomatous infiltrate. All three sections shown intense membrane cytokine inmunostaining in giant cells.

Figure 3. mRNA RANKL and OPG quantification. A) mRNA RANKL and B) mRNA OPG levels in temporal arteries from patients and controls. C) mRNA RANKL and D) mRNA OPG quantification in GCA-temporal arteries from patients with a weak and patients with a strong SIR at diagnosis.
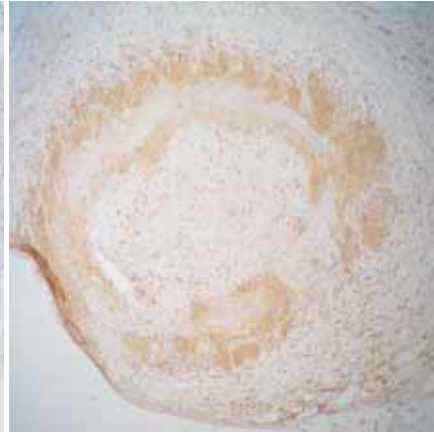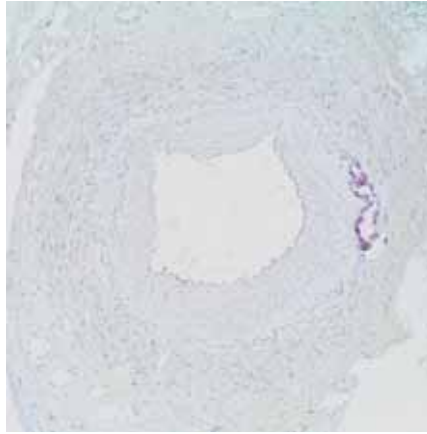
Figure 4. Correlation between mRNA OPG and mRNA MMP-9 (r = -0.45, p = 0.014) (A) and mRNA TIMP-1 (r = 0.706, p < 0.001) (B) levels in GCA-temporal arteries.
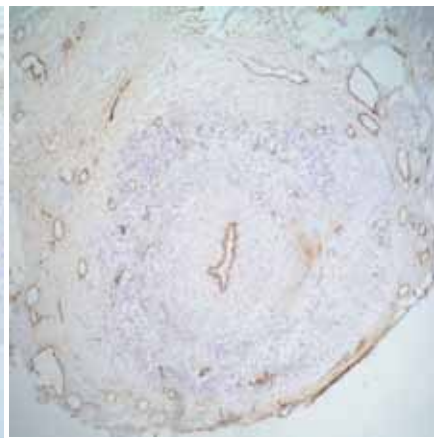
Figure 1
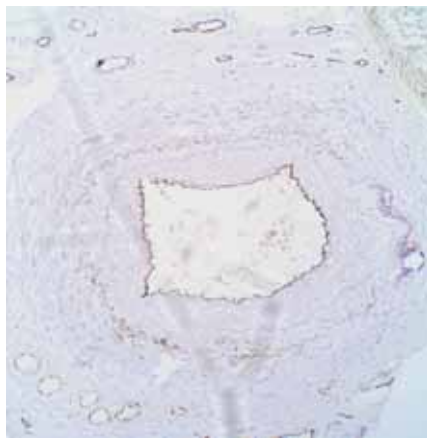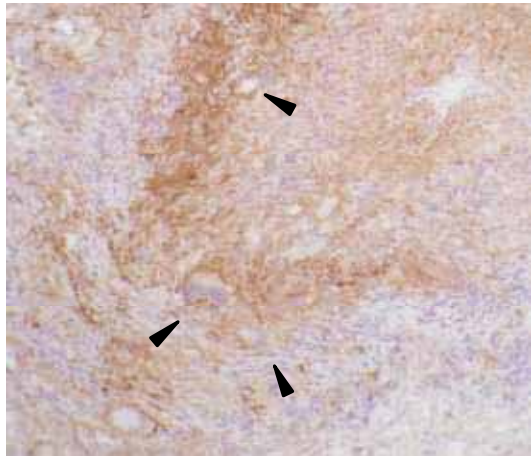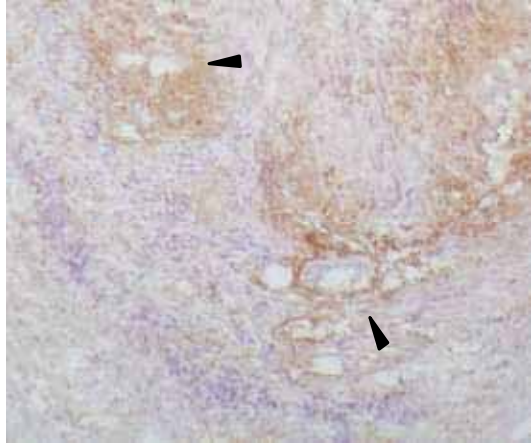

RANKL
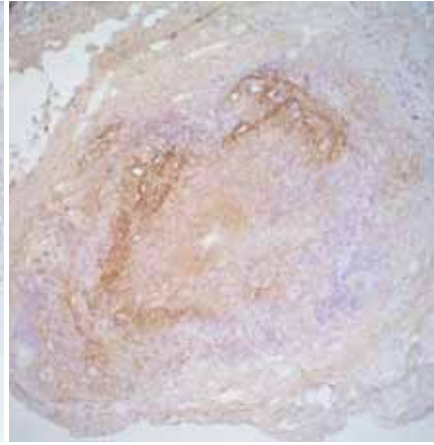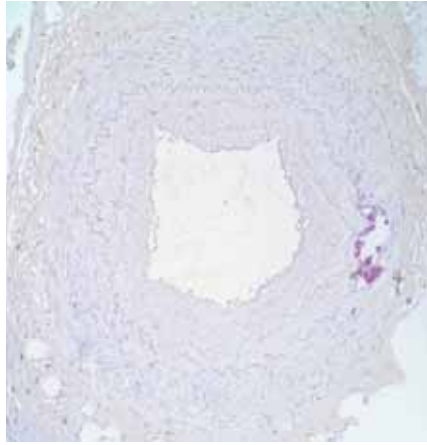
RANK

OPG

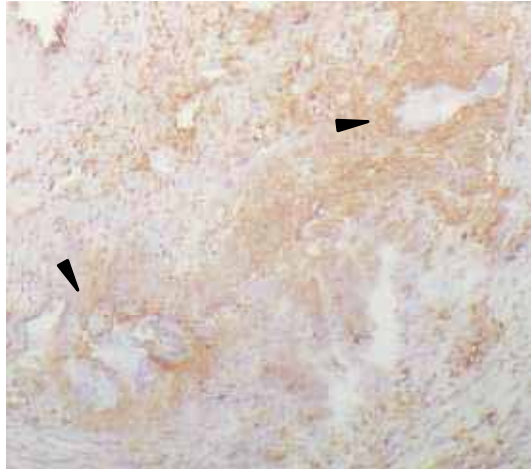CONTROLS   GCA
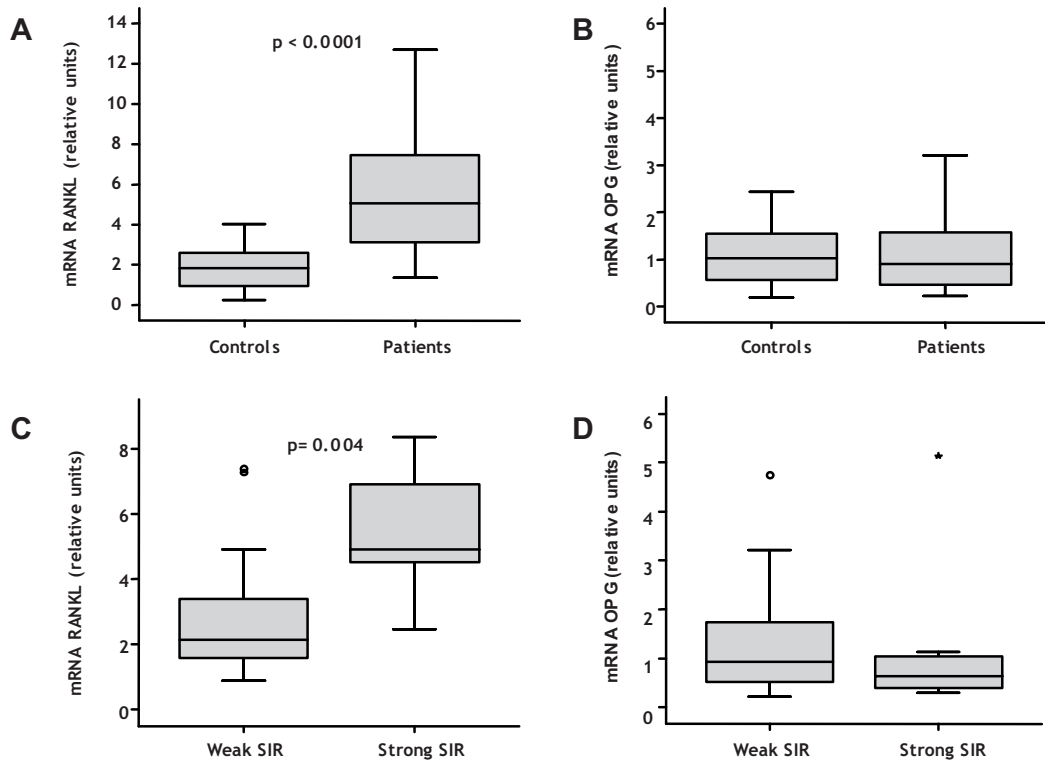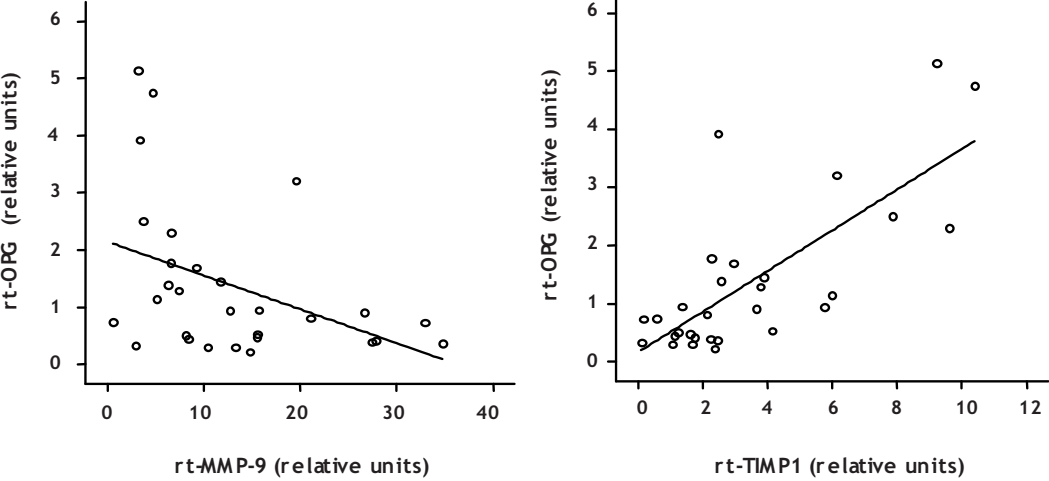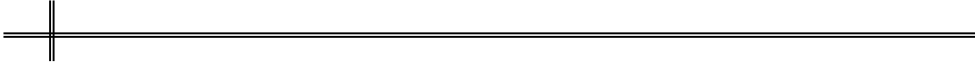
Figure 2

RANKL

RANK

OPG

Figure 3

Figure 4

V. DISCUSIÓN

Los pacientes con ACG pueden presentar niveles moderadamente elevados de IL-6 y otros biomarcadores inflamatorios durante los meses siguientes al diagnóstico a pesar de alcanzar la remisión clínica con el tratamiento corticoideo (*Weyand et al, 2000; Cid et al, 1996*). El primer trabajo de esta tesis evalúa por primera vez los niveles séricos de IL-6 y TNFα en pacientes en remisión con un periodo de seguimiento prolongado (mínimo de cuatro años) y confirma que ambas citocinas se mantienen elevadas en un porcentaje importante de pacientes. Cerca del 80% de los pacientes de la serie muestran niveles circulantes de IL-6 y TNFα por encima de los valores normales de referencia. La concentración sérica de ambas citocinas fue significativamente más elevada en pacientes que en un grupo de controles sanos de la misma edad. El resultado fue similar al comparar los controles con el grupo de pacientes que habían alcanzado una remisión definitiva y no precisaban tratamiento.

No se conoce el origen de dicha respuesta inflamatoria subclínica ni la repercusión que pueda tener sobre el paciente. Un posible origen podría ser la pared arterial de los grandes vasos, entre ellos la aorta. El estudio histológico de piezas quirúrgicas o necropsias, además de una importante desestructuración de la pared arterial, a veces muestra persistencia de infiltrados inflamatorios, incluso en pacientes tratados durante periodos de tiempo más o menos largos (*Lie, 1995*). No obstante, muchos de estos estudios no describen con detalle las características del infiltrado inflamatorio ni tampoco el régimen terapéutico recibido hasta el momento en que se obtuvo la muestra. Por tanto, no es posible concretar si el

infiltrado inflamatorio es residual o por actividad de la enfermedad, o si los especimenes aórticos fueron obtenidos en pacientes que se encontraban en aparente remisión o en pacientes con enfermedad activa. No obstante, Lie sugirió que las características del infiltrado inflamatorio se modifican a lo largo del tiempo, en función del grado de actividad de la enfermedad y la dosis de corticoides recibida. Las muestras que se obtienen de pacientes con diagnóstico reciente de ACG, suelen presentar lesiones granulomatosas activas y densas similares a las que se observan en la arteria temporal en el momento del diagnóstico mientras que, la intensidad y la extensión del infiltrado inflamatorio es menor en pacientes tratados durante periodos de tiempo más largos.

Es importante remarcar que las células inflamatorias no son la única fuente de citocinas circulantes. De hecho, las células miointimales procedentes de arterias temporales cultivadas producen importantes cantidades de IL-6 (*Lozano et al, 2008*). También hemos visto que el sobrenadante de arterias temporales cultivadas muestra unos niveles muy elevados de IL-6, tanto si la arteria procede de controles o de pacientes con ACG, aunque como era de esperar en los segundos los niveles de IL-6 fueron significativamente más elevados (resultados descritos en Vías de continuidad) (*García-Martínez et al, 2007*). Por tanto, la persistencia de actividad inflamatoria subclínica podría ser consecuencia tanto de la presencia de focos de inflamación residual en la pared arterial, como del fenómeno de remodelado vascular continuado a lo largo del tiempo. Cualquiera de estas dos hipótesis podría justificar el desarrollo de

complicaciones estructurales vasculares a largo plazo. Por otra parte, las diferencias que se observan entre individuos en los niveles de citocinas circulantes podrían explicarse por la existencia de determinados polimorfismos genéticos. De hecho, en pacientes con polimialgia reumática, la presencia del polimorfismo 174 C/C se asocia a unos niveles más elevados de IL-6 tanto al diagnóstico como durante la evolución, aunque no se correlaciona ni con la expresión clínica ni con el pronóstico de la enfermedad. En ese estudio se observó que entre los controles, los portadores del genotipo C/C también presentaban niveles más elevados de IL-6 (*Boiardi et al, 2006*).

En los últimos años, múltiples estudios señalan que algunas enfermedades inflamatorias crónicas se asocian a arteriosclerosis acelerada y enfermedad cardiovascular (*Roman et al, 2003, 2006; Van Leuven et al, 2006*). Es más, la presencia de niveles moderadamente elevados de biomarcadores inflamatorios en la población general también se asocia con un mayor riesgo de sufrir eventos cardiovasculares (*Tzoulaki et al, 2005; Ridker et al, 2008*). En nuestra serie, no observamos diferencias significativas en los niveles de citocinas/reactantes de fase aguda entre los pacientes que durante el seguimiento desarrollaron eventos vasculares y los que no. No obstante, estos datos deben ser analizados con cautela ya que sólo el 13% de los pacientes de la serie desarrolló complicaciones, cifra pequeña teniendo en cuenta la elevada prevalencia de enfermedad cardiovascular en este grupo de edad. Es posible que el estudio de una serie de pacientes más numerosa o durante un periodo de tiempo más largo pudiera evidenciar

una mayor prevalencia de eventos cardiovasculares en los pacientes con citocinas elevadas. La principal limitación en este sentido es la avanzada edad de los pacientes con ACG, que hace difícil plantear un estudio a más largo plazo. Tampoco pudimos determinar la contribución de la ACG frente a los tradicionales factores de riesgo cardiovascular en el desarrollo de estos eventos, debido al carácter retrospectivo del trabajo y la ausencia de un estudio histológico de los vasos afectados.

Aunque todos los pacientes estaban en remisión clínica en el momento en que se realizó la valoración, la concentración sérica de ambas citocinas después de años de evolución de la enfermedad, fue significativamente más elevada en pacientes que habían presentado alguna recidiva durante el seguimiento. Los niveles de IL-6 también fueron significativamente más elevados en pacientes que no habían podido suspender el tratamiento y precisaban dosis bajas de prednisona para el control de los síntomas. Podríamos pensar que en estos pacientes la enfermedad permanece activa en el momento de la valoración y por ello presentan niveles más elevados de citocinas proinflamatorias. Sin embargo, al comparar los pacientes que estaban en remisión estable sin necesidad de tratamiento con los controles, los niveles de ambas citocinas también fueron significativamente más elevados en los primeros. En este grupo la concentración de IL-6 y TNFα superaba el límite de normalidad en el 63 y 85% de los casos respectivamente. Es importante destacar, que salvo la correlación entre IL-6 y PCR, no había relación entre los niveles de citocinas circulantes y el resto de parámetros de fase aguda utilizados en la práctica

clínica diaria. Esto elimina el posible sesgo que se hubiese podido crear si hubiésemos realizado pautas de tratamiento más conservadoras en aquellos pacientes que tenían elevación de parámetros de fase aguda (VSG, PCR, haptoglobina) durante el seguimiento.

En estudios previos la expresión de TNFα en las lesiones en el momento del diagnóstico se correlacionaba con la persistencia de actividad de la enfermedad y los requerimientos terapéuticos (*Hernández-Rodríguez et al, 2004*). En el estudio actual, los niveles circulantes de IL-6 y TNFα tras un seguimiento prolongado también se relacionaron con un curso evolutivo más refractario y necesidades de tratamiento más elevadas. A pesar de que el TNFα persiste elevado en los pacientes refractarios, su bloqueo con infliximab no facilitó el mantenimiento de la remisión inducida por corticoides en un grupo de pacientes con ACG de reciente diagnóstico (*Hoffman et al, 2007*). A pesar de que el TNFα puede tener utilidad como marcador de actividad de la enfermedad, no significa que intervenga en el mantenimiento de la misma o que sea la única fuente de dicha actividad inflamatoria. Los mecanismos que perpetúan la respuesta inflamatoria en la ACG son complejos y pueden serlo cada vez más, ya que continuamente se van descubriendo nuevos mediadores inflamatorios. Es posible que las citocinas estudiadas simplemente sean un reflejo de la persistencia de actividad inflamatoria aunque no formen parte de los mecanismos que la perpetúan a lo largo del tiempo. Es por ello que, el bloqueo de un marcador biológico de forma aislada no aporta beneficios al tratamiento estándar con corticoides. La IL-6 está considerada como uno de los principales

inductores de la respuesta de fase aguda. Sin embargo, modelos animales incapaces de sintetizar IL-6, presentan una respuesta de fase aguda normal tras ser estimulados con lipopolisacáridos bacterianos, lo que sugiere que existen otras vías adicionales que permiten la activación de dicha respuesta (*Fattori et al, 1994*). El bloqueo de la IL-6 no se ha probado en la ACG aunque, a la vista de los resultados, es posible que el resultado fuese similar al del bloqueo del TNFα. En este sentido, se hace recomendable analizar el posible beneficio de un tratamiento en estudios funcionales, antes de ser utilizado en pacientes.

A pesar de que los niveles de IL-6 y TNFα se correlacionaron con mayores requerimientos de corticoides, esto no se tradujo en una mayor prevalencia de complicaciones derivadas del tratamiento. El carácter retrospectivo del estudio limita el valor de estas conclusiones, ya que es posible que no se hayan recogido todos los eventos adversos. Una de las principales complicaciones de los corticoides es el desarrollo de osteoporosis. En nuestro estudio, sólo se tuvieron en cuenta las fracturas sintomáticas pero no se investigó de forma sistemática el colapso vertebral asintomático que con el tiempo puede dar lugar a alteraciones estructurales esqueléticas. De todas formas, el desarrollo de efectos secundarios no sólo depende de la dosis de corticoides recibida, sino que existen factores individuales que favorecen su aparición, como la presencia de osteopenia o de síndrome metabólico en el momento del diagnóstico (*Queralt et al, 2000; Uzu et al, 2007*).

A pesar de no dar lugar a complicaciones significativas, la persistencia de actividad inflamatoria subclínica podría asociarse a síntomas inespecíficos generales (ej. astenia), lo que podría deteriorar la calidad de vida de los pacientes. Sorprendentemente, en este estudio la calidad de vida percibida por nuestros pacientes fue elevada teniendo en cuenta la avanzada edad y las limitaciones que ésta comporta. La puntuación obtenida mediante la escala de valoración de calidad de vida no se relacionó con los niveles de citocinas circulantes, a pesar de que éstos últimos fueron significativamente más elevados en pacientes que tenían una enfermedad más refractaria y habían presentado más recidivas clínicas. De todas formas, la escala utilizada, a pesar de ser sencilla y fácil de entender para los pacientes, no ha sido validada en grupos de población más amplios por lo que estos resultados tienen un valor limitado.

En este primer estudio hemos confirmado que los niveles de IL-6 y TNFα pueden permanecer moderadamente elevados durante periodos de tiempo prolongados, incluso en pacientes que están en remisión. A pesar del tiempo transcurrido desde el diagnóstico de la enfermedad, los niveles de ambas citocinas siguen correlacionándose con el número de recidivas y los requerimientos terapéuticos. Sin embargo, esto no parece tener relación con el desarrollo de complicaciones vasculares clínicamente significativas o con la aparición de efectos secundarios atribuibles a una mayor exposición a los corticoides.

Sobre esta misma serie de pacientes se realizó un estudio prospectivo con el objetivo de, analizar la prevalencia de alteraciones estructurales aórticas significativas, así como los factores relacionados con su desarrollo. El estudio sistemático de estos 54 pacientes demostró alteraciones estructurales aórticas en forma de aneurisma o dilatación en 12 de ellos (22.2% de la serie) tras una mediana de seguimiento de 5.4 años (rango 4-10.5 años). En el 42% de pacientes con aneurisma aórtico, las dimensiones del mismo hacían recomendable su reparación quirúrgica. La prevalencia de alteraciones estructurales aórticas fue superior a la descrita en estudios previos, todos ellos retrospectivos que incluyen pacientes diagnosticados durante periodos de tiempo más prolongados y con un tiempo de seguimiento también mayor. La aplicación de un protocolo diagnóstico inocuo y fácil de realizar en la práctica clínica diaria, permite detectar alteraciones estructurales en fases precoces que de otra manera podrían pasar desapercibidas. Todos los estudios, incluido el nuestro, muestran una mayor frecuencia de alteraciones a nivel de los segmentos torácicos de la aorta, a diferencia de los aneurismas de origen arteriosclerótico, que se desarrollan principalmente a nivel de la aorta abdominal. De hecho, en esta serie todos los pacientes tributarios de cirugía presentaban aneurismas a nivel de la aorta torácica ascendente y sólo un paciente presentaba un aneurisma de pequeñas dimensiones a nivel de la aorta abdominal.

La presencia de aortitis es frecuente en la ACG. Estudios necrópsicos describen una prevalencia de aortitis superior al 90% de los

pacientes y por técnicas de imagen se detectan datos sugestivos de aortitis entre el 50 y 75% de los casos. Sin embargo, no todos los pacientes con aortitis desarrollarán complicaciones estructurales aórticas clínicamente significativas. Además de la agresión inflamatoria inicial, es posible que otros factores determinen el desarrollo de estas complicaciones durante el seguimiento. Una de las hipótesis plantea la posibilidad de que la actividad inflamatoria inicial de la pared aórtica persista de forma residual aunque en menor grado durante los años posteriores al diagnóstico. Esta lesión inflamatoria residual no sería lo suficientemente intensa como para dar lugar a síntomas pero sí sería capaz de producir daño estructural progresivo en la pared aórtica. Los datos obtenidos de nuestra serie no sugieren que el desarrollo de aneurisma/dilatación aórticos esté relacionado con la persistencia de actividad inflamatoria o con una enfermedad más refractaria o de evolución más tórpida. Al contrario, las alteraciones estructurales aórticas fueron más frecuentes entre pacientes que mostraban una respuesta inflamatoria sistémica más débil en el momento del diagnóstico, pacientes que presentaron menos rebrotes clínicos durante el seguimiento y que en definitiva pudieron suspender antes el tratamiento corticoideo. Además, los niveles séricos de proteínas de fase aguda y citocinas proinflamatorias en el momento de la valoración también tendían a ser menores en aquellos pacientes que habían desarrollado aneurismas o dilatación aórticos. Tampoco el estudio de algunos pacientes mediante PET detectó aumento de captación de radiotrazador a nivel de la pared aórtica, que pudiera sugerir persistencia de actividad inflamatoria. Por otra parte, el análisis histológico de las

muestras aórticas procedentes de los dos pacientes sometidos a cirugía reparativa, evidenció algún foco aislado de células inflamatorias a nivel de la media arterial pero en ningún caso mostró un infiltrado inflamatorio denso como los descritos en casos de enfermedad activa. En ambos casos observamos una importante pérdida de fibras elásticas que aparecían completamente desorganizadas. Por tanto, podemos concluir que el desarrollo de alteraciones estructurales aórticas no parece debido fundamentalmente a la persistencia de actividad inflamatoria en la pared arterial. Es posible que estas alteraciones tengan un origen multifactorial. El daño inflamatorio inicial podría dar lugar a la pérdida de fibras elásticas y desestructuración de la pared aórtica. Sobre esta pared desestructurada actuarían otros factores que con el tiempo darían lugar a la progresiva dilatación del vaso. Factores locales que pudiesen modular de alguna manera tanto el tipo de respuesta inflamatoria que se produce en la pared arterial, como el fenómeno de remodelado vascular posterior. Esta regulación puede variar entre individuos y dar lugar a las diferentes expresiones clínicas de la enfermedad. Entre los factores que deberíamos estudiar se incluyen factores biológicos, bioquímicos o biomecánicos. En los siguientes párrafos se plantean algunas hipótesis sobre la posible implicación de algunos de estos factores en el desarrollo de aneurismas aórticos.

La mayoría de alteraciones estructurales aórticas en pacientes con ACG se desarrollan a nivel de la aorta torácica ascendente. La composición y estructura del árbol vascular varía en función del territorio analizado.

Algunos autores sugieren que las propias características del tejido aórtico podrían determinar la extensión inicial de la enfermedad y el desarrollo de alteraciones posteriores. El predominio de manifestaciones clínicas a nivel del territorio cráneo-cervical sugiere que la enfermedad podría tener un tropismo especial por la aorta proximal y sus ramas. Sin embargo, tanto los estudios necrópsicos como las técnicas de imagen han demostrado aortitis en toda la extensión de la pared aórtica y no únicamente en segmentos torácicos (*Ruddy et al, 2008; Hoffman, 2005, 2008*). Los segmentos torácico y abdominal de la aorta son diferentes tanto en el diámetro de la luz del vaso, como en el grosor de la pared arterial, la densidad de *vasa vasorum*, la cantidad de fibras elásticas y de colágeno así como la susceptibilidad para desarrollar arteriosclerosis o vasculitis inducida por agentes infecciosos (*Hoffman, 2003*). Se han observado diferencias entre la aorta torácica y la abdominal tanto en mecánica vascular, como en el perfil proteolítico o las vías de señalización celular, todos ellos, factores que podrían estar relacionados con el desarrollo de aneurismas aórticos. Para empezar, los diferentes segmentos aórticos tienen un origen embriológico distinto. Mientras que la mayor parte del árbol vascular deriva del mesodermo, el ectodermo da lugar a las estructuras vasculares de cabeza y cuello, así como raíz y cayado aórticos, tronco de la arteria pulmonar y segmentos proximales de los troncos supraórticos. Se han observado diferencias entre las células musculares lisas vasculares de origen ectodérmico y las que derivan del mesodermo (*Ruddy et al, 2008*). Es posible que esta distinción contribuya a que la respuesta vascular a la inflamación se regule de forma diferente en función del segmento aórtico analizado y que por tanto, se

favorezca el desarrollo de alteraciones estructurales en unos territorios pero no en otros.

Por otra parte, varios estudios sugieren el papel de algunos sistemas proteolíticos, principalmente gelatinasas y sus inhibidores naturales (TIMPs), en la desestructuración de la pared aórtica (*Koullias et al, 2004; Longo et al, 2002; Ikonomidis et al, 2004; Lesauskaite et al, 2006*). La elastina y el colágeno (la mayoría fibras de tipo I y tipo III) son los elementos estructurales más abundantes de la pared aórtica. El colágeno aporta resistencia mientras que las fibras elásticas se encargan de mantener la estructura de la pared vascular frente al estrés hemodinámico. Ambos elementos pueden ser degradados por la acción de enzimas proteolíticas (*Dobrin y Mrkvicha, 1994; Barbour, Spinale e Ikonomidis, 2007*). MMP2 y MMP9, gelatinasas con actividad elastinolítica, degradan la elastina y las membranas basales y se han relacionado con la formación de aneurismas aórticos en distintos modelos. La MMP12, elastasa con capacidad para degradar fibras elásticas, también se ha relacionado con la formación de aneurismas aórticos. La principal fuente de MMP12 son los macrófagos infiltrantes. Durante el desarrollo del aneurisma se produce la fragmentación de las fibras elásticas y disminuye la concentración de elastina. De hecho, la pérdida de fibras elásticas es uno de los primeros hallazgos que se observan en la formación de un aneurisma. Las arterias temporales de pacientes con ACG suelen presentar un infiltrado inflamatorio panarterítico que invade las tres capas de la arteria. En estos casos, la lámina elástica interna aparece fragmentada, a diferencia de los

pacientes en los que el infiltrado inflamatorio queda limitado a la adventicia en los que la lámina elástica interna suele permanecer indemne. La expresión de gelatinasas (MMP2, MMP9 y MMP14) está aumentada en el área granulomatosa de arterias temporales de pacientes con ACG, así como la actividad gelatinolítica a ese nivel, sugiriendo un posible papel de estos sistemas en la destrucción de la lámina elástica interna y la posterior desestructuración de la pared arterial (*Segarra et al, 2007; Rodríguez-Pla et al, 2005*). El estudio histológico de la aorta de uno de los pacientes de nuestra serie mostró un pequeño infiltrado inflamatorio con positividad inmunohistoquímica para MMP2 y MMP9. Sin embargo, la zimografía apenas detectó actividad gelatinolítica de MMP9 mientras que sí detectaba actividad gelatinolítica de MMP2. La MMP9 es producida principalmente por los macrófagos infiltrantes. En cambio, la MMP2 es una gelatinasa constitutiva de las células musculares lisas de la pared arterial y podría estar relacionada con los mecanismos de reparación vascular que tienen lugar tras la agresión inflamatoria. Por tanto, la progresión del daño vascular a lo largo de los años podría estar relacionada con la presencia de un remodelado vascular activo, más que con la persistencia de actividad inflamatoria subclínica. Después del daño inflamatorio inicial, la pared aórtica puede quedar debilitada y, ante la acción de otros factores, sufrir una dilatación progresiva. Un estudio preliminar realizado en 22 pacientes (5 con aneurisma/dilatación y 17 con aorta no dilatada), sugiere que las arterias temporales de pacientes que desarrollan alteraciones estructurales aórticas muestran mayor expresión de enzimas proteolíticos (MMPs) y menor expresión de sus inhibidores naturales (TIMPs) que los pacientes en

los que la aorta no se dilata (resultados descritos en Vías de continuidad). Esto podría tener interés clínico ya que existen diversos agentes con capacidad para modular la actividad de estos sistemas proteolíticos que podrían ayudar a prevenir el desarrollo de aneurismas aórticos. Entre éstos se incluyen: Marimastat (inhibidor global de las MMPs), algunos antibióticos (tetraciclinas, doxiciclina), estatinas o inhibidores del receptor de la angiotensina II. Todos ellos han demostrado efectos beneficiosos en modelos animales de aneurismas aórticos, aunque por el momento no existen estudios que confirmen este beneficio en humanos (*Barbour, Spinale e Ikonomidis, 2007*).

La mayor prevalencia de aneurismas aórticos a nivel de la aorta torácica podría explicarse por la acción de factores hemodinámicos. Según la ley de Laplace, la tensión sobre la pared vascular depende de la presión intraluminal, el diámetro vascular y el grosor de la pared del vaso. La aplicación de esta tensión sobre un área determinada representa la presión de la pared arterial. En todas las especies de mamíferos, incluido el ser humano, la relación entre el diámetro del vaso y el grosor de la pared arterial se mantiene estable en toda la longitud de la aorta, manteniendo una presión sobre la pared arterial uniforme en cualquier segmento de la misma en condiciones normales. La capacidad de los vasos para modificar su diámetro en función de la presión y volver a la normalidad una vez pasada esa presión, se conoce como distensibilidad. La rigidez es la falta de distensibilidad o resistencia a la deformación del vaso. La pérdida de fibras elásticas y el depósito desorganizado de colágeno favorecería un aumento

de la rigidez y una menor distensibilidad. De hecho, en algunos estudios se ha visto que la pared de aortas aneurismáticas obtenidas de pacientes sometidos a cirugía, muestra una mayor rigidez que la pared aórtica no aneurismática (*Ruddy et al, 2008*). Margos y cols estudiaron la distensibilidad de la aorta torácica ascendente mediante ecocardiografía en un grupo de pacientes con ACG antes del inicio del tratamiento corticoideo y lo compararon con un grupo control de personas sanas de la misma edad y sexo. La distensibilidad aórtica estaba reducida en los pacientes con ACG en comparación a los controles sanos. Además, los pacientes con ACG presentaban un mayor diámetro a nivel de la raíz aórtica y de la aorta torácica ascendente (*Margos et al, 2005*). El mayor diámetro aórtico observado en los pacientes con ACG, podría ser una alteración precoz que con el tiempo diese lugar a dilataciones aórticas clínicamente significativas. De forma similar, la realización de una angio-TC a una serie de 30 pacientes con ACG en el momento del diagnóstico demostró una dilatación leve de la aorta torácica ascendente en un 12.5% de los pacientes (*Prieto et al, 2009*). No obstante, en este estudio la dilatación de la aorta torácica ascendente no coincidió con la presencia de aortitis a ese nivel, que fue más frecuente a nivel de la aorta torácica descendente, cayado aórtico y aorta abdominal. Los autores sugieren que la presencia de aortitis a nivel del cayado aórtico y la aorta descendente podría reducir la distensibilidad de la aorta a estos niveles, generando una mayor presión sobre segmentos aórticos proximales que tenderían a dilatarse con mayor facilidad. La aorta proximal está sometida a una mayor presión que los segmentos más distales. Si esta presión actúa sobre un vaso que ha perdido distensibilidad como

consecuencia del fenómeno inflamatorio, la onda de presión trasmitida sería mucho más elevada favoreciendo la dilatación progresiva del vaso.

Otro factor que podría influir en el desarrollo de alteraciones estructurales aórticas es el género ya que, en nuestra serie éstas fueron más frecuentes en hombres. Estudios experimentales realizados con modelos animales observaron que los animales de sexo masculino desarrollan aneurismas aórticos con mayor frecuencia que los animales de sexo femenino. Los primeros mostraban mayor infiltración de macrófagos y expresión de MMP9 a nivel aórtico que los animales de sexo femenino. El factor protector que presentaban los animales de sexo femenino, se perdía cuando la aorta se transplantaba en un animal masculino. Los autores observaron que el tratamiento con estradiol conllevaba una menor expresión de MMP-9 a nivel aórtico y menor incidencia de aneurismas (*Ailawadi et al, 2004*). En pacientes de edad avanzada como los que configuran nuestra serie, las diferencias en los niveles de estradiol entre ambos sexos posiblemente no sean muy importantes. No obstante, no podemos descartar que factores hormonales puedan modular la homeostasis vascular e intervenir en el desarrollo de complicaciones estructurales aórticas.

A diferencia de los datos proporcionados por estudios previos retrospectivos, en nuestra serie no se observó una mayor prevalencia de daño estructural aórtico en pacientes portadores de los clásicos factores de riesgo cardiovascular. Esto puede ser debido a la naturaleza prospectiva de

nuestro estudio, en el que el control estricto de los factores de riesgo vascular se realizó de forma sistemática en todos los pacientes. De forma sorprendente, observamos una mayor prevalencia de hipercolesterolemia entre los pacientes que no desarrollaron alteraciones estructurales aórticas. Dado que todos los pacientes en los que se detectó hipercolesterolemia realizaban tratamiento con estatinas, podríamos especular sobre el posible mecanismo protector de las estatinas en el desarrollo del daño estructural aórtico. Además de una acción hipolipemiante, las estatinas poseen capacidad anti-inflamatoria, antioxidante y son capaces de reducir la secreción de MMPs. Algunos estudios experimentales con modelos animales han demostrado que las estatinas son capaces de suprimir el desarrollo y progresión de aneurismas en la aorta abdominal (*Steinmetz et al, 2005; Kalyanasundaram et al, 2006; Shiraya et al, 2009; Miyake y Morishita, 2009*). En un sistema de cultivo *ex vivo* de aorta humana aneurismática, la administración de cerivastatina redujo la expresión tisular de MMP-9 de forma dosis-dependiente además de inhibir la activación de las células inflamatorias infiltrantes (*Nagashima et al, 2002*). Un estudio reciente, realizado en pacientes con aneurismas de aorta abdominal que habían de ser sometidos a cirugía reparativa demostró que, el tratamiento con 20 mg de atorvastatina durante un periodo de 4 semanas antes de la cirugía, se asociaba a una menor expresión de c-Jun N Terminal quinasa (JNK), considerada una molécula de señalización precoz de inflamación, así como una menor infiltración de la pared arterial por células inflamatorias (células dendríticas, linfocitos T y macrófagos) y una menor expresión de MMPs (MMP-2 y MMP-9) (*Kajimoto et al, 2009*). Además, diversos estudios

observacionales han demostrado efectos beneficiosos de las estatinas en pacientes con aneurismas a nivel de aorta abdominal (*Sukhija et al, 2006; Schouten et al, 2006*). A pesar de que todos estos estudios van dirigidos a determinar un posible beneficio de las estatinas en pacientes con aneurismas de aorta abdominal, con características y mecanismos patogenéticos distintos a los que desarrollan los pacientes con ACG, estas observaciones, junto con los resultados de nuestra serie, son una motivación importante para en un futuro, investigar el posible efecto protector de las estatinas en el desarrollo de aneurismas en pacientes con ACG.

En nuestra serie, las alteraciones estructurales aórticas fueron más frecuentes en pacientes con una respuesta inflamatoria sistémica menos intensa, menos recidivas clínicas y menos requerimientos terapéuticos. Podríamos especular con que la falta de datos objetivos de actividad de la enfermedad, tanto clínicos como biológicos, favoreció en estos pacientes un descenso más rápido de la dosis de corticoides. Suponiendo que en estos pacientes persistiera un cierto grado de actividad inflamatoria subclínica no detectable con las pruebas que se utilizan en la práctica habitual, la suspensión precoz del tratamiento podría haber favorecido el desarrollo de alteraciones estructurales aórticas. Otra posible explicación surge de algunos estudios realizados en modelos animales de aneurismas aórticos. En ellos se utilizan ratones a los que se les realiza un transplante alogénico de aorta con el objetivo de analizar el efecto que tienen IFNγ (prototipo de "citocina proinflamatoria" y principal citocina en la inducción de la

respuesta inmune Th1) e IL-4 (representante de la respuesta Th2 y prototipo de "citocina anti-inflamatoria") sobre la formación de aneurismas aórticos (*Shimizu et al, 2004*). El transplante alogénico genera una respuesta inflamatoria en la pared aórtica que, en animales normales, da lugar a una hiperplasia de la íntima y estenosis del vaso. Los animales knock-out para el receptor de IFNγ, en los que por tanto predomina una respuesta inmune Th2, desarrollan aneurismas aórticos. La formación de estos aneurismas se acompaña de una importante destrucción de fibras elásticas en la túnica media y de una mayor expresión de ciertas MMPs (entre ellas MMP9 y MMP12) y TIMPs, con respecto a los animales normales que no desarrollan aneurismas aórticos. Los autores también observaron que el bloqueo de IL-4 (mediante la administración de anticuerpos anti-IL-4 o mediante el uso de ratones genéticamente deficientes tanto para el receptor del IFNγ como para IL-4) reducía la formación de aneurismas y se asociaba a una menor fragmentación de las fibras elásticas y menor expresión de MMP9 y MMP12. Los autores concluyen que la IL-4 es capaz de aumentar la actividad elastolítica y que a su vez el IFNγ podría tener un papel protector en la formación de aneurismas aórticos al reducir dicha actividad. El mismo grupo observó que la pared arterial de aortas abdominales aneurismáticas mostraba una mayor expresión de citocinas Th2 (IL-4, IL-5 e IL-10) y una nula o mínima expresión de citocinas Th1, en particular la cascada de señales estimulada por el IFNγ (*Schönbeck et al, 2002*). En modelos animales de aortitis inducida por infecciones víricas (citomegalovirus y γ-herpesvirus 68) se observó que los ratones genéticamente deficientes para el receptor del IFNγ desarrollaban una

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mayor inflamación de la pared arterial que llegaba a ser transmural y en ocasiones producía la muerte por rotura de la pared aórtica (*Presti et al, 1998; Weck et al, 1997*). Clásicamente, los pacientes con ACG presentan una respuesta inmune Th1 donde predomina la secreción de citocinas proinflamatorias (IFNγ, IL-6, TNFα) que a su vez regulan la respuesta inflamatoria sistémica. En el estudio que hemos presentado, no parece que estas citocinas intervengan de forma significativa en el desarrollo de aneurismas aórticos. Es más, el desarrollo de aneurismas fue más frecuente en pacientes con una RIS débil. A la vista de estos resultados, creemos que podría tener interés estudiar el posible papel de las citocinas anti-inflamatorias (respuesta Th2) en el desarrollo de aneurismas aórticos en pacientes con ACG.

Uno de los principales objetivos para el futuro será profundizar en la historia natural de las alteraciones estructurales aórticas y los factores que favorecen su desarrollo, ya que ello puede ayudar a prevenirlas y/o detectarlas de forma precoz. Esto tiene un especial interés en los pacientes con ACG que, debido a su avanzada edad y comorbilidad, no pueden ser sometidos a cirugías reparativas, que por otra parte no están exentas de complicaciones. La morbimortalidad asociada al desarrollo de complicaciones aórticas es elevada y por tanto las actuaciones dirigidas a la prevención y/o detección precoz de las mismas seguramente tendrían un impacto significativo sobre la vida de los pacientes.

Conocer los mecanismos patogenéticos que dan lugar tanto al desarrollo, como a la persistencia de la enfermedad, puede ser de gran ayuda a la hora de entender la historia natural de las complicaciones vasculares. En la patogénesis de la ACG intervienen múltiples citocinas que conforman un entramado de mediadores complejo. Una misma citocina puede ser producida por distintos tipos de células. A su vez, una misma citocina puede actuar sobre distintos tipos celulares y producir efectos biológicos diferentes en cada uno de ellos. Además, las citocinas poseen efectos redundantes, lo que significa que distintas citocinas pueden producir funciones similares. En los últimos años hemos asistido a la descripción de nuevas familias de citocinas cuyas funciones aún están por determinar. Sin embargo, aunque el número de citocinas es cada vez más amplio y las funciones que ejercen muy variadas, la mayoría de ellas comparten mecanismos de acción y vías de señalización comunes. Tal es el caso de la superfamilia del TNFα, que engloba varias familias de proteínas con características estructurales similares al TNFα. La familia constituida por el RANKL/RANK/OPG, además de ser el principal factor regulador del metabolismo óseo, podría regular la respuesta inmune y la homeostasis vascular. En los últimos años se han generado terapias que modifican la actividad de estas citocinas y que han demostrado resultados prometedores en el tratamiento de la osteoporosis (*Cummings et al, 2009*). El tercer trabajo de esta tesis es el primero que describe la expresión de estas proteínas en una enfermedad vascular inflamatoria como es la ACG.

Hemos comprobado que la expresión vascular de RANKL y sus receptores RANK y OPG está aumentada en pacientes con ACG, principalmente a expensas del infiltrado inflamatorio que invade la pared arterial. Este aumento de expresión local no parece tener repercusión sistémica, pues los niveles circulantes de ambas citocinas no muestran diferencias significativas entre pacientes y controles. A pesar de que la expresión génica de RANKL a nivel arterial fue superior en pacientes que presentaban una RIS intensa en el momento del diagnóstico, tampoco esto se tradujo en una mayor severidad de la enfermedad ni en mayores requerimientos terapéuticos. Por tanto, es posible que estas citocinas actúen principalmente de forma autocrina-paracrina y carezcan de funciones sistémicas. Por otra parte, diversos órganos, además del sistema vascular, pueden contribuir a los niveles circulantes de RANKL y OPG, lo que podría justificar la ausencia de diferencias entre pacientes y controles. En este sentido, el tejido óseo podría tener un papel relevante en la secreción de estas citocinas lo que justificaría que los niveles circulantes de OPG se correlacionen con la edad de los pacientes. La mayor secreción de OPG podría traducir un intento por compensar el aumento de la resorción ósea que tiene lugar a lo largo de los años. Por otra parte, la OPG se ha relacionado con la existencia de enfermedad vascular de origen arterioesclerótico. Es posible que a medida que aumenta la edad, aumente también el grado de severidad de la enfermedad arterioesclerótica, lo que se traduciría en unos niveles progresivamente más elevados de OPG.

El estudio histológico de las arterias normales no evidenció expresión de RANKL o RANK mientras que en arterias de pacientes con ACG, la expresión de ambas proteínas se localizó principalmente en zonas invadidas por el infiltrado inflamatorio, constituido por linfocitos, macrófagos y células gigantes multinucleadas. Aunque no se ha demostrado, es posible que estas citocinas sean capaces de modular respuestas biológicas a nivel de las células gigantes, de forma similar a lo que hacen a nivel del tejido óseo, donde la interacción entre RANKL y RANK es necesaria para producir osteoclastos maduros a partir de células hematopoieticas de la línea monocítica-macrofágica. De hecho los osteoclastos maduros también son células multinucleadas aunque con funciones bien conocidas, a diferencia de las células gigantes multinucleadas de la ACG cuyas funciones no están bien definidas. Además de secretar factores de crecimiento y angiogénicos, se cree que intervienen en la digestión de detritus vasculares producidos durante el fenómeno inflamatorio. No obstante, es posible que estas células no tengan un papel determinante en el curso clínico de la enfermedad ya que, en primer lugar no se observan en todas las biopsias de pacientes con ACG y en segundo lugar, suelen localizarse alrededor de la lámina elástica interna en pacientes con afectación inflamatoria panarterítica y son menos frecuentes en aquellos en los que la inflamación queda circunscrita a la adventicia.

A diferencia de RANKL y RANK, que no se expresan en arterias de controles, observamos una marcada expresión proteica de OPG a nivel del

endotelio vascular, tanto en arterias de pacientes con ACG como en arterias controles. La OPG normalmente reside en la célula endotelial formando un complejo con el factor von Willebrand en el interior de los cuerpos de Weibel Palade y ambos son liberados a la circulación en respuesta a estímulos inflamatorios. Cabe destacar que la expresión de OPG fue igualmente intensa tanto en las células endoteliales del lumen principal como en los *vasa vasorum* o los neovasos formados en el interior de la pared arterial durante el fenómeno inflamatorio. Esto sugiere que la OPG es una molécula constitutiva de células endoteliales, tanto en estructuras vasculares maduras como en vasos recién desarrollados. Sin embargo, a diferencia del factor vonWillebrand, que había sido propuesto como marcador de actividad inflamatoria al estar elevado en pacientes con ACG, los niveles séricos de OPG no parecen tener la misma utilidad ya que no mostraron diferencias entre pacientes y controles. El papel exacto de la OPG a nivel de biología vascular se desconoce aunque estudios realizados en enfermedad arteriosclerótica sugieren funciones de protección vascular. Es posible que la expresión de OPG aumente ante la presencia de diversos estímulos capaces de provocar daño vascular. Hemos observado que la expresión génica de OPG se correlaciona con una ratio MMP9/TIMP-1 menor, lo que disminuiría la actividad proteolítica, necesaria para la degradación de las elásticas y el remodelado vascular. Los resultados son congruentes con estudios previos que muestran un aumento de la actividad proteolítica en cultivos de osteoclastos tratados con RANKL, lo que favorecería la resorción ósea. Esta hipotética capacidad para modular la actividad proteolítica y por tanto el remodelado vascular, podría tener

interés en la formación de alteraciones estructurales vasculares. En este sentido hemos observado que los pacientes que desarrollan aneurisma o dilatación de aorta durante el seguimiento tienen una menor expresión génica de OPG en arteria temporal en el momento del diagnóstico. Se trata de resultados preliminares, que no llegan a alcanzar significación estadística, probablemente debido a las pequeñas dimensiones de la muestra. Sería interesante en un futuro poder estudiar, en una serie mayor, este posible efecto protector de la OPG en el desarrollo de alteraciones estructurales aórticas (resultados descritos en Vías de continuidad).

Se ha sugerido que el sistema RANKL/OPG interviene en la patogénesis de la arteriosclerosis. Los pacientes con ACG pueden desarrollar patología vascular tanto por enfermedad arteriosclerótica como por presentar un remodelado vascular anómalo. A pesar de ello, esto no parece ser un problema significativo en los pacientes de nuestra serie ya que, como vimos en el primer trabajo de esta tesis, sólo una mínima parte de los pacientes desarrolló durante el seguimiento síntomas clínicos relacionados con enfermedad cerebrovascular, cardiovascular o vascular periférica. Además del infiltrado inflamatorio, también las células musculares lisas de la arteria inflamada expresan RANKL, RANK y OPG. Estas citocinas podrían modular la capacidad proliferativa, migratoria y secretora de estas células y de esa manera intervenir en el remodelado vascular. La expresión de RANKL observada en la íntima podría ser a consecuencia de la migración de células miointimales hacia el interior de la pared arterial, ya que esta expresión parece ser más importante en arterias

que presentan un mayor grado de hiperplasia de la íntima. El RANKL ha demostrado capacidad migratoria sobre otros tipos celulares por lo que no sería de extrañar que también tuviese este efecto sobre células musculares lisas de la pared vascular. Por el contrario, la expresión de OPG a nivel de la íntima parecía más intensa en arterias con poca hiperplasia intimal ya que en este caso, la OPG podría inhibir el efecto de RANKL y por tanto la migración de las células miointimales.

El estudio muestra una correlación entre los niveles génicos de OPG y de IL-6 a nivel de arteria temporal. Es posible que algún estímulo común sea capaz de regular en paralelo la expresión de ambas citocinas. De hecho, estudios previos han demostrado que diversos mediadores inflamatorios pueden aumentar la expresión tanto de RANKL como de OPG a nivel vascular. Por otra parte, sabemos que la IL-6 posee actividad angiogénica y que su expresión a nivel arterial se relaciona con una mayor formación de neovasos y menor prevalencia de eventos isquémicos. La OPG se expresa de forma tan nítida a nivel endotelial que puede compararse a una tinción específica de células endoteliales como la *Ulex Aeropaeus*, por lo que las secciones teñidas con anticuerpos anti-OPG pueden ser útiles para mirar la cantidad de neovasos formados a nivel de la pared arterial (Resultados no mostrados). En este sentido, la mayor expresión de IL-6 daría lugar a mayor formación de neovasos y por tanto mayor expresión de OPG almacenada en las células endoteliales.

Este es el primer estudio que intenta describir la expresión de RANKL/RANK/OPG en pacientes con ACG. De entrada, no parece que esta familia de citocinas tenga efectos clínicos significativos sobre los pacientes, a pesar de que la expresión de RANKL está aumentada en aquellos con una RIS intensa en el momento del diagnóstico. De todas formas, no podemos descartar que intervengan en la regulación de respuestas locales y en el remodelado vascular que tiene lugar tras la agresión inflamatoria. Si esto fuese cierto, la expresión de estas citocinas podría determinar el desarrollo de complicaciones vasculares a largo plazo. Por otra parte, sería interesante conocer si además la expresión de estas citocinas se relaciona con una mayor prevalencia de osteoporosis y fracturas óseas. En la actualidad disponemos de tratamientos capaces de modular el sistema RANKL/RANK/OPG que podrían tener utilidad en la prevención de osteoporosis en pacientes con ACG.

VI. VÍAS DE CONTINUIDAD
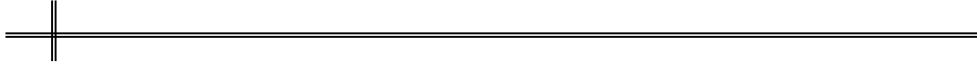
En las siguientes páginas se presentan otros estudios en curso y algunos datos adicionales que se han derivado de los trabajos que constituyen el núcleo central de esta tesis. Algunos de estos datos son potencialmente relevantes y en el futuro se intentará llevar a cabo nuevos estudios que permitan determinar la importancia clínica de los mismos.

Conocer la historia natural de las alteraciones estructurales aórticas será fundamental para realizar un diagnóstico precoz. En estos momentos se está realizando un nuevo estudio para detectar alteraciones estructurales aórticas en pacientes con ACG que tienen un seguimiento muy prolongado (superior a 8 años). La serie de pacientes estudiada y el protocolo diagnóstico son los mismos que los descritos en el segundo trabajo de esta tesis. Nuestro objetivo consiste en saber cómo evolucionan las alteraciones estructurales aórticas detectadas en la primera valoración y saber si aparecen alteraciones que no estuviesen presentes previamente. A falta de completar el estudio, hemos detectado algunos pacientes que, teniendo una aorta aparentemente normal en la primera valoración, han desarrollado criterios de alteración estructural aórtica en la segunda valoración, por lo que parece recomendable realizar valoraciones periódicas de la aorta en estos pacientes, incluso años después del diagnóstico.

Los estudios que pretendemos llevar a cabo en un futuro estarán principalmente dirigidos a estudiar los mecanismos patogenéticos de dichas alteraciones, entre ellos investigar si el grado de actividad proteolítica en la pared vascular puede determinar el desarrollo de

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dilatación o aneurisma aórtico. Por otra parte estamos desarrollando un método de cultivo de arteria temporal que permita realizar intervenciones terapéuticas preclínicas. De forma breve, ambos proyectos se presentan en las siguientes páginas.

1. Creación de un modelo de cultivo de arteria temporal

Tras el fracaso del bloqueo del TNFα con infliximab intentamos crear un modelo de estudio que permitiese realizar intervenciones terapéuticas y observar los cambios funcionales que se producen tras dichas intervenciones. Con este objetivo se creó este modelo de cultivo de arteria temporal. Se realizan secciones de arteria temporal de 0.5 a 1 mm que se incluyen en distintos pocillos de una placa sobre una base de Matrigel. Añadimos a cada muestra un medio de cultivo que incluye RPMI y 10% de suero bovino. De cada arteria temporal se plantearon 5 condiciones: arteria temporal sin tratamiento y arteria temporal tratada con un anticuerpo monoclonal anti-TNFα (1μg/ml), OPG recombinante (200 ng/ml), antagonista del receptor de la IL-1 recombinante (500 ng/ml) y dexametasona (0.5 μg/ml). Las muestras permanecieron cinco días a 37º y a un 5% de CO2. Comprobamos que bajo estas condiciones se conserva tanto la estructura de la pared arterial como el infiltrado inflamatorio (Figura 5).

A los cinco días se recuperaron los sobrenadantes y se guardaron a -80º hasta su utilización. En un segundo tiempo se determinó la concentración de diversas citocinas proinflamatorias (IL-1, IL-6), metaloproteasas (MMP-2, MMP-9) y factores fibrogénicos (PDGF) en los sobrenadantes.

Figura 5. Arteria temporal tras ser sometida a las condiciones de cultivo
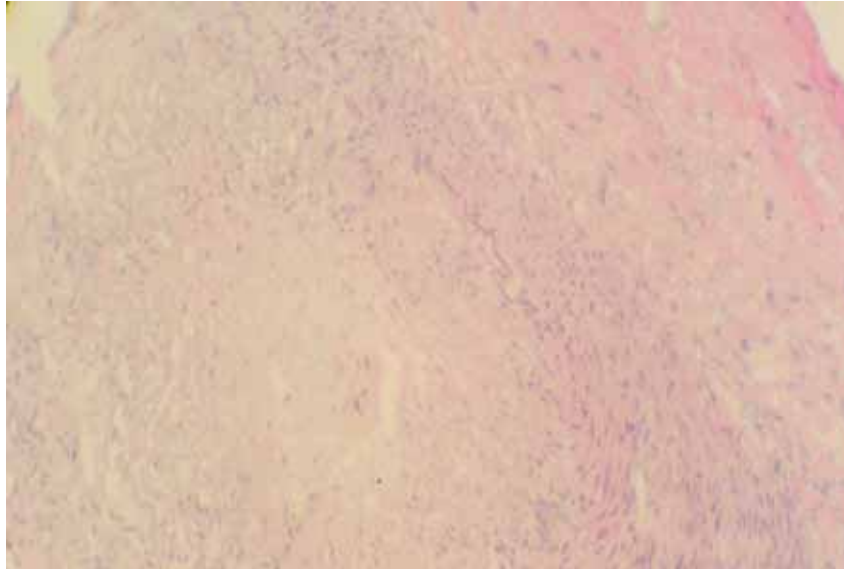
Tinción de hematoxilina/eosina de arteria temporal cultivada durante 14 días. Se observa que tanto la estructura de la pared arterial como el infiltrado inflamatorio están preservados.

En total se estudiaron 12 arterias temporales, 8 de pacientes con ACG y 4 procedentes de controles. En situación basal observamos que la concentración de la mayoría de mediadores estudiados era más elevada en el sobrenadante de las arterias positivas que en el de las arterias control. En la tabla 3 se muestran las concentraciones de cada una de las moléculas analizadas.

Tabla 3. Concentración de distintos factores en el sobrenadante del cultivo de arteria temporal.

	IL-6 (ng/ml)	IL-1β (pg/ml)	MMP-2 (ng/ml)	MMP-9 (ng/ml)	PDGF (pg/ml)
PACIENTES	42	6	39	49	23
CONTROLES	13	0.1	15	8	41
p	NS	NS	NS	0.048	NS

A excepción del PDGF, la concentración del resto de mediadores fue superior en los sobrenadantes de las arterias patológicas en comparación a los controles. El análisis de muestras pequeñas, probablemente limita la obtención de diferencias estadísticamente significativas entre ambos grupos. No obstante, llama la atención la elevada producción de IL-6 que presentan las arterias controles a pesar de carecer de infiltrado inflamatorio. Es posible que esta producción provenga de las células musculares lisas que reaccionan ante la agresión producida por la escisión de la muestra. En un estudio previo observamos que el sobrenadante obtenido de cultivos de células musculares lisas procedentes de arterias temporales también muestra una elevada concentración de IL-6 (*Lozano et al, 2008*).

Cuando seleccionamos únicamente las arterias temporales de pacientes con ACG y valoramos el efecto que tienen las distintas intervenciones terapéuticas sobre la producción de citocinas y factores de crecimiento

obtuvimos los siguientes resultados representados en forma de gráficas. Los asteriscos marcan los resultados estadísticamente significativos (p<0.05).

Las concentraciones de IL-6 en el sobrenadante disminuyeron al tratarlas con el antagonista del receptor de la IL-1 (p=0.023) y sobretodo con dexametasona (p=0.016). Sin embargo el tratamiento con OPG y con un anticuerpo bloqueante del TNFα, no produjo cambios significativos. También se observó un descenso en la concentración de IL-1β de las arterias tratadas con dexametasona (p=0.016). El resto de tratamientos no provocó cambios significativos.
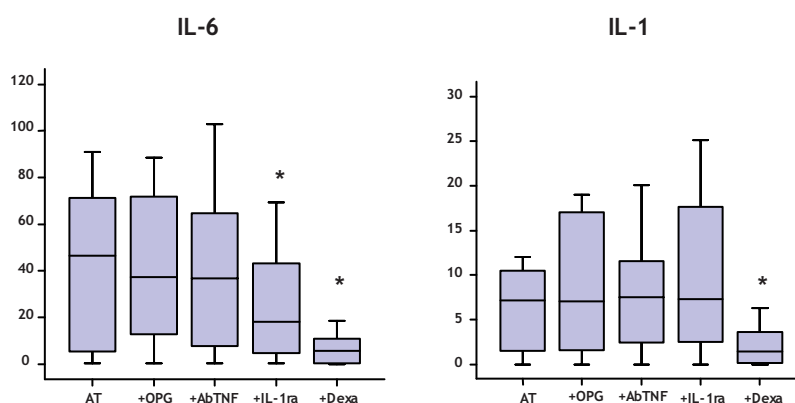

Respecto a las metaloproteasas, el tratamiento con el antagonista del receptor de la IL-1 redujo de forma casi significativa las concentraciones de MMP-2 en el sobrenadante (p=0.05). La concentración de MMP-9 se redujo de forma significativa tras la administración de dexametasona (p=0.003). El

tratamiento con un bloquante del TNFα disminuyó la concentración de MMP-9, aunque en este caso las diferencias no llegaron a ser significativas (p=0.058).

MMP-2

MMP-9
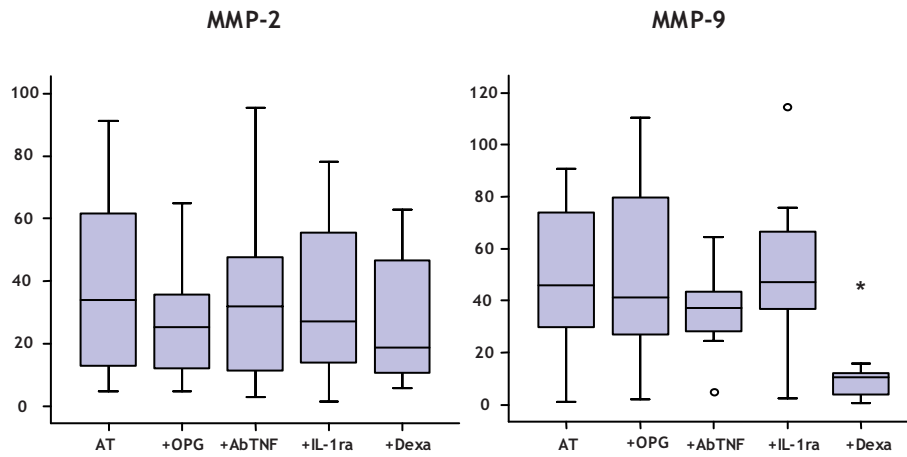
Las concentraciones de PDGF no se vieron modificadas por ninguno de los tratamientos utilizados.

PDGF
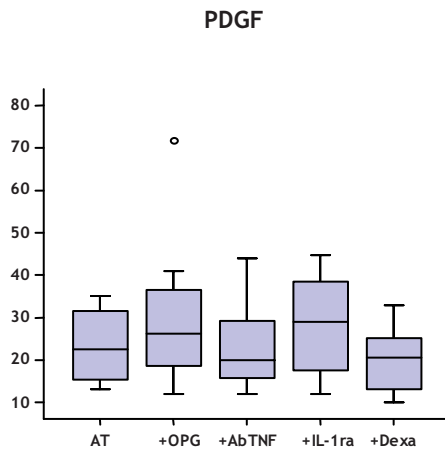
Hemos comprobado que la arteria temporal se mantiene viable tras varios días en medio de cultivo. Este sistema podría ser útil para realizar una valoración preclínica de posibles nuevos tratamientos antes de aplicarlos en humanos. No obstante, este es un estudio preliminar y en la actualidad se están llevando a cabo nuevos experimentos que permitirán optimizar la técnica. Algunos de estos resultados ya se han presentado en congresos internacionales.

➤ A García-Martínez, E Lozano, M Segarra, J Hernández-Rodríguez, G Espígol, S Prieto, MC Cid. Human temporal artery culture on matrigel: a useful method for preclinical assessment of functional changes after intervention. American College of Rheumatology. 71st Annual Scientific Meeting. Boston, MA, USA. Arthritis Rheum 2007;56(suppl):S497.

2. Factores relacionados con el desarrollo de alteraciones estructurales aórticas.

Diversos estudios han demostrado la relación entre el aumento de actividad proteolítica y el desarrollo en aneurismas aórticos, aunque ninguno de ellos fue realizado en pacientes con ACG. Obtener tejido aórtico para estudios de investigación es difícil, aunque cabe la posibilidad de que la arteria temporal sea un fiel reflejo de los procesos que tienen lugar en otros territorios vasculares. A lo largo de los últimos años hemos estudiado a nivel de arteria temporal la expresión de diversos biomarcadores de inflamación en la ACG. Uno de nuestros objetivos es el estudio de factores relacionados con un remodelado vascular anómalo que puede dar lugar a la aparición de alteraciones estructurales con consecuencias clínicas. Es posible que la activación de sistemas proteolíticos sea un factor importante en el desarrollo de estas alteraciones. De hecho, en arterias temporales de pacientes con ACG, la expresión de MMP2, MMP9 y MMP12 se relaciona con la destrucción de la lámina elástica (*Segarra et al, 2007; Rodríguez-Pla et al, 2005*). La degradación de fibras elásticas es a su vez un factor fundamental en el desarrollo de aneurismas aórticos por lo que la expresión de enzimas proteolíticos podría jugar un papel importante en el desarrollo de estas complicaciones.

Un análisis preliminar realizado en una serie pequeña de pacientes parece sustentar esta hipótesis. Entre los pacientes que habían sido sometidos al protocolo diagnóstico para detectar dilatación o aneurisma de

aorta, seleccionamos 22 en los que se había valorado la actividad proteolítica en un estudio previo (*Segarra et al, 2007*). En estos pacientes se había determinado la expresión génica de MMP-2, MMP-9 y sus inhibidores naturales TIMP1 y TIMP2 en arteria temporal mediante PCR a tiempo real. Los pacientes que desarrollaron alteraciones aórticas durante el seguimiento tenían tendencia a presentar un mayor status proteolítico en arteria temporal en el momento del diagnóstico.

Se observó una mayor expresión de MMP2 en los pacientes que con el tiempo desarrollaron alteraciones estructurales aórticas. La MMP2 es producida principalmente por las células musculares lisas y se relaciona con el fenómeno de remodelado vascular. La mayor producción de MMP2 podría favorecer el desarrollo de alteraciones estructurales aórticas en estos pacientes. Estos datos indican que el riesgo de desarrollar estas complicaciones podría estar presente ya en fases iniciales de la enfermedad, aunque éstas se manifiesten clínicamente años después del diagnóstico. También observamos que los pacientes que desarrollaban complicaciones aórticas presentaban una menor producción de TIMP1. El TIMP1 es el inhibidor natural de la MMP9, gelatinasa producida principalmente por las células inflamatorias. En la Figura 6 se muestran las gráficas con los resultados.

Figura 6. Cuantificación de mRNA de MMP2 y MMP9 y sus inhibidores naturales, TIMP2 y TIMP1 respectivamente, así como la ratio entre ellos *(AEA: alteración estructural aórtica)*
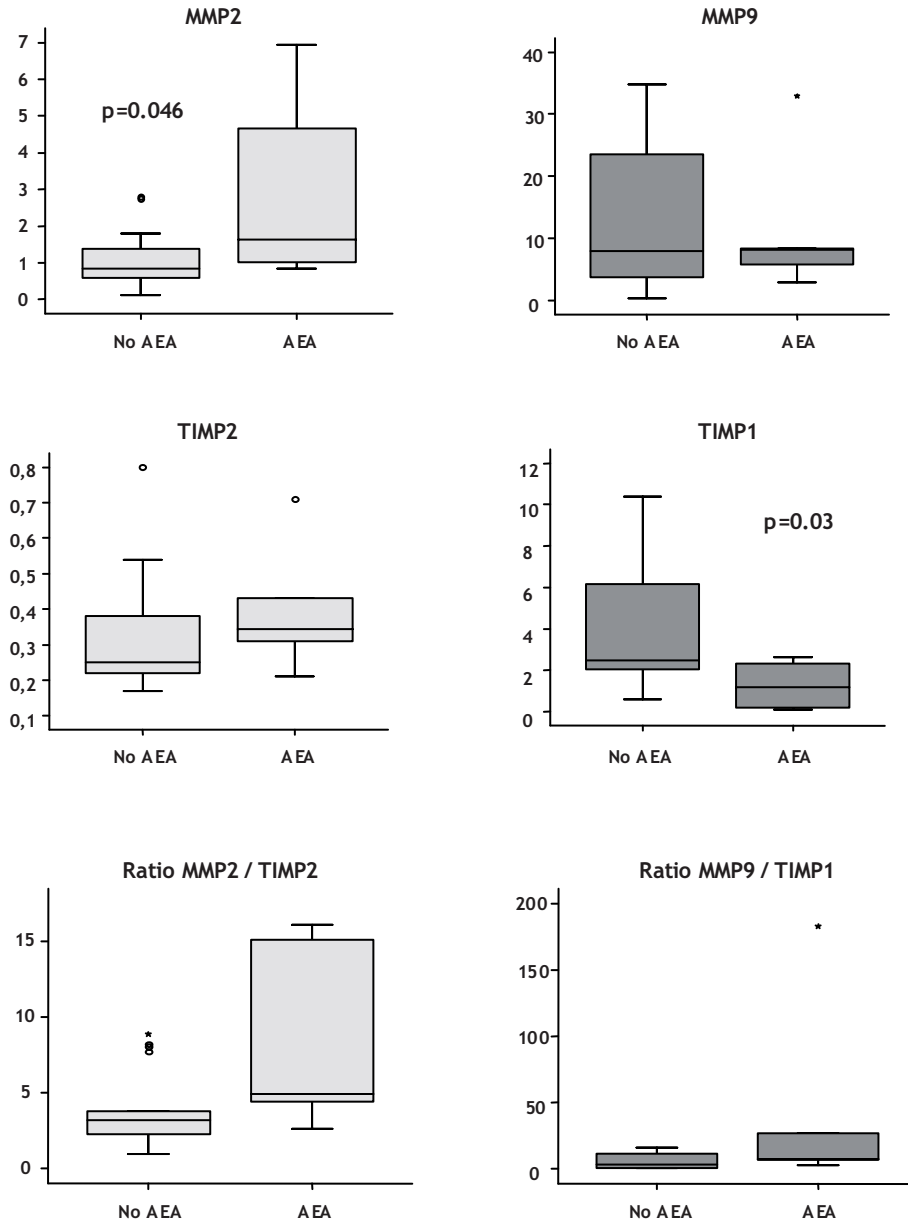
A pesar de que estos resultados son preliminares la tendencia observada sugiere que el aumento de actividad proteolítica a nivel arterial podría ser uno de los mecanismos que intervienen en el desarrollo de alteraciones estructurales aórticas en pacientes con ACG.

Siguiendo en esta misma línea quisimos estudiar el posible papel de la OPG en el desarrollo de estas complicaciones. Desde su descubrimiento en 1997, muchos estudios han demostrado la relación entre la OPG y el sistema vascular. Los datos más significativos hasta el momento son los que demuestran la relación entre niveles de OPG y la severidad de la enfermedad vascular de causa arteriosclerótica. Sin embargo, el papel de la OPG en biología vascular sigue siendo una incógnita. En la actualidad no se conoce si la OPG es simplemente un marcador de ateromatosis o participa en la progresión de la enfermedad vascular. Otra posibilidad es que la OPG sea un factor de protección vascular, que se produciría ante ciertos estímulos lesivos con el objetivo de limitar el daño vascular.

Es posible que el sistema RANKL/RANK/OPG ejerza funciones autocrinas-paracrinas a nivel arterial e intervenga en el remodelado vascular que tiene lugar tras la agresión inflamatoria. Un remodelado vascular ineficaz puede dar lugar a alteraciones estructurales vasculares, por lo que podría tener interés estudiar la relación de este sistema de citocinas con el desarrollo de aneurisma o dilatación aórticos.

Se analizó la expresión sérica y tisular de OPG en algunos pacientes que habían sido sometidos al estudio para detectar dilatación o aneurisma aórticos. En total, se determinó la concentración sérica de OPG en 32 pacientes (25 sin y 7 con alteraciones estructurales aórticas) mediante ELISA, y en 24 pacientes (19 sin y 5 con alteraciones estructurales aórticas), se determinó la expresión génica de OPG en arteria temporal mediante PCR a tiempo real. La Figura 7 muestra los resultados obtenidos.

Figura 7. Expresión de OPG en suero y arteria temporal de pacientes sin y con alteraciones estructurales aórticas
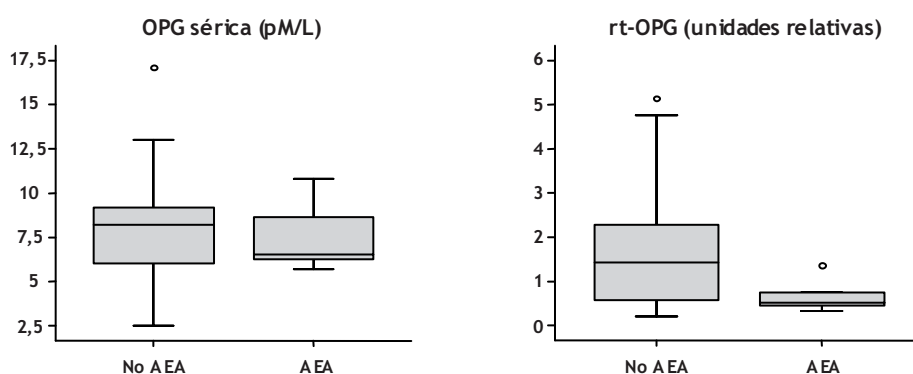
Mientras que no hubo diferencias en los niveles séricos de OPG entre ambos grupos, observamos que la expresión génica de OPG a nivel arteria temporal fue menor en los pacientes que desarrollaron dilatación o aneurisma de aorta durante el seguimiento. Estos datos sugieren que la OPG sí que podría jugar un papel en el remodelado vascular como factor de protección vascular. Un posible mecanismo de este efecto podría ser la regulación de ciertos sistemas proteolíticos, a través de disminuir la

expresión de metaloproteasas y/o aumentando la expresión de sus inhibidores naturales. De esta manera, la OPG reduciría la actividad proteolítica e inhibiría el desarrollo de complicaciones aórticas en pacientes con ACG. Creemos que sería interesante ampliar la serie de pacientes para confirmar estos datos.

VII. CONCLUSIONES
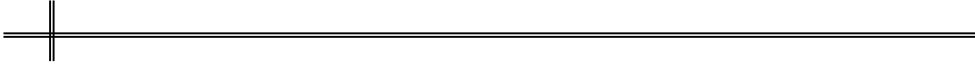
Los niveles de citocinas proinflamatorias circulantes pueden persistir elevados durante largos periodos de tiempo, incluso en pacientes que han alcanzado la remisión clínica y no precisan tratamiento. Los niveles de TNFα e IL-6 durante el seguimiento también se correlacionan con un mayor número de recidivas clínicas y mayores necesidades terapéuticas, aunque esto no parece repercutir negativamente sobre el estado clínico de los pacientes. Los niveles de citocinas circulantes no se correlacionaron, ni con la calidad de vida percibida por los propios pacientes ni, con el desarrollo de complicaciones vasculares o efectos secundarios atribuibles al tratamiento crónico con corticoides. Tampoco el desarrollo de aneurisma o dilatación aórticos, fenómeno relativamente frecuente en nuestra serie, se relacionó con la persistencia de actividad inflamatoria. No obstante, el número de pacientes estudiado fue pequeño y el número de complicaciones vasculares no aórticas también, por lo que no podemos descartar que en una serie más amplia o tras un periodo de seguimiento más prolongado pudiésemos encontrar un mayor número de complicaciones o alguna relación con la persistencia de actividad inflamatoria.

Del segundo trabajo es importante remarcar que la aplicación de un protocolo de estudio sencillo y fácilmente accesible en la práctica clínica diaria ha demostrado la presencia de dilatación o aneurisma aórtico en más del 20% de los pacientes y en casi la mitad de ellos lo suficientemente importante como para requerir reparación quirúrgica. La potencial gravedad de las complicaciones derivadas del daño estructural aórtico hace

necesaria una vigilancia continua, tanto clínica como radiológica, de los pacientes con ACG. Las complicaciones aórticas parecen tener un origen multifactorial. Probablemente, la agresión inflamatoria inicial produce la pérdida de fibras elásticas y células musculares lisas así como la desorganización de la pared aórtica, provocando un cambio en las propiedades físicas del vaso. La pared aórtica se vuelve más rígida y menos distensible y esto favorece que la acción de factores hemodinámicos produzca la dilatación progresiva del vaso. No obstante, el principal factor hemodinámico conocido es la presión arterial. En nuestros pacientes la presión arterial se controla de forma estricta en todos ellos, por lo que creemos que no es el único factor que interviene en el desarrollo de estas complicaciones. Por lo que hemos visto y por los datos publicados por otros autores, es posible que existan factores que actuarían a nivel local modulando tanto el tipo de respuesta inflamatoria como el fenómeno de remodelado vascular posterior. Entre ellos, podríamos mencionar factores hormonales o genéticos, factores relacionados con el tipo de respuesta inflamatoria local, expresión de sistemas proteolíticos o factores secretados durante el fenómeno de reparación vascular. El segundo trabajo de esta tesis, más que aportar conclusiones sobre los mecanismos que intervienen en el desarrollo de dichas complicaciones, deja abierta la puerta a futuras investigaciones. Será fundamental comprender la historia natural y los factores relacionados con el desarrollo del daño estructural aórtico ya que esto permitirá realizar el diagnóstico precoz de estas complicaciones tan graves y quizás un tratamiento más específico.

Por último, se ha descrito, por primera vez en pacientes con ACG, la expresión del sistema RANKL/RANK/OPG. A pesar de no demostrar efectos sistémicos significativos es posible que estas citocinas tengan funciones reguladoras locales en la pared vascular. Se ha observado que la expresión de RANKL está aumentada en pacientes con ACG con respecto a los controles y que esta expresión depende fundamentalmente del infiltrado inflamatorio vascular. En cambio, no observamos diferencias significativas en la expresión génica de OPG entre pacientes y controles lo que sugiere que la OPG probablemente es una proteína constitutiva de las células vasculares que se libera ante ciertos estímulos lesivos para el vaso. De hecho, la expresión inmunohistoquímica de OPG en arterias de pacientes con ACG es mucho más tenue que en caso del RANKL. La expresión proteica de OPG es muy manifiesta a nivel endotelial, tanto en arterias de pacientes como controles, lo que también va a favor de que la OPG sea un elemento constitutivo de la pared vascular. No podemos descartar que el sistema RANKL/OPG intervenga en los mecanismos de remodelado vascular que tienen lugar tras la agresión inflamatoria por lo que podría regular el desarrollo de alteraciones estructurales vasculares en pacientes con ACG. Además, sería interesante analizar si la expresión del sistema RANKL/RANK/OPG se relaciona con la aparición de complicaciones clínicas derivadas de la pérdida de masa ósea. La importancia radica en que en la actualidad disponemos de tratamientos capaces de modificar la actividad de esta familia de citocinas, por lo que podríamos incidir en ambos aspectos clínicos.

VIII. BIBLIOGRAFÍA

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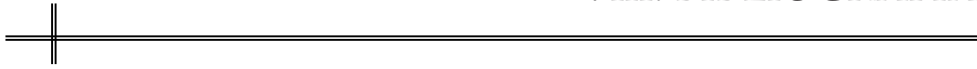
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