ANTIBODY PROFILES IN PATIENTS TREATED WITH TUMOR NECROSIS FACTOR-ALPHA ANTAGONISTS: NEW FINDINGS

PhD THESIS

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MD

To my family and

to dr Piercarlo Sarzi-Puttini

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We hereby certify that the thesis entitled "**ANTIBODY PROFILES IN PATIENTS TREATED WITH TUMOR NECROSIS FACTOR-ALPHA ANTAGONISTS: NEW FINDINGS**" and presented by Fabiola Atzeni was written under our supervision, and believe that it satisfies the requirements for a PhD in Immunology.

Prof. Piercarlo Sarzi-Puttini

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Dell' Acqua D, de Portu S, Cecchini G, Cruini C,

Carrabba M, Meroni PL.

Arthritis Res Ther 2005; *In press*

for Crohn's disease: a prospective cohort study. **Atzeni F,**

Ardizzone S, Sarzi-Puttini P, Colombo E, Maconi G,

De Portu S, Carrabba M, Bianchi Porro G.

Aliment Pharmacol Ther 2005;22:453-61

FIGURES

1. ABBREVIATIONS

aCL: anticardiolipin antibodies

ACR: American College of Rheumatology

ANA: antinuclear antibody

Anti-β2GPI: anti-beta2 glycoprotein antibodies

Anti-CCP: anti-cyclic citrullinated peptide antibodies

Anti-dsDNA: anti-double stranded (ds) DNA

aPL: antiphospholipid antibodies

AR: rheumatoid arthritis

AS: ankylosing spondylitis

CD: Crohn's disease

CDAI: Crohn's Disaese Activity Index

CHF: Congestive heart failure

CLIFT: IIF using C. Luciliae

CRP: C-reactive protein

DAS-28:   Disease Activity Score

DMARDs: disease-modifying antirheumatic drugs

ELISA: enzyme-linked immunosorbent assay

ENA: anti-nuclear extractable antigens

HPA axis: hypothalamic - pituitary - adrenal axis

I.V.:   intravenous

ESR: erythrocyte sedimentation rate

FDA: Food and Drug Administration

Fig.:	figure
Ig:	immunoglobulin
IL:	interleukin
IIF:	indirect immunofluorescence
IFN:	interferon
JRA:	juvenile rheumatoid arthritis
MMPs:	metalloproteinases
MRI:	magnetic resonance imaging
MTX:	methotrexate
NKT:	natural killer T Cells
ReAct:	Adalimumab Research in Active RATrial
RF:	rheumatoid factor
SAP:	serum amyloid P
S.C.:	subcutaneous
SpA:	spondyloarthritis
SLE:	systemic lupus erythematosus
Th:	T helper
TB:	tuberculosis
TNF-α:	tumor necrosis factor-alpha

2. ACKNOWLEDGEMENTS

This thesis is the result of a long journey between four large cities that individually represent various aspects of my life. They have each contributed to my professional, cultural and personal formation.

1) Cagliari: My city! The home of my family, and all of the affections of those I love. A city that represents my eternal nostalgia.

2) Barcelona: My second native land. The home of my University, my friends and my inner peace.

3) Milan: The city of difficult tasks, publications, solitude, and inner frustrations.

4) New York: My dream! The city of English- my nightmare. Also a place of hard work and fatigue.

This thesis is not just a journey between different worlds and realities. It is essentially a representation of various encounters with people. Though each encounter differed, each was equally important and significant. I want to thank everyone who supported and encouraged me to continue to climb the "mountain of ice" during those times when I felt tempted to top and turn back.

Particularly I want to thank Dr. Piercarlo Sarzi-Puttini, someone who has been both a guide and a friend simultaneously. He stood by me even as he faced his own hardships through life. He believed in me and encouraged me to continue down the sometimes torturous road of medicine. Essentially he helped me to realized "my dream." A dream that has caused my life to be spread between Milan and Barcelona, between loneliness

and "movida." I wish to thank him for giving me the opportunity to speak in public, even when I wasn't yet at my peak. Above all, I want to extend my thanks for correcting my work even when it was truly disastrous.

I would like to thank my father and mother because they continuously believed in me. They never doubted my professional or personal capacity. In the countless moments when I would have gladly abandoned everything to return home, they always managed to feel nearby even when the distances that separated us were enormous.

To my sisters Simona and Ilenia who supported me even when I became like a broken record and repeated the same phrases again and again. I want to thank them for being supportive during those times when I would complain about my own problems in life while ignoring theirs.

I also want to thank the group at the Hospital Luigi Sacco of Milan. Particularly I want to thank Prof. Mario Carrabba who accepted me in his group during the years spent in Milan.

I wish to acknowledge the Independent University of Barcelona, and I particular Prof. Paz Martinez for his availability, patience and suggestions.

And to my colleagues in New York. Particularly Kleio, Jing and Lorenzo.

2. RIGRANZIAMENTI

Questa tesi è il risultato di un lungo viaggio che ha coinvolto quattro grandi città, che rappresentano per me realtá diverse e che hanno contribuito alla mia formazione professionale, culturale e personale.

1. Cagliari: la mia cittá, la mia famiglia, gli affetti piú cari, la mia eterna nostalgia;
2. Barcelona: la mia seconda patria, l'Universitá, gli amici, la mia pace;
3. Milano: il duro lavoro, le pubblicazioni, la solitudine, la mia frustazione interiore;
4. New York: il mio sogno, l'inglese: il mio incubo, la dura fatica;

Il risultato di questa tesi non é solo un viaggio tra mondi e realtá diverse, ma soprattutto quello di un incontro con persone, tutte importanti, anche se in maniera diverse, che voglio ringraziare per essermi state accanto anche quando mi sembrava di scalare una "montagna di ghiaccio" e volevo fermarmi o ritornare indietro.

In modo particolare desidero ringraziare il dr Piercarlo Sarzi-Puttini, capo ed amico allo stesso tempo, per essermi stato accanto anche quando lui stesso é stato messo alla prova dai duri eventi della vita, per aver creduto in me, per avermi incoraggiato a continuare in questo tortuoso cammino della medicina e avermi aiutato nella realizzazione forse di "un sogno". Un sogno che mi ha portato a vivere tra Barcelona e Milano, tra la solitudine e la movida. Desidero ringraziarlo per avermi permesso di parlare in pubblico anche se ben consapevole di non essere ancora all'altezza e soprattutto per aver corretto miei lavori anche quando erano un vero disastro.

Ringrazio mio padre e mia madre perché hanno creduto sempre in me senza, dubbitare mai delle mie capacitá professional e personali, per essemi stati accanto, anche se le

distanze che ci separavano erano enormi, nei "mille" momenti in cui volentieri avrei abbonadonato tutto e sarei ritornata nella mia terra.

Mie sorelle, Simona e Ilenia, per avermi ascolatato anche quando sembrava che il "disco mi fosse incantato" e continuavo a ripetere sempre le stesse frasi, e a dire quando fosse complessa la mia vita, ignorando i lori problemi.

Il gruppo dell' Ospedale Luigi Sacco di Milano e in particolare il professor Mario Carrabba, per avermi accettato nel suo gruppo in questi anni trascorsi a Milano.

L' Universitá Autonoma di Barcelona e in particolare alla prof Paz Martinez per la sua disponibilitá, pazienza e per i consigli.

La Cornell University e lo Hospital for Special Surgery, in New York, la professoressa Mary Crow per avermi permesso di partecipare alla loro vita accademica, per avermi accolto a New York.

Il dottor Richard Cervera per la sua disponibilitá, per i suoi consigli, per aver contribuito alla mia formazione clinica e alla conoscenza della storia di Catalunia.

Il professor Jose Font per la sua disponibilitá e per accogliermi nel suo gruppo quando mi reco a Barcelona.

Il professor Maurizio Cutolo e il professor Roberto Caporali per la loro disponibilitá, per aver accettato di venire qui a Barcelona, per partecipare ad un evento per me importante.

Il professor Pierluigi Meroni per aver contribuito alla realizzazione di uno dei lavori piú importanti di questa tesi.

Gli amici sardi, in particolare Antonello, Marco e Loris per la loro pazienza, il sostegno morale e la loro amicizia.

Gli amici spagnoli, in particolare Charon e Maite, che sempre hanno rappresentato il mio punto di riferimento in Barcelona, per la loro disponibilita´ e amicizia.

I colleghi di New York, in particolre Kleio, Jang e Lorenzo.

3. INTRODUCTION

It has been reported that patients with rheumatoid arthritis (RA), Crohn's disease (CD) and spondyloarthritis (SpA) treated with selective tumor necrosis factor-alpha (TNF-α) inhibitors develop autoantibodies such as antinuclear antibodies (ANA) and anti-double stranded DNA (anti-dsDNA) antibodies [1-12]. TNF-α is a pro-inflammatory cytokine that is produced by multiple cell types, including blood monocytes, macrophages, mast cells and endothelial cells, and plays multiple complex functional roles within the immune system, including the stimulation of inflammation, cytotoxicity, the regulation cell adhesion and the induction of cachexia [13,14]. There are therefore a number of potential mechanisms by means of which anti-TNF-α treatment could be beneficial in RA, CD and other diseases [15-20].

RA is a chronic inflammatory disease that primarily affects the peripheral joints and often leads to tissue degradation and the destruction of bone and cartilage. As organic joint damage is irreversible, it is important to recognise and treat RA early with the aim of halting its progression [21,22]. Clinical trials have demonstrated that TNF-α blocking agents are highly beneficial for most patients with RA refractory to classic treatment with disease-modifying antirheumatic drugs (DMARDs), but a significant number also fail to respond to anti-TNF-α therapy [23]. No reliable predictive markers of a clinical response have been identified, although a recent report suggests that reduced rheumatoid factor (RF) and anti-cyclic citrullinated peptide (anti-CCP) antibody levels may be useful adjuncts when assessing treatment efficacy [24].

 A reduction in IgM-RF titres was initially described by Charles *et al*. [2] in a small series of patients receiving infliximab, but the subsequent findings were inconsistent. Two recent studies have found decreased RF and anti-CCP antibody titres in RA patients

treated with infliximab. In both cases, decrease paralleled the improvements in the disease activity scores, but one group reported a return to baseline levels when the follow-up was extended to 54 and 78 weeks [3,25]. However, De Rycke et al. [26] showed that RF, but not anti-CCP antibodies, is modulated by infliximab in RA and our findings support the existing evidence that RF and anti-CCP antibodies are independent autoantibody systems in RA.

CD is a chronic inflammatory disease of the gastrointestinal tract whose variable clinical course is characterised by segmental transmural inflammation and granulomatous changes of unknown origin [27]. Infliximab is a chimeric IgG1 monoclonal anti-TNF-α antibody that represents a significant advance in the treatment of CD [28]. Controlled clinical trials have demonstrated its effectiveness in rapidly induces and maintains remission in patients with moderate/severe refractory CD, healing endoscopic lesions, and treating draining perianal (PA) fistulae in the short and long term [13,20]. Moreover, audit data from North America and Europe have shown that its efficacy in clinical practice is comparable with that observed in clinical trials.

Trials have shown that CD and RA patients develop ANAs and anti-dsDNA antibodies: according to the reported safety data, respectively 63.8% and 49.1% of patients develop newly positive ANAs during infliximab treatment, and respectively 13% and 21.5% develop newly positive anti-dsDNA antibodies [29]. Two important papers have described the risk of immunogenicity induced by infliximab treatment in CD patients: Baert *et al*. [30] showed that the development of antibodies against infliximab leads to infusion reactions and a shorter treatment response to treatment and, as concomitant immunosuppressive therapy reduces the magnitude of the immunogenic response,

concluded that it is necessary to combine methotrexate (MTX) and infliximab in order to reduce the risk of the appearance of anti-idiotypic autoantibodies [31]; and Vermeire *et al*. [4] found a cumulative 24-month incidence of ANAs in 71/125 patients (56.8%), almost half of whom developed ANAs after the first infusion and >75% became ANA-positive after fewer than three infusions. However, lower percentages have been reported in patients treated with etanercept. Interestingly, these autoantibodies have been only anecdotally associated with clinical manifestations suggesting drug-induced systemic lupus erythematosus (SLE) [32-35].

It was thought that the absence of such an association was related to the IgM or IgA isotypes of anti-dsDNA antibodies, as well as low antibody affinity (in contrast with the widely accepted relationship between SLE and the high affinity IgG isotype) [2]. However, the occurrence of these autoantibodies is now considered a drug class-related side effect, despite the higher prevalence of ANAs and anti-dsDNA antibodies in patients treated with infliximab than in those treated with etanercept, and the absence of a flare when etanercept therapy was started in a patient with previous infliximab-induced SLE.

Finally, anti-phospholipid antibodies (aPL), which are mainly detectable by means of anti-cardiolipin assay (aCL), have also been reported in RA patients receiving TNF-α blockers. In some cases, their appearance was related to concomitant infectious processes, but no clear correlation was found with the specific clinical manifestations of anti-phospholipid syndrome, although one paper suggests that they may predict a poor clinical outcome [11,12].

The aim of this thesis is to evaluate prospectively the auto-antibody profiles of CD and RA patients treated with infliximab and adalimumab, and their relationships to clinical outcomes.

In particular, the aim of the first one-year prospective study was planned to evaluated: a) the clinical efficacy of adalimumab; b) whether the prevalence and titres of RA-associated auto-antibodies, such as RF and anti-CCP antibodies correlate with treatment effect; and c) whether non organ-specific auto-antibodies are induced by adalimumab like other TNF-α blocking agents.

The aim of the second study was evaluated: the frequency and correlation of autoantibody development at standardised timepoints in refractory/inflammatory and fistulising CD patients in a routine clinical setting. Finally, the findings were related to disease status before the start of infliximab treatment, the response to infliximab treatment and the onset of adverse clinical events.

The first part of this thesis considers the role of TNF-α in RA and CD, and the efficacy of anti-TNF-α agents in inducing auto-antibodies (including two recent reviews by our group and a recently published case report); the second evaluates the mechanisms involved in auto-antibody development in CD and RA patients receiving anti-TNF-α treatment; and the third briefly summarises two original studies, together with their discussion and conclusions.

4. TUMOR NECROSIS FACTOR- ALPHA (TNF-α)

TNF-α, a soluble 17-kD protein consisting of three subunits, is a potent cytokine produced by multiple cell types (including monocytes, macrophages, B and T cells, and fibroblasts) that plays a key role in CD and RA. It is an autocrine stimulator and potent paracrine inducer of other cytokines, including interleukin (IL)-1, IL-6 and IL-8, and granulocyte-monocyte colony-stimulating factor, and also promotes inflammation by stimulating fibroblasts to express adhesion molecules, such as intercellular adhesion molecules, which interact with their respective ligands on the surface of leukocytes and lead to increased leukocyte transport to inflammatory sites [36-39,13,14].

TNF-α indirectly down-regulates inflammation by stimulating the pituitary release of corticotropin , which stimulates the adrenal cortex release of cortisol, a direct inhibitor of inflammation (see Fig.1) [40].

Fig. 1 Tumor necrosis factor (TNF)-α
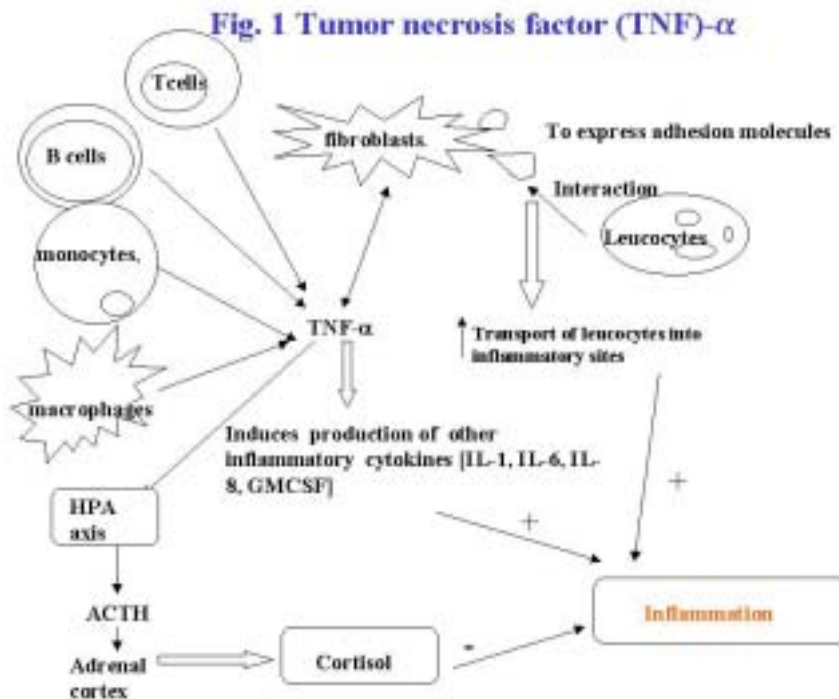
4.1. TNF-α AND RHEUMATOID ARTHRITIS

RA is the most common inflammatory joint disease, affects approximately 1% of adults worldwide, and is a major cause of disability, morbidity and mortality [21,41,42]. It is characterised by synovial pannus formation leading to cartilage destruction and bone erosions.

Although its pathogenesis is unknown, pro-inflammatory cytokines such as TNF-α and a IL-1 play a key role [43]. The synovial membrane of RA patients shows hyperplasia, increased vascularity and the infiltration of inflammatory cells. These are primarily CD4+ T cells which, when activated by an unknown antigen, stimulate monocytes, macrophages and sinovial fibroblasts to produce IL-1, IL-6 and TNF-α, and to secrete matrix metalloproteinases (MMPs) as a result of cell-surface signaling by means of CD 69 and CD118, as well through the release of soluble mediators such as interferon (IFN)-

γ, IL-17, IL-1, IL-6 and TNF-α [43-45]. Activated CD4+T cells also stimulate B cells by means of cell-surface contacts and the binding of α sub 1 and β sub 2 integrin, CD 40 ligand and CD28 to produce immunoglobulin (Ig), including RF. The precise role of RF is unknown, but it may be involved in activating complement as a result of the formation of immune complexes. Activated CD4+ T cells express osteoprogesterin ligands that stimulate osteoclastogenesis, and the activated macrophages, lymphocytes and fibroblasts can also stimulate the angiogenesis that is responsible for the synovial vascularity found in RA patients [43]. Synovium endothelial cells are activated and express adhesion molecules that promote the joint recruitment of inflammatory cells. Finally, TNF-α induces production of several cytokines, and stimulates fibroblasts to express adhesion molecules that interact with the ligands on the surface of leukocytes, thus increasing the number of inflammatory cells in the joint (see Fig.2).

Fig. 2 Pathogenesis of rheumatoid arthritis (RA)

Antigen unknown

HLA DBB1*0404/ DBB*10401.5

APC

Synovia

Macrophages

T cell CD4+

Synovial fibroblasts

Mononuclear cells

CD118   CD69,   **integrins**

TNF- α, IF-γ, IL-17, IL-1, IL-6

Osteoprogesterin ligand

CD28, CD40 ligand

B cells

Angiogenesis

Endotelial cells

Condrocytes

Osteoclasts

Ig, Rheumatoid Factor

Immunocomplex

TNF-α, IL-1, IL-6,

Granulocites

inhibitor of MMPS

↑MMPs

Bone destruction

Joint damage

4.2. TNF-α AND CROHN'S DISEASE

CD is a chronic inflammatory disease of the gastrointestinal tract that is characterised by segmental transmural inflammation and granulomatous changes, and is now one of the major gastrointestinal health problems in industrialised countries [27]. Its etiology is unclear, but known to involve genetic predisposition, infections agents, cell-mediated immunity and dietary factors [46]. The lipids presented by CD1 molecules are mainly recognised by a CD4/CD-8 double-negative subset of T lymphocytes called natural killer

T (NKT) cells, which resemble cytolytic T cells and are capable of producing large amounts of IFN-γ [47-53].

IFN-γ plays a role in macrophage activation and the subsequent release of a whole cascade of inflammatory cytokines, including IL-1, IL-12, IFN-γ, TNF-α and its regulatory counterpart IL-10, which promotes delayed hypersensitivity and granulomatous transformation – and one of the characteristic histological features of CD is non-caseating granulomas containing epithelioid and giant cells (see Fig.3, 4) [52].

Fig. 3 Pathogenesis of Crohn's disease (CD)

GALT

Glycolipids (M. food additives)   Intestinal epithelium

APC   CD8

NKT

IF-γ   Macrophages

IL-1, IL-12, TNF-α, IL-10

Granulomatous inflammation
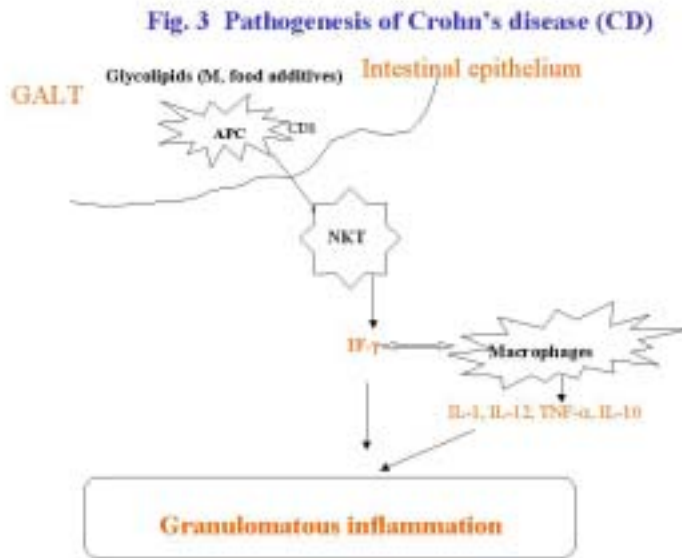
Fig. 4 Histological aspects of Crohn's disease (CD)
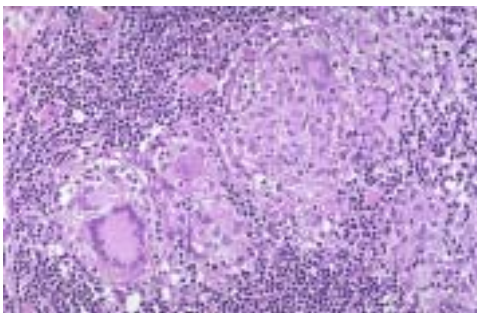
High-magnification image showing the granulomatous nature of the inflammation of Crohn's disease, with epithelioid cells, giant cells and many lymphocytes. Special stains for organisms are negative**.**

4.3. ANTI-TNF-α AGENTS

The fact that TNF-α plays a key role in the pathogenesis of chronic inflammatory diseases, including CD and RA, a new class of drugs have been developed in an attempt to neutralise its biological activities [54-56]. The three currently available TNF-α-neutralising agents are etanercept, infliximab and adalimumab; however, only infliximab and adalimumab induce the remission of CD (see Fig.5).

4.3.1. Etanercept

Etanercept is a fully human recombinant molecule consisting of two soluble TNF receptor (p75) subunits fused to the Fc portion of human IgG1. It binds and neutralises soluble and membrane-bound TNF as well as the related lymphotoxin-α molecule known as TNFβ. Infliximab and adalimumab only bind TNF-α, which explains why non-responders to another anti-TNF blocking agent may benefit from switching to etanercept because it is possible that subsets of patients have TNF-β- rather than TNF-α-dependent disease: for example, significant TNF-β levels are detected in patients with juvenile rheumatoid arthritis (JRA), for which only etanercept is efficacious [57-60].

Etanercept is administered twice weekly in the form of 25 mg subcutaneous (s.c.) injections. It is approved for use in treatment-resistant RA, and a 20% improvement in the ACR score has been found in 60-70% of RA patients after 12 weeks' therapy [57-59].

4.3.2. Infliximab

Infliximab is a chimeric monoclonal antibody with variable murine and constant human IgG1 regions [61-63] that binds soluble TNF-α. In RA, it is administered intravenously *(i.v.)* using three fortnightly doses of 3 mg/kg, followed by one 3 mg/kg dose every eight weeks. Like adalimumab, infliximab mediates the lysis of TNFα-expressing cells via

complement- or antibody-dependent cytotoxicity mechanisms [64-66]. Its specific binding to monocytes induces apoptosis by activating the capsase 8 and mitochondrial pathways *in vitro*, and it has been shown to induce the apoptosis of activated T cells [64]. Its powerful anti-inflammatory effect in CD has been explained by the recent finding that it induces apoptosis in peripheral blood monocytes and lamina propria T lymphocytes and, in clinical trials, it has led to a 20% improvement in the ACR score in more than 50% of patients with active AR [55,61-63,67].

4.3.3. Adalimumab

Adalimumab is a fully human therapeutic monoclonal antibody that specifically binds to circulating and cell-surface TNF-α, and blocks its interaction with p55 and p75 cell-surface TNF receptors [32,54]. In the presence of complement, it leads to the *in vitro* lysis of cells expressing high levels of surface membrane TNF-α. It does not bind or inactivate lyphotoxin (TNF-β), but it does modulate the biological responses induced by TNF, including changes in the levels of the adhesion molecules responsible for leukocyte migration (ELAM-1, VCAM-1 and ICAM-1) [32,68].

In RA patients, adalimumab treatment rapidly decreases C-reactive protein (CRP) levels, the erythrocyte sedimentation rate (ESR) and IL-6 levels, and induces changes in the hypothalamic-pituitary-adrenal (HPA) axis [32,69-71]. The recommended dose in adults with RA is 40 mg administered as a single dose s.c. every 2 weeks [72].

In CD patients, a loading dose of 80 mg s.c. is recommended, followed by 40 mg every two weeks; in particular, adalimumab seems to be an alternative in non-responders to infliximab treatment or in patients who had infusion reactions [73]. Baert *et al*. [30] have reported that a high proportion of CD patients treated with infliximab develop antibodies

against it, which shorten the duration of their clinical response and increase the incidence of infusion reactions; although patients treated with adalimumab develop antibodies against it, the incidence is low [32,74]. Clinical trials have shown that adalimumab alone or in combination with methotrexate (MTX) is effective in RA and a 20% improvement in the ACR score was observed in 67% of the patients receiving 12 weeks' therapy in the open-label, multicentre, multinational Phase IIIb Adalimumab Research in Active RA (ReAct) Trial conducted primarily in Europe [75].

Given the above, treatment with anti-TNF-α blocking agents has significant benefits in inflammatory diseases, such as RA, CD, ankylosing spondylitis (AS) and a variety of autoimmune and non-autoimmune diseases. The following review is an example of this use.

4.3.4. POTENTIAL OFF-LABEL USE OF INFLIXIMAB IN AUTOIMMUNE AND

NON-AUTOIMMUNE DISEASES: A REVIEW.

Atzeni F, Sarzi-Puttini P, Doria A, Iaccarino L, Capsoni F.

Potential off-label use of infliximab in autoimmune and nonautoimmune diseases: a review

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Abstract

TNF-α is a crucial cytokine in the establishment and maintenance of inflammation in multiple autoimmune and nonautoimmune disorders. A number of large placebo-controlled trials have shown that infliximab, a chimeric monoclonal antibody against TNF-α, is effective and well-tolerated in patients with Crohn's disease and rheumatoid arthritis (RA) and has become a widely used treatment for these diseases. More recent controlled trials have also shown the effectiveness of TNF-α blockers in psoriasis, psoriatic arthritis, and ankylosing spondylitis. The results of clinical trials, open-label studies, and case studies indicate that TNF inhibitors (alone or in combination with other protocols) look very promising for the treatment of a variety of other conditions, including uveitis, sarcoidosis, Sjögren's syndrome (SS), Behçet's syndrome, vasculitis, and graft versus host disease. There is a rationale for using TNF blockade even in systemic lupus erythematosus, a prototype of autoantibody-mediated disease, and a pilot study seems to confirm this potential effective approach. The neutralisation of TNF might therefore play a role in the treatment of many autoimmune and nonautoimmune disorders other than Crohn's disease or RA.

We here review the current and prospective roles of infliximab in the treatment of autoimmune diseases and other conditions that do not currently have FDA or EMEA approval.
© 2004 Published by Elsevier B.V.

Keywords: TNF-α; Infliximab; Autoimmune and nonautoimmune disorders; TNF inhibitors; biologic agents

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28 **Contents**

1. Introduction

TNF-α is a crucial cytokine in the establishment and maintenance of inflammation in many autoimmune diseases. A number of large placebo-controlled trials have shown that infliximab, a chimeric monoclonal antibody against TNF-α, is effective and well tolerated, and it has now become a widely used treatment for Crohn's disease [1]. In 1998, the FDA approved infliximab in the treatment of moderate to severe active Crohn's disease with the aim of reducing signs and symptoms in patients inadequately responding to conventional therapies [1]; it was also indicated for the treatment of patients with fistulising Crohn's disease in order to reduce the number of draining enterocutaneous fistulae. In November 1999, the FDA approved its use in rheumatoid arthritis (RA) with methotrexate and expanded this indication in December 2000 [2]; in June 2003, the European Union expanded its indication to include the treatment of ankylosing spondylitis [3].

The results of clinical trials, open-label studies, and case studies indicate that TNF inhibitors (alone or in combination with other protocols) look very promising for the treatment of a variety of other conditions, including uveitis, sarcoidosis, Sjőgren's syndrome (SS), Behçet's syndrome, vasculitis, and graft versus host disease. There is a rationale for using TNF blockade even in systemic lupus erythematosus, a prototype of autoantibody-mediated disease, and a pilot study seems to confirm this potential effective approach.

We here review the current and prospective roles of infliximab in the treatment of autoimmune diseases and other conditions that do not currently have FDA or EMEA approval.

2. Infliximab

Infliximab is an intravenously administered chimeric IgG 1K monoclonal antibody consisting of a constant human regions and variable murine regions that specifically binds human TNF-α with an association constant of 1010 M1. The recommended dose for the treatment of severe active Crohn's disease in refractory patients is 5 mg/kg as a single dose [1]; in the treatment of RA, it is given at a dose of 3 mg/kg week 0, 2, and 6, with a maintenance dose of 3 mg/kg every 8 weeks (6/year).

96 The infusion of infliximab induces a rapid and
97 clinically highly efficacious TNF blockade, with a
98 remission rate of 30–40% after a single dose. A lack
99 of response appears to be a stable trait even after
100 repeated infusions, thus suggesting that it may be
101 genetically determined; however, extensive studies of
102 TNF-α gene mutations as predictors of response have
103 led to various results, and it is known that poly-
104 morphisms in the TNF-R1 and TNF-R2 receptors are
105 not associated with treatment response [2].

106 *2.1. Biological effects of infliximab*

107 TNF-α induces proinflammatory cytokines such as
108 IL- 1 and IL-6, which enhance leukocyte migration by
109 increasing endothelial layer permeability and the
110 expression of adhesion molecules by endothelial cells
111 and leukocytes, activating neutrophil and eosinophil
112 functions, and inducing acute phase and other liver
113 proteins. Infliximab neutralises the biological activity
114 of TNF-α by binding with high affinity to its soluble
115 and transmembrane forms, and inhibiting the binding
116 of TNF-α to its receptors [2]; the related TNF-β
117 (lymphotoxin a) cytokine, which uses the same
118 receptors as TNF-α, is not neutralised by infliximab.
119 It has also been found that infliximab down-regulates
120 IL-18, but not IL-12 or IL-13 [4], up-regulates CXC-
121 chemokine receptor type II expression, magnifies the
122 proliferative activity of CXC-chemokines in human
123 melanocytes, and, in Crohn's disease, decreases the
124 levels of IL-10 [5]. In brief, the TNF-α blocking
125 effects of infliximab are beneficial in the case of
126 diseases that involve the aberrant effects of lympho-
127 cytes, macrophages, and neutrophils.

128 **3. Psoriasis**

129 TNF-α plays a fundamental role in the onset and
130 persistence of skin inflammation in psoriasis. The best
131 evidence of the essential activity of this cytokine in
132 the pathogenesis of psoriasis comes from the fact that
133 selective TNF-α blockers are therapeutically highly
134 efficacious [6]. The TNF-α inhibitors, infliximab and
135 etanercept, have been successfully used in the treat-
136 ment of moderate to severe psoriasis and psoriatic
137 arthritis in randomised controlled trials [7]. In a trial
138 involving 33 psoriatic patients, infliximab 5 or 10 mg/

139 kg or placebo was given at weeks 0, 2 and 6, and 82%
140 in the 5 mg/kg group and 73% in the 10 mg/kg group
141 achieved at least a 75% reduction in the Psoriasis Area
142 and Severity Index (PASI) by week 10 [8]. In a
143 different study, the mean PASI score decreased from
144 19.04 to 4.91, once again an improvement of about
145 75% [9]. A 54-week, open-label, compassionate-use
146 study of 10 patients who received intravenous
147 infliximab (5 mg/kg in weeks 0, 2, and 6, with
148 individualised doses after week 10) and continued
149 with their current therapy (stable dose) until week 10,
150 experienced a 71.3% improvement in their PASI
151 scores and a mean 82.5% reduction in joint inflam-
152 mation from baseline as assessed by magnetic
153 resonance imaging [9].

154 An open-label trial of a single infliximab dose
155 given to seven patients found a 69% PASI improve-
156 ment after 2 weeks, which was sustained in the fourth
157 until the tenth weeks [10]. Infliximab can also be used
158 with good effects in methotrexate-resistant psoriasis,
159 with a median time to response of 4 weeks. Anti-TNF-
160 α biological agents induce rapid disease resolution
161 and long-lasting remission, thus suggesting that they
162 may alter the natural course of the disease; further
163 studies are warranted in order to establish more
164 precisely the biological bases of this action, the
165 subgroup of patients who may benefit most from
166 treatment, and the modalities of combination therapy
167 with other antipsoriatic agents [10]. Many other TNF-
168 α inhibitors have been developed, but none of them
169 has yet been used in the treatment of psoriasis.

170 The variants of psoriasis are also responsive to
171 infliximab; even severe cases of pustular psoriasis
172 respond rapidly [10]; its efficacy in three patients
173 indicate that it is a promising medication in the
174 treatment of psoriatic arthritis [7]. It is also useful in
175 treating recalcitrant erythrodermic psoriasis [11].
176 Infliximab will probably also be a useful agent in
177 treating palmoplantar psoriasis, because etanercept, a
178 similar medication, can be effective in this regard
179 [12]. It was also found to be helpful in treating an
180 HIV-positive patient with Reiter's syndrome [13].

181 The main limitations concerning the use of
182 selective TNF-α blockers in psoriasis include the
183 reactivation of latent tuberculosis, the risk of oppor-
184 tunistic infections, the development of specific anti-
185 bodies (which is associated with a shorter duration of
186 treatment response), and the fact that their high cost

187 has given rise to a host of reimbursement issues [14].
188 Feldman et al. [15] say that narrow-band ultraviolet B
189 (UVB) phototherapy seems to be the best first-line
190 agent for the control of psoriasis in terms of efficacy,
191 safety, and cost-effectiveness, and infliximab should
192 be considered a second-line agent.

4. Other cutaneous diseases

194   Many cutaneous diseases involve a prominent
195 infiltrate of neutrophils with or without eosinophils,
196 and blocking TNF-α breaks the inflammatory cascade
197 by decreasing neutrophil proliferation and function.
198 Some researchers have pointed out that this could be
199 useful in treating bullous dermatoses (e.g., pemphi-
200 gus) and pyoderma gangrenosum, and it is known to
201 be highly effective in treating recalcitrant subcorneal
202 pustular dermatosis (Sneddon–Wilkinson disease;
203 [16]). A sustained response to infliximab has been
204 found in patients with SAPHO syndrome [17], and
205 severe hidradenitis suppurativa without comorbid
206 conditions is also responsive to infliximab.

5. Sjőgren's syndrome

208   There is currently no effective treatment for
209 patients with primary Sjőgren's syndrome (SS), but,
210 as TNF-α may be a key element in its pathogenesis, a
211 multicentre, randomised, double-blind, placebo-con-
212 trolled trial randomly assigned 103 patients with
213 primary SS to receive infliximab 5 mg/kg or placebo
214 in week 0, 2, and 6 [18]. The patients were then
215 followed up for 22 weeks. All of the patients fulfilled
216 the new American-European Consensus Group cri-
217 teria for SS and had active disease as determined by
218 values of >50 mm on two out of three 0–100 mm
219 visual analogue scales (VAS) that evaluated joint pain,
220 fatigue, and buccal, ocular, skin, vaginal, or bronchial
221 dryness [18]. By week 10, 26.5% of the patients
222 receiving placebo and 27.8% of those treated with
223 infliximab had a favourable overall response
224 (P=0.89); the corresponding figures at week 22 were
225 20.4% and 16.7% (P=0.62). Thus, there was no
226 difference between the two groups in terms of any of
227 the secondary endpoints (e.g., tender and swollen
228 joints, the basal salivary flow rate, the results of the

229 Schirmer test for lacrimal gland function, the focus
230 score on labial salivary gland biopsy) during the 22
231 weeks of the trial. This trial did not provide any
232 evidence of the efficacy of infliximab in primary SS
233 (18).

6. Adult Still's disease

235   There is no consensus concerning the treatment of
236 corticosteroid-resistant adult-onset Still's disease
237 (ASD). In a French trial involving 20 patients
238 unresponsive to the usual therapy (15 treated with
239 infliximab, 10 with etanercept, and 5 with both drugs
240 consecutively), 18 of whom were concurrently receiv-
241 ing steroids and 17 an immunosuppressant, a partial
242 response was observed in 16 cases: 7 treated with
243 etanercept and 9 with infliximab [19]. There were four
244 treatment failures (two on each anti-TNF-α agent).
245 These data showed that anti-TNF-α therapy may be
246 helpful for some patients with refractory ASD,
247 although most patients achieve only a partial remis-
248 sion [19].

7. Vasculitis

250   Infliximab can remove the conditions which
251 facilitate the development of vasculitis. It is useful
252 for treating rheumatoid and relapsing vasculitis and
253 has been found to be efficacious in treating giant cell
254 arteritis. It has also been effectively used to treat RA-
255 associated vasculitis and cryoglobulinemia and can be
256 useful in treating Wegener's granulomatosis and
257 Churg–Strauss syndrome [20–22]. Cogan's syndrome,
258 idiopathic keratoscleritis, and lymphomatous tracheo-
259 bronchitis have been successfully treated with inflix-
260 imab, which has also been found helpful in the
261 treatment of refractory polymyalgia rheumatica [23].
262   Endothelial vasomotor dysfunction and the
263 markers of systemic inflammation are independent
264 determinants of cardiovascular risk, but the link
265 between clinical inflammation and endothelial dys-
266 function is unclear. Chandesris et al. [22] used
267 infliximab to treat 14 patients with active antibodies
268 associated vasculitis. They showed that vasculitis is
269 associated with endothelial dysfunction and that anti-
270 TNF-α therapy (alone or in combination with standard

271 treatment) leads to clinical remission, reduced inflam-
272 mation, and improved endothelium-dependent vaso-
273 motor responses [20–23].

8. Kawasaki's disease

275 Kawasaki's disease (KD) is a multisystem vascu-
276 litis of unknown etiology. Coronary artery aneurysms
277 occur in 25% of untreated cases, but in only 4% of
278 patients receiving conventional treatment with intra-
279 venous immunoglobulin (i.v. IG) and high-dose
280 aspirin (ASA). Unresponsive children are a challenge,
281 and TNF-α levels peak during the acute and subacute
282 phase of KD, especially in the children who develop
283 coronary artery aneurysms. There is only one pub-
284 lished case of a child treated with infliximab: a 3-year-
285 old male with giant coronary artery aneurysms
286 unresponsive to multiple doses of i.v. IG and
287 methylprednisolone, who defervesced and showed
288 improved laboratory measures after the first dose of
289 infliximab [24].

9. Behçet's disease

291 Infliximab has been used to treat a variety of
292 manifestations of Behçet's disease, including severe
293 and pediatric cases, and has induced remission. The
294 manifestations successfully treated with infliximab
295 include panuveitis (five patients), gastrointestinal
296 disease, ileocolitis, cervical esophageal perforation,
297 and recalcitrant orogenital ulceration [25]. Infliximab
298 can be used to treat Behçet's disease in cases that
299 have failed to respond to etanercept [26]. Our group
300 has recently described a case of a patient with a
301 severe Behçet's who failed to respond to the usual
302 therapy showed a sustained improvement on etaner-
303 cept [27].

10. Uveitis and scleritis

305 The efficacy and safety of infliximab have been
306 assessed in treatment-resistant uveitis and scleritis
307 [28]. In a retrospective, noncomparative interventional
308 case series of seven patients with noninfectious ocular
309 inflammatory disease refractory to alternative immu-

310 nosuppression, infliximab 200 mg was given at
311 intervals of 4–8 weeks depending on the clinical
312 response [28]: six patients clinically improved, with
313 five achieving remission and significant reduction in
314 immunosuppression, one patient showed an initial
315 response but developed a delayed hypersensitivity
316 response that precluded further treatment. No other
317 adverse effects occurred. In conclusion, infliximab
318 seems to be an effective and safe treatment for
319 noninfectious uveitis and scleritis and may be
320 indicated as rescue therapy for relapses of ocular
321 inflammation or as maintenance therapy when con-
322 ventional immunosuppression has failed [28]. Further
323 investigation of the use of infliximab for treatment-
324 resistant scleritis and uveitis is warranted.

11. Systemic lupus erythematosus

326 Systemic lupus erythematosus (SLE) is an auto-
327 immune disease characterized by numerous autoanti-
328 bodies and clinical involvement in multiple organ
329 systems. However, it is the consequent systemic
330 inflammatory activity that is responsible of organ
331 damage. In fact, several studies suggest that this
332 inflammatory tissue damage can be prevented even in
333 the presence of autoantibodies and despite the
334 deposition of immune complexes and the activation
335 of complement [29].

336 TNF-α is increased in the blood of SLE patients
337 and is correlated with disease activity. Even sTNFR
338 serum levels are increased in SLE patients compared
339 to normal subjects, and both receptors (sTNFR I and
340 sTNFR II) may correlate with disease activity [30].
341 However, it is worthy to note that the increased TNF-
342 α found in SLE sera is bioactive [31], and it has been
343 suggested that it plays a role in the pathogenesis of the
344 disease.

345 Moreover, kidney sections from patients with lupus
346 glomerulonephritis contain a significant amount of
347 TNF-α, and its expression correlates with histological
348 activity [32].

349 We recently investigated the expression of TNF-α
350 in cutaneous lesions of patients with SLE using an
351 immunohistochemical approach. We observed that
352 lesional skin of SLE patients was characterized by an
353 increased expression of TNF-α when compared with
354 normal skin obtained from the same patient.

Therefore, TNF-α seems to play a significant role in the tissue inflammation, and these results support the idea that the anti-TNF-α therapy may represent an interesting candidate for the treatment of some SLE manifestations.

Anti-TNF-α was effectively used in lupus prone mice, and in most cases, the autoantibody titre in the serum was reduced [33].

Up to now, only two observational studies on the use of infliximab in SLE patients have been published in form of abstracts. Aringer et al. [34] carried out an open study on safety and efficacy of TNF blockade in SLE. Six patients (three with nephritis, one with nephritis and arthritis, and two with arthritis) were treated with infliximab in combination with azathio-prine or methotrexate and/or low-dose corticosteroids. Anti-TNF blockade was safe and effective either in patients with nephritis or in those with arthritis. However, arthritis tended to relapse when therapy was stopped. It is worthy to note that in two patients, antidouble-stranded DNA antibodies increased during treatment but without concomitant SLE flare.

Katz et al. [35] treated with infliximab nine SLE patients with polyarthritis. Three patients improved, among them one discontinued infliximab due to pneumonia and one due to cerebrovascular accident. Six patients did not improve and discontinued treatment because of the severity of infusion reactions.

Further studies are needed to clarify the efficacy and toxicity of this treatment in SLE patients, particularly the potential damage due to the increase of autoantibody production.

12. Miscellaneous conditions

12.1. Acute alcoholic hepatitis

TNF-α may contribute to the progression of acute alcoholic hepatitis (AAH). A recent study evaluating the efficacy of the combination of infliximab and prednisolone in patients with severe AAH (Maddrey score ≥32) compared intravenous infusions of inflix-imab 10 mg/kg in weeks 0, 2, and 4 with placebo administered at the same times; all of the patients received prednisolone (40 mg/day) for 28 days [36]. After the randomisation of 36 patients, 7 patients in the infliximab group and in the placebo group died within 2 months; the probability of death within 2 months was nonsignificantly higher in the infliximab group ($39\pm11\%$ vs. $18\pm9\%$), and the study was stopped by the Follow-up Committee and the sponsor (Assistance Publique-Hopitaux de Paris). The frequency of severe infections within 2 months was also higher in the infliximab group ($P<0.002$). In conclusion, three infusions of infliximab 10 mg/kg in combination with prednisolone may be harmful in patients with severe AAH because of the high prevalence of severe infections [36].

12.2. Cap polyposis

Cap polyposis is a disorder characterised by bloody diarrhea with rectosigmoid polyps covered by a cap of fibropurulent exudate. Its pathogenesis is unknown, but histological findings suggest that mucosal pro-lapse may play a role. Drug therapies are usually unsuccessful, and treatment requires sigmoid resec-tion or, if the disease recurs after initial surgical resection, panproctocolectomy. There is one published case of a patient unresponsive to treatment with mesalamine, antibiotics, lidocaine enemas, and corti-costeroids who, after four infliximab infusions at 8-week intervals, had normal rectum and sigmoid colon endoscopy results, and biopsies showed the complete histological resolution of the inflammatory process [37]. It was concluded that infliximab is an effective therapy for cap polyposis and avoids the requirement for surgery. No clinical evidence was obtained to support mucosal prolapse as a causative factor, but the response to infliximab suggests that TNF-α plays a role in the pathogenesis of this disorder [37].

12.3. Graft-versus-host disease

Acute and chronic graft-versus-host diseases (GVHDs) remain the major obstacles to successful hematopoietic cell transplantation. The induction of GVHD can be divided into three phases: (i) recipient conditioning, (ii) donor T cell activation, and (iii) effector cells mediating GVHD. It has been shown that cytokines are extremely important in initiating and propagating GVHD [38], and it is worth noting that IL-2 and TNF-α lead to cell activation as well as local tissue damage. The last few years have seen major advances in the development of monoclonal

443 antibodies that target cytokines; drugs targeting the
444 IL-2 receptor (daclizumab and basiliximab) are now
445 widely used to prevent renal transplant rejection [38],
446 and drugs targeting TNF-α (infliximab, etanercept,
447 and adalimumab) are currently used in rheumatoid
448 arthritis and Crohn's disease but are also being tested
449 in a number of other autoimmune diseases. These
450 agents are very selective immunosuppressants, whose
451 mechanisms of action are different from those of
452 calcineurin inhibitors; they are therefore potentially
453 promising for the treatment or prevention of GVHD
454 [38]. In a case of acute GVHD with lupus anti-
455 coagulant, the lupus anticoagulant disappeared follow-
456 ing immunosuppressive therapy with a combination of
457 steroids and infliximab [39]. Infliximab was also found
458 to be useful in the treatment of acute GVHD in a series
459 of three patients, and other reports indicate that it can
460 be used to treat especially steroid-refractory cases of
461 GVHD.

462 Many authors have presented updated results
463 concerning the use and development of anticytokine
464 therapy for GVHD [39]. The most effective approach
465 to GVHD prevention will probably be a combination
466 regimen capable of disrupting the three phases of the
467 GVHD cascade. Once GVHD has occurred, all three
468 phases are activated. The development of combination
469 therapies for the treatment of both acute and chronic
470 GVHD will probably yield better results than mono-
471 therapy. The numerous new treatment modalities
472 should improve the outlook for acute and chronic
473 GVHD.

474 *12.4. Chronic sciatica*

475 One early study reported good results in patients
476 with sciatica and disc herniation of up to 3 months'
477 duration. Atcheson and Dymeck [40] described a new
478 case of a man who had failed extensive treatment for
479 several months (including three epidural injections),
480 after 8 months of unrelenting sciatica, he received a
481 single infliximab infusion, and 1 week later reported a
482 >50% reduction in back and leg pain. Six months after
483 treatment, his back and leg pain had decreased by,
484 respectively, 89% and 86%. Magnetic resonance
485 imaging 3 months after the infliximab infusion
486 showed a 50% reduction in herniation and the
487 disappearance of a previously noted S1 root com-
488 pression. This report extends the potential use of

489 infliximab to patients with more chronic sciatica and
490 those who have previously received epidural steroids.

13. Conclusions
491

492 TNF neutralisation seems to be a useful target in a
493 variety of autoimmune and other disorders. Larger
494 randomised trials are warranted to confirm the
495 preliminary data and case reports.

Take-home messages
496
497

- TNF-α is a crucial cytokine in the establishment
  and maintenance of inflammation in multiple
  autoimmune and nonautoimmune disorders.
- The results of recent clinical trials and case studies
  indicate that TNF inhibitors (alone or in combina-
  tion with other protocols) look very promising for
  the treatment of a variety of other conditions,
  including uveitis, sarcoidosis, Sjögren's syndrome,
  Behçet's syndrome, vasculitis, and graft versus
  host disease.
- There is a rationale for using TNF blockade even
  in systemic lupus erythematosus, a prototype of
  autoantibody mediated disease, and a pilot study
  seems to confirm this potential effective approach.
- Infliximab and other biologic agents may play a
  significant role in the treatment of autoimmune
  diseases and other conditions that do not currently
  have FDA or EMEA approval.

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References
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518

[1] Comerford LW, Bickston SJ. Treatment of luminal and
fistulizing Crohn's disease with infliximab. Gastroenterol Clin
North Am 2004;33:387–406.
[2] Maini R, St Clair EW, Breedveld F, Furst D, Kalden J,
Weisman M, et al. Infliximab (chimeric anti-tumour necrosis
factor alpha monoclonal antibody) versus placebo in rheuma-
toid arthritis patients receiving concomitant methotrexate: a
randomised phase III trial. ATTRACT Study Group. Lancet
1999;354:1932–9.
[3] Braun J, Brandt J, Listing J, Zink A, Alten R, Burmester G,
et al. Long-term efficacy and safety of infliximab in the
treatment of ankylosing spondylitis: an open, observational,
extension study of a three-month, randomized, placebo-
controlled trial. Arthritis Rheum 2003;48:2224–33.

519
520
521
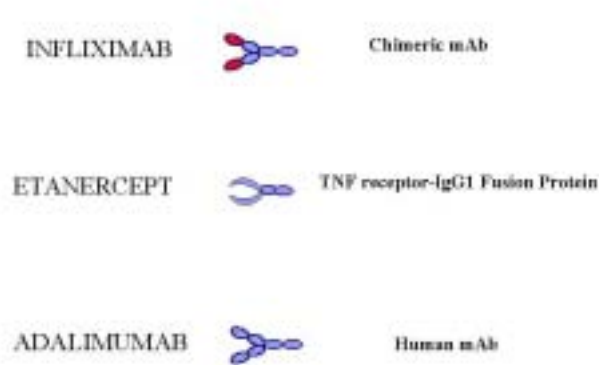522
523
524
525
526
527
528
529
530
531
532

[4] Pittoni V, Bombardieri M, Spinelli FR, Scrivo R, Alessandri C, Conti F, et al. Anti-tumour necrosis factor (TNF) alpha treatment of rheumatoid arthritis (infliximab) selectively down regulates the production of interleukin (IL) 18 but not of IL12 and IL13. Ann Rheum Dis 2002;61:723–5.

[5] Norgauer J, Dichmann S, Peters F, Mockenhaupt M, Schraufsttter I, Herouy Y. Tumor necrosis factor alpha induces upregulation of CXC-chemokine receptor type II expression and magnifies the proliferative activity of CXC-chemokines in human melanocytes. Eur J Dermatol 2003;13:124–9.

[6] Gottlieb AB. Infliximab for psoriasis. J Am Acad Dermatol 2003;49(2 Suppl.):S112–7.

[7] Cauza E, Spak M, Cauza K, Hanusch-Enserer U, Dunky A, Wagner E. Treatment of psoriatic arthritis and psoriasis vulgaris with the tumor necrosis factor inhibitor infliximab. Rheumatol Int 2002;22:227–32.

[8] Gottlieb AB, Li S, Evans R, Menter A. Infliximab in the treatment of psoriasis: results from the first 10 weeks of a phase II trial. Poster presented at: American Academy of Dermatology 61st Annual Meeting; March 21–26, 2003; San Francisco, CA.

[9] Antoni C, Dechant C, Hanns-Martin Lorenz PD, Wendler J, Ogilvie A, Lueftl M, et al. Open-label study of infliximab treatment for psoriatic arthritis: clinical and magnetic resonance imaging measurements of reduction of inflammation. Arthritis Rheum 2002;47:506–12.

[10] Gisondi P, Gubinelli E, Cocuroccia B, Girolomoni G. Targeting tumor necrosis factor-alpha in the therapy of psoriasis. Curr Drug Targets Inflamm Allergy 2004;3:175–83.

[11] Rongioletti F, Borenstein M, Kirsner R, Kerdel F. Erythrodermic, recalcitrant psoriasis: clinical resolution with infliximab. J Dermatol Treat 2003;14:222–5.

[12] Weinberg JM. Successful treatment of recalcitrant palmoplantar psoriasis with etanercept. Cutis 2003;72:396–8.

[13] Gaylis N. Infliximab in the treatment of an HIV positive patient with Reiter's syndrome. J Rheumatol 2003;30:407–11.

[14] Rabinovich M, Fainaru M. Infliximab for the treatment of extensive plaque psoriasis-regulation, cost and reimbursement. Isr Med Assoc J 2003;5:817–8.

[15] Feldman SR, Garton R, Averett W, Balkrishnan R, Vallee J. Strategy to manage the treatment of severe psoriasis: considerations of efficacy, safety and cost. Expert Opin Pharmacother 2003;4:1525–33.

[16] Voigtlander C, Luftl M, Schuler G, Hertl M. Infliximab (antitumor necrosis factor alpha antibody): a novel, highly effective treatment of recalcitrant subcorneal pustular dermatosis (Sneddon–Wilkinson disease). Arch Dermatol 2001;137:1571–4.

[17] Wagner AD, Andresen J, Jendro MC, Hulsemann JL, Zeidler H. Sustained response to tumor necrosis factor alpha-blocking agents in two patients with SAPHO syndrome. Arthritis Rheum 2002;46:1965–8.

[18] Mariette X, Ravaud P, Steinfeld S, Baron G, Goetz J, Hachulla E, et al. Inefficacy of infliximab in primary Sjogren's syndrome: results of the randomized, controlled Trial of Remicade in Primary Sjogren's Syndrome (TRIPSS). Arthritis Rheum 2004;50:1270–6.

[19] Fautrel B, Sibilia J, Mariette X, Combe B, Cri CR. Tumor necrosis factor{alpha} blocking agents in refractory adult Still's disease: an observational study of 20 cases. Ann Rheum Dis 2004 (Jun 7) [Electronic publication ahead of print].

[20] Lamprecht P, Arbach O, Voswinkel J, Lilienthal T, Nolle B, Heller M, et al. Induction of remission with infliximab in therapy-refractory Wegener's granulomatosis—follow-up of six patients. Dtsch Med Wochenschr 2002;127:1876–80.

[21] Hellmich B, Gross WL. Recent progress in the pharmacotherapy of Churg–Strauss syndrome. Expert Opin Pharmacother 2004;5:25–35.

[22] Chandesris MO, Gayet S, Schleinitz N, Doudier B, Harle JR, Kaplanski G. Infliximab in the treatment of refractory vasculitis secondary to hepatitis C-associated mixed cryoglobulinaemia. Rheumatology (Oxford) 2004;43:532–3.

[23] Salvarani C, Cantini F, Niccoli L, Catanoso MG, Macchioni P, Pulsatelli L, et al. Treatment of refractory polymyalgia rheumatica with infliximab: a pilot study. J Rheumatol 2003;30:760–3.

[24] Weiss JE, Eberhard BA, Chowdhury D, Gottlieb BS. Infliximab as a novel therapy for refractory Kawasaki disease. J Rheumatol 2004;31:808–10.

[25] Haugeberg G, Velken M, Johnsen V. Successful treatment of genital ulcers with infliximab in Behcet's disease. Ann Rheum Dis 2004;63:744–5.

[26] Estrach C, Mpofu S, Moots RJ. Behcet's syndrome: response to infliximab after failure of etanercept. Rheumatology (Oxford) 2002;41:1213–4.

[27] Sarzi-Puttini P, Atzeni F, Randisi G, Vulpio L, Marrazza MG, Antivalle M. Successful treatment of resistant Behcet's disease with etanercept. Ann Rheum Dis 2004;532 [abstract AB0100].

[28] Murphy CC, Ayliffe WH, Booth A, Makanjuola D, Andrews PA, Jayne D. Tumor necrosis factor alpha blockade with infliximab for refractory uveitis and scleritis. Ophthalmology 2004;111:352–6.

[29] Clynes R, Dumitru C, Ravetch JV. Uncoupling of immune complex formation and kidney damage in autoimmune glomerulonephritis. Science 1998;279:1052–4.

[30] Studnicka-Benke A, Steiner G, Petera P, Smolen JS. Tumor necrosis factor alpha and its soluble receptors parallel clinical disease and autoimmune activity in systemic lupus erythematosus. Br J Rheumatol 1996;35:1067–74.

[31] Aringer M, Feierl E, Steiner G, Stummvoll GH, Hofler E, Steiner CW, et al. Increased bioactive TNF in human systemic lupus erythematosus: associations with cell death. Lupus 2002;11:102–8.

[32] Aringer M, Zimmermann C, Graninger WB, Petera P, Stöckl S, Steiner G, et al. TNF is an essential mediator in lupus nephritis. Arthritis Rheum 2002;46:S418–9 [abstract].

[33] Segal R, Dayan M, Zinger H, Mozes E. Suppression of experimental systemic lupus erythematosus (SLE) in mice via TNF inhibition by an anti-TNFalpha monoclonal antibody and by pentoxiphylline. Lupus 2001;10:23–31.

[34] Aringer M, Zimmermann C, Graninger WB, Petera P, Steiner G, Smolen JS. Anti TNF therapy of SLE is feasible and apparently effective. Arthritis Rheum 2003;48:S378 [abstract].

[35] Katz RS, Holt-Daly N, MacDonald PA. Frequent infusion reaction associated with infliximab treatment in patients with

647 polyarthritis related to systemic lupus erythematosus. Arthritis
648 Rheum 2003;48:S379 [abstract].
649 [36] Naveau S, Chollet-Martin S, Dharancy S, Mathurin P, Jouet P,
650 Piquet MA, et al. Foie-Alcool group of the Association
651 Francaise pour l'Etude du Foie. A double-blind randomized
652 controlled trial of infliximab associated with prednisolone in
653 acute alcoholic hepatitis. Hepatology 2004;39:1390–7.
654 [37] Bookman ID, Redston MS, Greenberg GR. Successful treat-
655 ment of cap polyposis with infliximab. Gastroenterology
656 2004;126:1868–71.
667

657 [38] Jacobsohn DA, Vogelsang GB. Anti-cytokine therapy for the
658 treatment of graft-versus-host disease. Curr Pharm Des
659 2004;10:1195–205.
660 [39] Couriel DR, Saliba R, Hicks K, Ippoliti C, De Lima MJ,
661 Hosing C, et al. Tumor necrosis factor alpha blockade for the
662 treatment of steroid-refractory acute GVHD. Blood 2004
663 (Apr 6) [Electronic publication ahead of print].
664 [40] Atcheson SG, Dymeck T. Rapid resolution of chronic sciatica
665 with intravenous infliximab after failed epidural steroid
666 injections. Spine 2004;29:E248–50.

UNCORRECTED PROOF

Fig. 5 Anti-Tumor necrosis factor (TNF)-α agents

INFLIXIMAB   Chimeric mAb

ETANERCEPT   TNF receptor-IgG1 Fusion Protein

ADALIMUMAB   Human mAb

4.4. TOXICITY PROFILE AND AUTOIMMUNITY

Anti-TNF-α agents have had a marked impact on the treatment of RA and CD insofar as they have proved to be efficacious in reducing disease activity in patients incompletely responding to conventional therapy [56,57,76,77]. The controlled phase III trials conducted during their clinical development did not reveal any increase in serious adverse events in comparison with active control drugs, but do not cover a sufficient time or a sufficient number numbers of patients to detect unusual adverse events: since their completion, post-marketing reports of tuberculosis (TB), opportunistic infections and lymphomas have led the American Food and Drug Administration (FDA) to require label changes [77].

The safety issues relating to all TNF-α antagonists are infections (including *Mycobacterium TB* and other opportunistic infections), demyelinating disorders, autoimmune syndromes (including lupus-like syndrome), heart failure and administration reactions.

4.4.1. Infections

It is well known that serious infections occur in untreated RA patients and patients treated with traditional DMARDs, but there is evidence to suggest that TNF-α antagonists increase the risk of developing certain infections [78].

The incidence of TB seems to have increased the most: up until December 2002 in the USA, TB was reported in 39 patients treated with etanercept (with a least one fatal outcome) and in 335 treated with infliximab, with at least 12 deaths; during the course of adalimumab clinical trials, 13 cases of TB were observed in 2,468 patients, most of which developed before the implementation of TB surveillance [78,79]. The majority of TNF-α antagonist-associated cases are believed to be the result of the reactivation of latent disease, and nearly 50% were due to extra-pulmonary and/or disseminated disease: given these data, routine tuberculin skin testing has been recommended before starting treatment [80]. However, there are unpublished data confirming the effectiveness of isoniazide prophylaxis in patients with a positive tuberculin skin test before treatment with TNF-α antagonists.

Up until June 2002, opportunistic infections were reported in 337 patients treated with infliximab or etanercept for various reasons, leading to at least 21 deaths; the reported organisms included other mycobacteria, fungi such as *Histoplasma capsulatum*, and *Coccidioides immitis*, *Pneumocystis jiroveci* (*carinii*), and yeasts such as *Cryptococcus neoformans* and *Candida* species, mold such as *Aspergillus*. In terms of the underlying mechanisms, one recent *in vitro* study has demonstrated a decreased T helper 1 immune response against *H. capsulatum* by host defence cells treated with infliximab; however, as TNF-α also plays a role in granuloma formation, cytokines and adhesion molecule

production, enzyme release, and the migration and maturation of inflammatory cells, its neutralisation may have contributed to an increased susceptibility to infections [81, 77,13].

4.4.2. Lymphomas

A 2 to 25 times increase in the incidence of lymphomas among RA patients had been reported even before the introduction of TNF-α antagonists, and an important concern raised at the FDA Advisory Committee meeting in March 2003 was the absence of lymphomas in the comparative groups used in the clinical trials of etanercept, infliximab and adalimumab [77, 82,83]. Although this suggests that these biological agents increase the risk of lymphomas, further data (including careful longitudinal assessments of treated patients) are required and are currently being collected to clarify the issue [83].

4.4.3. Congestive heart failure

Congestive heart failure (CHF), mortality and hospitalisations due to heart failure were increased in the clinical trials of infliximab [34,84,85]. An FDA warning of cases of heart failure in patients treated with etanercept or infliximab led Wolfe *et al*. [86] to review the National Data Bank for cases of heart failure disease in patients with RA: the most relevant information gleaned was that there were no incidents of heart failure in 1,569 patients aged less than 50 years treated with TNF-α antagonists. Furthermore, heart failure seems to be a rare event as only 47 cases were reported to the FDA among the approximately 270,000 patients exposed to TNF-α blocking agents [34]. As TNF-α is important for viral clearance, one possible explanation for CHF in patients without a history of heart disease is that the myocardial decompensation is secondary to viral myocarditis, and one study of TNF-α-deficient mice has demonstrated decreased survival

after infection with encephalomyocarditis virus due to defects in viral clearance from the myocardium [84,85,87]. These findings suggest that evaluating viral infection may help characterise any new cases of heart failure among patients treated with TNF-α blocking agents [88].

4.4.4. Demyelination

Twenty cases of patients developing neurological symptoms with accompanying demyelination revealed by magnetic resonance imaging (MRI) have been reported to the FDA database as TNF-α antagonist-associated adverse events [77,89]. Although this complication has been attributed to the possible precipitation of a multiple sclerosis-like demyelinating syndrome, a brain biopsy from one index case demonstrated the presence of leukoencephalopathy [89]. Any future cases of " demyelination syndrome" will require careful analysis to determine the etiology of the symptoms, and greater scrutiny is necessary to exclude progressive multifocal leukoencephalopathy [89].

4.4.5. AUTOIMMUNITY AND ANTI-TNF-α AGENTS. REVIEW

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Autoimmunity and Anti–TNF-α Agents

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**ABSTRACT: Treatment of rheumatoid arthritis (RA) patients with anti-tumor
necrosis factor-alpha (anti–TNF-α) biologic agents has been associated with a
reduction in the levels of specific autoantibodies, such as rheumatoid factor
(RF) and anticyclic citrullinated peptide (anti-CCP), and the induction of non-
organ-specific autoantibodies (antinuclear antibodies [ANAs], anti-dsDNA,
and antiphospholipid antibodies [aPLs]). The mechanisms by which the block-
ade of anti–TNF-α decreases the generation of specific autoantibodies, such as
anti-CCP and RF, are not yet known. However, it has been shown that these
agents can downregulate the production of several inflammatory cytokines and
mediators and that these anti-inflammatory effects may account for reduced
autoantibody generation, particularly in the synovial compartment. Infliximab
treatment leads to the induction of ANAs in 63.8% of RA patients and 49.1%
of Crohn's disease (CD) patients, and anti-dsDNA antibodies in 13% of RA
patients and 21.5% of CD patients, respectively. The development of ANAs and
anti-dsDNA antibodies has also been described after etanercept therapy in
11% and 15% of RA patients, respectively. In the controlled trials, increases in
ANA and anti-dsDNA titers were observed in 5.3% and in 12.9% of adali-
mumab-treated RA patients. Only limited data on the induction of aPL anti-
bodies during TNF-α blocking treatment are available.**

**KEYWORDS: infliximab; etanercept; adalimumab; autoantibodies; rheumatoid
factor; anticyclic citrullinated peptide antibodies (anti-CCP); autoimmunity**

INTRODUCTION

Treatment with biologic agents directed against anti-tumor necrosis factor-alpha
(TNF-α), such as infliximab, etanercept, and adalimumab, has significant clinical

TABLE 1. Pharmacologic characteristics of TNF-α inhibitors

Pharmacologic characteristic	Infliximab	Etanercept	Adalimumab
Half-life	8–10 days	3–5.5 days	14 days
Binding affinity	1.8×10^9	10^{10}	2.3×10^{10}
Target binding	TNF	TNF, lymphotoxin	TNF
Structure	chimeric mAb	TNF receptor-IgG1 fusion protein	Human mAb
Complement	+	−	+
Cell lysis cytotoxicity	+	−	+
Administration and dose	i.v. 4–8 q wk 3–10 mg/kg	s.c. 2 q wk 25 mg	s.c. 2 q mo 40 mg

benefits in inflammatory diseases such as Crohn's disease (CD), ankylosing spondylitis (AS), rheumatoid arthritis (RA), and a variety of autoimmune and non-autoimmune diseases.[1–3]

Infliximab

Infliximab, a recombinant chimeric antibody produced by mouse myeloma cells (TABLE 1), contains sequences from human IgG1 constant and mouse variable regions, is specific for the membrane-bound or secreted or extracellular space TNF-α of humans and chimpanzees,[3–8] and prevents TNF from binding to its membranous and soluble receptors. After initial parenteral administration, its serum half-life is approximately 8.9 days and is maintained by dosing every 8 weeks thereafter. Its intravenous administration ensures that maximum serum concentrations are reached within 1 hour. Multiple clinical studies have confirmed that 30 weeks of treatment lead to a 20% improvement in the American College of Rheumatology (ACR) score in more than 50% of patients with active RA as against only 20% of patients treated with placebo.[9] The same effect is generally maintained after 54 weeks.[10–13]

Infliximab is registered by the Food and Drug Administration (FDA) and the European Medicines Evaluation Agency (EMEA) as therapy for treatment-resistant RA, treatment-resistant moderate to severe CD, and CD with fistulas.[8,10–14] Recently, its efficacy was demonstrated in seronegative spondyloarthritis (SS).[15,16]

Etanercept

Etanercept, a fusion protein (decoy receptor) secreted by Chinese ovary (CHO) cells, combines the ligand binding portion of human TNF receptor 2 (TNFR2=p75=CD120b) with sequences of human IgG1 (TABLE 1). Unlike TNFR1 (p55=CD120a), TNFR2 is a constitutive membrane receptor that is inducible upon stimulation and can be found on the surface of almost all cells except red blood cells and resting lymphocytes.[4] Because its affinity for TNF-α is greater than that for TNFR1, it can capture the cytokine more easily. Inasmuch as active TNFR2 is a trimer, the recombinant decoy receptor need not be a monomer to reach the required

bioactivity in competing with natural receptors.[3,4,17] Etanercept is administered twice weekly in 25-mg subcutaneous injections, its median half-life in serum is 4.8 days, and its maximum concentration is reached within 3 days. A 20% improvement in the ACR score was achieved in 60–75% of RA patients on 12 weeks of etanercept therapy as against 14–33 % of those on placebo.[17–21] Reports suggest that the benefit of etanercept treatment is maintained for years.

Etanercept is approved by the FDA and the EMEA for use in treatment-resistant RA as well as for severe active and progressive RA and treatment-resistant polyarticular juvenile chronic arthritis. It is also approved by the FDA for treatment-resistant psoriatic arthritis,[17–20,22,23] and it has a beneficial effect in AS.[24,25]

Adalimumab

Adalimumab is a recombinant human immunoglobulin G1 monoclonal antibody that is specific for human TNF. It specifically binds to circulating and cell surface TNF-α and blocks its interaction with p55 and p75 cell surface TNF receptors[26] (TABLE 1).

The first fully human anti–TNF-α monoclonal antibody, it was approved for the treatment of moderate to severe RA by the FDA in 2002 and by the EMEA in September 2003.[27,28] The standard dose is 40 mg subcutaneously every other week, and the drug can be used alone or in combination with disease-modifying antirheumatic drugs (DMARDs) such as methotrexate (MTX).[26] After a single 40-mg subcutaneous dose to a healthy adult, it reaches a maximum serum concentration of 4.7 ± 1.6 g/ml within 131 ± 5 hours. The average absolute bioavailability of adalimumab, estimated from three studies, is 64%. Population pharmacokinetic analyses have revealed a trend towards a greater apparent clearance in the presence of anti-adalimumab antibodies and a lower clearance with increasing age in patients 40 to >75 years. A 20% improvement in ACR score was achieved in 67% of RA patients on 12 weeks of adalimumab in the ReaAct Trial and a moderate European League Against Rheumatism (Eular) response in 81%.[29,30]

TOXICITY PROFILE AND AUTOIMMUNITY

The safety issues relating to all current TNF antagonists are infections (including *Mycobacterium tuberculosis* and other opportunistic infections), demyelinating dis-

TABLE 2. Serious adverse events correlate with anti-TNF agents

Adverse events	Refs.
Infections (sepsis, mainly candida, aspergillus, cytomegalovirus, cryptococcus, mycobacterium)	31,32,26
Hematology (anemia, neutropenia, lymphoma)	31,32,26
Neurology (demyelination)	31,32,26
Bone-muscle-connective tissue (lupus-like syndrome)	31,32,26,47–49
Anti-infliximab antibodies and infusion/injection reaction	31,32,26,39

TABLE 3. Effects on immunogenicity of biologic agents: review of the literature

Authors	Year	Anti-TNF agent	Pts (n)	ANA (%)	Anti– ds-DNA (%)	aCl (%)	Refs.
Charles *et al.*	2000	Infliximab	156	–	5-7	–	42
Antivalle *et al.*	2002	Infliximab	91	54.9	20.9	–	45
Ferraccioli *et al.*	2002	Etanercept	8	–	–	62.5 (post-infections)	64
De Richie *et al.*	2003	Infliximab	62	82.3	16.7	–	43
Jonsdottir *et al.*	2004	Infliximab Etanercept	67 57	–	–	28 25	65
Ferrero *et al.*	2004	Infliximab	24			87.5	54
Bobbio-Pallavicini *et al.*	2004	Infliximab	30	80	3.3	6.7	35
Allanore *et al.*	2004	Infliximab	59	63.8	32	–	46
Keystone *et al.*	2004	Adalimumab	–	12.9	5.3	–	26
Atzeni *et al.*	In press	Adalimumab	57	28	3.5	1.7	37

orders, autoimmune syndromes (i.e., systemic lupus erythematosus), congestive heart failure, and administrative reactions[26,31,32] (TABLE 2).

Antibody formation can follow the administration of biologic agents such as the TNF antagonists including the development of neutralizing and non-neutralizing antibodies[4,26,33] (TABLE 3).

Rheumatoid Factor and Anticyclic Citrullinated Peptide

Specific autoantibodies such as rheumatoid factor (RF) and anticyclic citrullinated peptide (CCP) are related to the severity of the rheumatoid process and could be reduced by effective TNF-α–blocking agents.[34]

Bobbio-Pallavicini *et al.*[35] found a significant decrease in the titers of both anti-CCP antibodies and RF after 30 weeks of infliximab therapy, which suggests that serial evaluations of these antibodies may be useful in monitoring the clinical course of RA patients undergoing treatment with infliximab. However, they observed a different evolution of RF and anti-CCP antibody titers during long-term therapy: after 54 and 78 weeks, there was a progressive decrease with the former but not the latter despite the persistence of clinical improvement indicated by DAS 28.[35] Alessandri *et al.*[36] reported that infliximab treatment led to a decrease in the serum titers of RF and anti-CCP antibodies in RA patients showing improvement, suggesting that these measurements may be a useful adjunct in assessing treatment efficacy. In a study of 57 patients with RA,[37] we found that 46 (80.7%) were positive for anti-CCP antibodies at baseline, and 43 (75%) were positive for RF. Although none of these patients became negative after adalimumab treatment, the serum titer of anti-CCP antibodies and RF decreased significantly after 12 months of treatment ($P < .001$ for both). When the patients were grouped on the basis of their clinical response, a significant decrease in serum levels of anti-CCP antibody and RF was observed only in

those who showed clinical improvement according to ACR 20 and ACR 50 criteria (both *P* < .001) (data submitted for publication).[9,37]

The mechanisms by which the infliximab-induced blockade of anti–TNF-α decreases the generation of specific autoantibodies such as anti-CCP and RF are not yet known; however, it has been shown that infliximab can downregulate the production of several inflammatory cytokines and mediators and that these anti-inflammatory effects may account for reduced autoantibody generation, particularly in the synovial compartment.[38]

Citrullination represents a post-translational modification of proteins involved in apoptosis. As citrullinated fibrin is one of the major citrullanated proteins in rheumatoid synovium, it is an important antigenic target of anti-fillaggrin antibodies.[38] Anti–TNF-α treatment can modulate apoptotic processes (as in inflammatory bowel disease), so that regulation of apoptosis after TNF-α blockade may at least partially explain this observation.[38]

Neutralizing Antibodies

Infliximab therapy can lead to the formation of anti-infliximab antibodies[32,39] whose presence was associated with infusion reactions in 6.9–19% of patients. Infusion reactions are important immunologic events induced by the presence of substantial serum concentrations of such antibodies.[31,32,39] Thereafter, infliximab rapidly disappears from the serum and becomes undetectable within 4 weeks. Once an infusion reaction has occurred, the duration of the response to subsequent infusions decreases, but, although the relation between the duration of response and infliximab concentrations was clear, it was no stronger than the correlation with antibody concentrations. As antibodies develop soon after the initial infusion in most patients, Baert *et al.*[39] believe that immunosuppressive therapy should be started before the administration of infliximab to prevent antibody formation and improve the duration of the drug response; however, concomitant treatment with immunomodulators in ACCENT I (a Crohn's disease clinical trial evaluating infliximab in a new long-term treatment regimen) did not substantially increase the benefit of the three-dose induction regimen and 8-week maintenance therapy.[40]

It remains to be established whether any of the immunosuppressive agents (azathioprine, mercaptopurine, or MTX) provides greater protection against immunogenicity than the others. A drug interaction between MTX and infliximab has been proposed because, although maximum serum infliximab levels remain the same with or without concomitant MTX therapy, one study found that the rate of disappearance of infliximab was slower among patients taking MTX than among those receiving infliximab alone.[41]

In conclusion, it is clear that immunosuppressive treatment prevents the formation of antibodies against infliximab, thus reducing the incidence of infusion reactions and increasing the duration of response. When necessary, anti-TNF therapy can temporarily be discontinued and restarted without the loss of a clinical response or an increased risk of anaphylaxis or immunologic reaction.

Adalimumab. As might be expected with infusion of any immunoglobulin, a proportion of patients develop human anti-idiotypic antibodies specific for adalimumab that, in clinical trials, have been observed in 12% of patients receiving monotherapy and 1% of those receiving combination therapy with MTX.[26,28] These antibodies

probably account for the somewhat lower ACR responses observed in the monotherapy study; a significantly better response was observed with adalimumab 40 mg weekly, and it is estimated that 5–15% of patients may require weekly doses while receiving monotherapy.[26,28] There is no evidence that weekly adalimumab dosing may be beneficial in patients receiving concomitant MTX therapy or that the anti-idiotypic antibodies affect the safety profile of adalimumab.

Antinuclear, Anti-dsDNA Antibodies, and Lupus-Like Syndrome

Infliximab treatment has led to the induction of ANAs in 63.8% of RA patients and 49.1% of CD patients and to anti-dsDNA antibodies in 13% of RA patients and 21.5% of CD patients, respectively.[33,42–46] The development of ANAs and anti-dsDNA antibodies has also been described after etanercept therapy in 11% and 15% of RA patients, respectively.[33] Clinical studies of the effect of adalimumab in RA have reported the appearance of autoantibodies, including ANAs (12.9%) and anti–dsDNA antibodies (5.3%),[26] and one case of a lupus-like syndrome in which serositis and arthritis resolved upon discontinuation of therapy.[26] We[37] found that 4 of 57 adalimumab-treated RA patients (7.2%) and 5 of 55 MTX-treated patients with RA (9%) were ANA positive at baseline; after 12 months of therapy, ANA induction was observed in 12 adalimumab-treated RA patients and 6 MTX-treated RA patients, with the total number of ANA-positive patients being 16 (28%) in the adalimumab group and 8 (14.5%) in the control group. The difference in ANA positivity before and after follow-up was statistically significant only in the adalimumab group ($P <.001$), and the difference in induction between the two treatment groups was also significant ($P <.001$) (data in press). However, despite the development of ANAs and anti-dsDNA antibodies in a fairly high proportion of patients during anti–TNF-α therapy, the incidence of possible lupus-like syndromes was low. Some cases of lupus-like symptoms (with or without autoantibodies) in patients receiving infliximab or etanercept treatment have been reported, but it is difficult to determine whether they were related to anti–TNF-α treatment, the underlying disease, or other concomitant drugs or whether they occurred coincidentally.[47–49] The identification of drug-induced SLE in RA patients is especially difficult because of overlaps between the two diseases. Charles *et al.*[42] described the induction of anti-dsDNA antibodies in 7% of RA patients treated with infliximab (with or without MTX therapy) and noted that their isotypes differed from those of IgG. De Rychie *et al.*[43] confirmed the high prevalence of ANAs and anti-dsDNA antibodies in RA patients after infliximab treatment. Moreover, increased ANA titers and induction of anti-dsDNA antibodies are more pronounced in SA than in RA.[43] However, ANAs may be more likely in untreated RA that in untreated SA, and the absence of associated MTX therapy in SA may contribute to the observed differences. Of interest is the observation by Boehm *et al.*[50] that MTX therapy can lead to a decrease in circulating autoantibodies in patients with cutaneous lupus.The diagnostic and prognostic value of anti-dsDNA antibodies remains controversial, because lupus-associated anti-dsDNA antibodies are classically of the IgG isotype, whereas IgM or IgA anti-dsDNA antibodies may occur in other diseases as well (mixed connective tissue disease, primary Sjögren's syndrome, scleroderma, RA, AS, chronic active hepatitis, and primary biliary cirrhosis).[42,51] Other ANA reactivities have not been systematically studied in relation to infliximab therapy. One study found an increase in the number of patients

with antinucleosome antibodies before and after infliximab treatment, but this was not statistically significant.[52,53] Antihistone antibodies are particularly common in SLE and drug-induced lupus (with respective sensitivities of 50–70% and 90–95%), but they may also occur in other diseases (RA, juvenile chronic arthritis, auto-immune chronic hepatitis, and chronic infection) or even in healthy controls.[53] Some infliximab-treated patients with RA or SA have shown the newly developed anti-histone antibodies.[43,54]

Mechanism of Antinuclear and Anti-DNA Antibody Induction

Autoantibody induction may be a predictable consequence of anti–TNF-α block-ade, which could promote humoral autoimmunity by inhibiting the induction of the cytotoxic T-lymphocyte response that normally suppresses autoreactive B cells.[55] Infliximab may also neutralize the biologic activity of TNF-α by binding the soluble forms and thus preventing its interaction with its p55 and p75 cell receptors. Inflix-imab also binds the transmembrane form of TNF-α and could induce antibody or complement-dependent cytotoxicity in the cells expressing it. Furthermore, it increases the number of apoptotic T lymphocytes in the lamina propria and apoptotic monocytes in peripheral blood in Crohn's disease.[55,56] In this case, one hypothesis concerning the development of autoimmune diseases such as SLE is that increased apoptosis may promote the release of numerous autoantigens, thus leading to the development of autoantibodies against cytoplasmic and nuclear compounds such as ANA and dsDNA, especially if their production is no longer suppressed by the action of infliximab on the suppressor T-cell population.[56] This process may not occur in organ-specific cells such as thyrocytes, because they do not harbor TNF-α receptors, which may partially explain the absence of organ-specific autoantibodies associated with autoimmune vasculitis, hepatitis, or endocrine diseases. Charles *et al.*[42] and Ferrero *et al.*[54] demonstrated that most of the anti-dsDNA autoantibodies detected during the treatment of RA were of the IgM isotype. Furthermore, Ferrero *et al.*[54] showed that most of the detected aPL autoantibodies were also of IgM isotype. The role of IgM in the development of autoimmune diseases remains to be elucidated, but natural autoreactive IgM autoantibodies may suppress autoimmunity by induc-ing B-cell tolerance and thus participating in the negative selection of autoreactive B cells. The larger pool of autoantibodies of the IgM isotype observed during inflix-imab treatment could be due to a higher production of natural autoreactive IgM, but it may also be an induced population that can further switch to IgG with a well-known pathogenic effect.[42,54] The high frequency of IgM may also be caused by TNF blockade, as demonstrated in a murine model of collagen-induced arthritis in which anti–TNF-α monoclonal antibodies reduced isotype switching to IgG in the local draining lymph node.[57]

Antiphospholipid Autoantibodies

Antiphospholipid (but not aCL) autoantibodies are found in 2–6% of healthy blood donors and up to 12% in an aging population.[58] In RA patients, aCLs have been found at even higher frequencies.[59] aCL antibodies associated with recurrent thromboembolic disease and fetal loss, but their clinical significance in RA is uncer-tain and their presence has been considered a nonspecific marker of activation of the

immune system.[60,61] Only limited data on the induction of aPL antibodies during TNF-α blocking treatment have been reported. Elliott et al.[62] found aCL antibodies (cA2) in 1 of 20 RA patients treated with anti–TNF-α in an 8-week open trial. Rankin et al.[63] measured the serologic effects of repeated doses of the humanized anti–TNF-α antibody CDP 571 in patients with RA and found that some develop positive aCL (IgG). Ferraccioli et al.[64] showed variations in aCL titers over time in etanercept-treated patients with concomitant bacterial infection in whom reduced titers were seen after treatment with antibiotics. In a recent paper, Jonsdottir et al.[65] described a correlation between treatment with TNF-α antagonists (infliximab and etanercept), the appearance of aCL antibodies, and a worse clinical outcome; however, two other studies did not confirm the association between aCL antibodies and clinical outcome. We[37] found that none of 57 RA patients before adalimumab treatment and none of 55 MTX-treated RA patients was positive for aCL or anti–β-$_2$GPI autoantibodies at baseline, and after 12 months, aCL levels were not significantly different between both groups (2 of 57 vs. 2 of 55) (data in press).

CONCLUSIONS

This review indicates that anti-TNF agents induced a decrease in RF and anti-CCP levels and an increase in the production of neutralizing and non-neutralizing autoantibodies. The possible mechanisms underlying these phenomena and their correlation with clinical manifestations remain to be elucidated. Although the fact that the production of ANAs and anti-dsDNA antibodies does not correlate with clinical features suggestive of autoimmune diseases is reassuring in terms of treatment safety, longer-term observational studies of anti-dsDNA–positive patients are essential and the mechanism of anti-dsDNA antibodies by anti–TNF-α therapy must still be clarified.

REFERENCES

1. SKAPENKO, A., J.R. KALDEN & H. SCHULZE-KOOPS. 2001. Treatment of rheumatoid arthritis in the third millennium. Scand. J. Rheumatol. **30:** 249–254.
2. MCLAIN, D.A. 2001. Rheumatoid arthritis: update on new therapies. South Med. J. **94:** 893–895.
3. CALABRESE, L.H. 2003. Molecular differences in anticytokine therapies. Clin. Exp. Rheumatol. **21:** 241–248.
4. BENCSATH, M., A. BLASKOVITS & J. BORVENDEG. 2003. Biomolecular cytokine therapy. Pathol. Onc. Res. **9:** 25–29.
5. SHARMA, P.K., D. HOTA & P. PANDHI. 2004. Biologics in rheumatoid arthritis. Japi **52:** 231–236.
6. FELDMANN, M., F.M. BRENNAN & R.N. MAINI. 1996. Role of cytokines in rheumatoid arthritis. Ann. Rev. Immunol. **14:** 397–440.
7. VASSALLI, P. 1987. The pathophysiology of tumor necrosis factors. N. Engl. J. Med. **316:** 379–385.
8. ELLIOTT, M.J., R.N. MAINI & M. FELDMANN. 1994. Randomized double-blind comparison of chimeric monoclonal antibody tumor necrosis factor alpha (cA2) versus placebo in rheumatoid arthritis. Lancet **344:** 1105–1110.
9. FELSON, D.T., J.J. ANDERSON, M. BOERS, et al. 1993. The American College preliminary core set of disease activity measures for rheumatoid arthritis clinical trials. Arthritis Rheum. **36:** 729–740.

10. KAVANAUGH, A., E.W. ST, CLAIR, W.J. MCCUNE, *et al*. 2000. Chimeric anti-tumor necrosis factor-alpha monoclonal antibody treatment of patients with rheumatoid arthritis receiving methotrexate therapy. J. Rheumatol. **27:** 841–850.
11. MAINI, R., E.W. ST. CLAIR, F. BREEDVELD, *et al*. 1999. Infliximab versus placebo in rheumatoid arthritis patients receiving concomitant methotrexate: a randomised phase III trial. Lancet **354:** 1932–1939.
12. LIPSKY, P.E., D.M. VAN DE HEIJDE, E.W. ST. CLAIR, *et al*. 2000. 102-week clinical and radiological results from the ATTRACT trial: a 2 year, randomized, controlled, phase 3 trial of infliximab in patients with active RA despite MTX. Arthritis Rheum. **43:** abstr. 269.
13. LIPSKY, P.E., D.M. VAN DER HEIJDE, E.W. ST. CLAIR, *et al*. 2000. Infliximab and methotrexate in the treatment of rheumatoid arthritis. Anti-Tumor Necrosis Factor Trial in Rheumatoid Arthritis with Concomitant Therapy Study Group. N. Engl. J. Med. **343:** 1594–1602.
14. PRESENT, D.H., P. RUTGEERTS, S. TARGAN, *et al*. 1999. Infliximab for the treatment of fistulas in patients with Crohn's disease. N. Engl. J. Med. **340:** 1398–1405.
15. BRANDT, J., H. HAIBEL, D. CORNELY, *et al*. 2000. Successful treatment of active ankylosing spondylitis with the anti-tumor necrosis factor alpha monoclonal antibody infliximab. Arthritis Rheum. **43:** 1346–1352.
16. BRAUN, J., J. BRANDT, J. LISTING, *et al*. 2002. Treatment of active ankylosing spondylitis with infliximab: a randomised controlled multicentre trial. Lancet **359:** 1187–1193.
17. MORELAND, L.W., G. MARGOLIES, L.W. HECK, *et al*. 1996. Recombinant solubile tumor necrosis factor receptor (p80) fusion protein: toxicity and dose finding trial in refractory rheumatoid arthritis. J. Rheumatol. **23:** 1849–1855.
18. MORELAND, L.W., S.B. COHEN, S.W. BAUMGARTNER, *et al*. 2001. Long-term safety and efficacy of etanercept in patients with rheumatoid arthritis. J. Rheumatol. **28:** 1238–1244.
19. MORELAND, L.W., M.H. SCHIFF, S.W. BAUMGARTNER, *et al*. 1999. Etanercept therapy in rheumatoid arthritis. A randomized controlled trial. Ann. Intern. Med. **130:** 478–486.
20. MARTIN, R., E. RUDERMAN, R. FLEISCHMANN, *et al*. 2000. A phase III trial of etanercept vs methotrexate (MTX) in early rheumatoid arthritis. Ann. Rheum. Dis. **59:** 48.
21. GENOVESE, M.C., J.M. BATHON, R.W. MARTIN, *et al*. 2002. Etanercept vs methotrexate in patients with early rheumatoid arthritis: two-year radiography and clinical outcomes. Arthritis Rheum. **46:** 1443–1450.
22. KIETZ, D.A., P.H. PEPMUELLER & T.L.MOORE. 2001. Clinical response to etanercept in polyarticular course juvenil arthritis. J. Rheumatol. **28:** 360–362.
23. MEASE, P.J., B.S. GOFFE, J. METZ, *et al*. 2000. Etanercept in the treatment of psoriatic arthritis and psoriasis: a randomised trial. Lancet **356:** 385–390.
24. BRANDT, J., J. LISTING, J. SIEPER, *et al*. 2004. Development and presentation of criteria for short term improvement after TNF alpha treatment in ankylosing spondylitis. Ann. Rheum. Dis. **63:** 1438–1444.
25. MEASE, P.J. 2003. Disease-modifying anthireumatic drugs therapy for spondyloarthropathies: advances in treatment. Curr. Opin. Rheumatol. **15:** 12–25.
26. KEYSTONE, E. & B. HARAOUI. 2004. Adalimumab therapy in rheumatoid arthritis. Rheum. Dis. Clin. N. Am. **30:** 349–364.
27. WEINBLATT, M.E., E.C. KEYSTONE, D.E. FURST, *et al*. 2003. Adalimumab, a fully human antitumor necrosis factor–monoclonal antibody, for the treatment of rheumatoid arthritis in patients taking concomitant methotrexate. The ARMADA trial. Arthritis Rheum. **48:** 35–39.
28. FURST, D.E., E. SCHIFF, R. FLEISCHMAN, *et al*. 2002. Safety and efficacy of adalimumab, a fully human anti-TNF-α monoclonal antibody, given in combination with stardard antirheumatic therapy: safety trial of adalimumab in rheumatoid arthritis (STAR). Arthritis Rheum. **46:** S572.
29. VAN GESTEL, A.M., M.L. PREVOO, T. VAN HOFMA, *et al*. 1996. Development and validation of the European League Against Rheumatism response criteria for rheumatoid arthritis. Comparison with preliminary American College of Rheumatology and World Health Organization/International League Against Rheumatism criteria. Arthritis Rheum. **39:** 34–40.

30. BOMBARDIERI, S., A.G. TZIOUFAS, F. MCKENNA, *et al*. 2004. Efficacy evaluation of adalimumab (Humira) in patients with a single and multiple prior biologics in the ReAct Trial. Arthritis Rheum. **50** (suppl.9): abstr. 365.
31. DAY, R. 2002. Adverse reactions to TNF-alpha inhibitors in rheumatoid arthritis. Lancet **359:** 540–541.
32. ANTONI, C. & J. BRAUN. 2002. Side effects of anti-TNF therapy: current knowledge. Clin. Exp. Rheumatol. **20:** 152–157.
33. ZIOLKOWSKA, M. & W. MASLINSKI. 2003. Laboratory changes on anti-tumor necrosis factor treatment in rheumatoid arthritis. Curr. Opin. Rheumatol. **15:** 267–273.
34. MEYER, O., C. LABARRE, M. DOUGADOS, *et al*. 2003. Anticitrullinated peptide antibody assays in early rheumatoid arthritis for predicting five years radiographic damage. Ann. Rheum. Dis. **42:** 677–680.
35. BOBBIO-PALLAVICINI, F., C. ALPINI, R. CAPORALI, *et al*. 2004. Autoantibody profile in rheumatoid arthritis during long-term inflimab treatment. Arthritis Res. Ther. **6:** 264–272.
36. ALESSANDRI, C., M. BOMBARDIERI, N. DEL PAPA, *et al*. 2004. Decrease of anti-cyclic citrullinated peptide antibodies and rheumatoid factor following ant-TNF therapy (infliximab) in rheumatoid arthritis is associated with clinical improvement. Ann. Rheum. Dis. **63:** 1218–1221.
37. ATZENI, F., P. SARZI-PUTTINI, D. DELL'ACQUA, *et al*. 2005. Adalimumab clinical efficacy is associated with rheumatoid factor and anti-cyclic citrullinated peptide antibody titre reduction and anti-nuclear antibody appearance: a one year prospective study. Submitted for publication.
38. REPARON-SCHIJT, C.C., W.J. VAN ESCH, C. VAN KOOTEN, *et al*. 1988. Functional analysis of rheumatoid factor-producing B cells from the synovial fluid of rheumatoid arthritis patients. Arthritis Rheum. **41:** 2211–2220.
39. BAERT, F., M. NOMAN, S. VERMEIRE, *et al*. 2003. Influence of immunogenicity on the long-term efficacy of Infliximab in Crohn's disease. N. Engl. J. Med. **348:** 601–608.
40. HANAUER, S.B., B.G. FEAGAN, G.R. LICHTENSTEIN, *et al*. 2002. Maintenance infliximab for Crohn's disease: the ACCENT I randomised trial. Lancet **359:** 1541–1549.
41. MAINI, R.N., F.C. BREEDVELD, J.R. KALDEN, *et al*. 1988. Therapeutic efficacy of multiple intravenous infusions of anti-tumor necrosis factor alpha monoclonal antibody combined with low-dose weekly methotrexate in rheumatoid arthritis. Arthritis Rheum. **41:** 1552–1563.
42. CHARLES, P.J., R.J. SMEENK, J. DE JONG, *et al*. 2000. Assessment of antibodies to double-stranded DNA induced in rheumatoid arthritis patients following treatment with infliximab, a monoclonal antibody to tumor necrosis factor alpha: findings in open-label and randomized placebo-controlled trials. Arthritis Rheum. **43:** 2383–2390.
43. DE RYCKE, L., E. KRUITHOF, N. VAN DAMME, *et al*. 2003. Antinuclear antibodies following infliximab treatment in patients with rheumatoid arthritis or spondylarthropathy. Arthritis Rheum. **48:** 1015–1023.
44. VERMEIRE, S., M. NOMAN, G. VAN ASSCHE, *et al*. 2003. Autoimmunity associated with anti-tumor necrosis factor alpha treatment in Crohn's disease: a prospective cohort study. Gastroenterology **125:** 32–39.
45. ANTIVALLE, M., G. RANDISI, M. MARRAZZA, *et al*. 2002. Clinical relevance of the induction of antibodies to double-stranded DNA during long-term treatment of rheumatoid arthritis with Infliximab. Ann. Rheum. Dis. Abstr. 0166.
46. ALLANORE, Y., J. SELLAM, F. BATTEUX, *et al*. 2004. Induction of autoantibodies in refractory rheumatoid arthritis treated by infliximab. Clin. Exp. Rheum. **22:** 756–758.
47. DE BANDT, M.J., V. DESCAMPS & O. MEYER. 2001. Two cases of etanercept-induced systemic lupus erythematosus in patients with rheumatoid arthritis. Ann. Rheum. Dis. **60:** 175.
48. SHAKOOR, N., M. MICHALSKA, C.A. HARRIS, *et al*. 2002. Drug-induced systemic lupus erythematosus associated with etanercept therapy. Lancet **359:** 579–580.
49. SARZI-PUTTINI, P., S. ARDIZZONE, G. MANZIONNA, *et al*. 2003. Infliximab-induced lupus in Crohn's disease: a case report. Dig. Liver Dis. **35:** 814–817.

50. BOEHM, I.B., G.A. BOEHM & R. BAUER. 1998. Management of cutaneous lupus erythematosus with low-dose methotrexate: indication for modulation of inflammatory mechanisms. Rheumatol. Int. **18:** 59–62.
51. MILTENBURG, A.M.M., A. ROOS, L. SLEGTENHORST, *et al.* 1993. IgA anti-dsDNA antibodies in SLE: occurrence, incidence and association with clinical and laboratory variables of disease activity. J. Rheumatol. **20:** 53–58.
52. AMOURA, Z., S. KOUTOUZOV & J.C. PIETTE. 2000. The role of nucleosomes in lupus. Curr. Opin. Rheumatol. **12:** 369–373.
53. BERNSTEIN, R. 1996. Autoantibodies to histones, Sm and ubiquitins. *In* Manual of Biological Markers of Disease. W.J. Van Venrooij & R.N. Maini, Eds. C2.: 1–7. Kluwer Academic Publishers. Dordrecht, the Netherlands.
54. FERRERO-PEYRET, C., F. COURY, J.G. TEBIB, *et al.* 2004. Infliximab therapy in rheumatoid arthritis and ankylosing spondylitis-induced specific antinuclear autoantibodies without autoimmune clinical manifestations: a two-year prospective study. Arthritis Res. Ther. **6:** 535–543.
55. LUGERING, A., M. SCHMIDT, N. LUGERING, *et al.* 2001. Infliximab induces apoptosis in monocytes from patients with chronic active Crohn's disease by using a caspase-dependent pathway. Gastroenterology **121:** 1145–1157.
56. BELL, D.A. & B. MORRISON. 1991. The spontaneous apoptotic cell death of normal human lymphocytes *in vitro*: the release of, and immunoproliferative response to, nucleosomes *in vitro*. Clin. Immunol. Immunopathol. **60:** 13–26.
57. CAMPBELL, I.K., K. O'DONNELL, K.E. LAWLOR, *et al.* 2001. Severe inflammatory arthritis and lymphadenopathy in the absence of TNF. J. Clin. Invest. **107:** 1519–1527.
58. FIELDS, R.A., H. TOUBBEH, R.P. SEARLES, *et al.* 1989. The prevalence of anticardiolipin antibodies in a healthy elderly population and its association with antinuclear antibodies. J. Rheumatol. **16:** 623–625.
59. KEANE, A., R. WOODS, V. DOWDING, *et al.* 1987. Anticardiolipin antibodies in rheumatoid arthritis. Br. J. Rheumatol. **26:** 346–350.
60. WOLF, P., J. GRETLER, F. AGLAS, *et al.* 1994. Anticardiolipin antibodies in rheumatoid arthritis: their relation to rheumatoid nodules and cutaneous vascular manifestations. Br. J. Dermatol. **131:** 48–51.
61. KAPIOTIS, S., W. SPEISER, I. PABINGER-FASCHING, *et al.* 1991. Anticardiolipin antibodies in patients with venous thrombosis. Haemostasis **21:** 19–24.
62. ELLIOTT, M.J., R.N. MAINI, M. FELDMANN, *et al.* 1993. Treatment of rheumatoid arthritis with chimeric monoclonal antibodies to tumor necrosis factor-α. Arthritis Rheum. **36:** 1681–1690.
63. RANKIN, E.C., E.H. CHOY, D. KASSIMOS, *et al.* 1995. The therapeutic effects of an engineered human anti-tumour necrosis factor alpha antibody (CDP571) in rheumatoid arthritis. Br. J. Rheumatol. **34:** 334–342.
64. FERRACCIOLI, G., M. MECCHIA, E. DI POI, *et al.* 2002. Anticardiolipin antibodies in rheumatoid patients treated with etanercept or conventional combination therapy: direct and indirect evidence for a possible association with infections. Ann. Rheum. Dis. **61:** 358–361.
65. JONSDOTTIR, T., J. FORSLID, M.A. VAN VOLLENHOVEN, *et al.* 2004. Treatment with tumour necrosis factor alpha antagonists in patients with rheumatoid arthritis induces anticardiolipin antibodies. Ann. Rheum. Dis. **63:** 1075–1078.

4.4.6. INFLIXIMAB-INDUCED LUPUS IN CROHN'S DISEASE: A CASE REPORT.

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4.5. POSSIBLE MECHANISMS INVOLVED IN THE DECREASE IN RHEUMATOID FACTOR AND ANTI-CITRULLINATED PROTEIN ANTIBODIES, AND THE PRODUCTION OF NEUTRALISING AND NON-NEUTRALISING AUTOANTIBODIES. DURING ANTI-TNF-α TREATMENT

Two important problems associated with anti-TNFα agents are immunogenicity and autoimmunity [90,34].

4.5.1. Autoantibodies against anti-TNF-α agents

Structural differences in anti-TNF-α agents affect their immunogenicity, but the development of antibodies against them can also be influenced by concomitant infection [90]. The development of anti-drug antibodies is T cell dependent and, in "normal' healthy individuals, antigen-presenting dendritic cells do not express adequate levels of co-stimulatory molecules such as CD80 to activate T cells and stimulate subsequent B cell differentiation; under these conditions, only highly immunogenic polypeptides trigger an immune response. However, in the setting of acute bacterial infections, dendritic cells are induced to express high levels of co-stimulatory molecules that lower the threshold of T cell activation and, under these conditions, an immune response can be triggered by even weakly immunogenic polypetides [91]. This means that the production of autoantibodies against anti-TNF agents may vary in different disease states: i.e. the immunogenicity may be greater in patients with CD (in whom there is a prominent component of bacterial triggers) than in those with RA, in whom there is not (see Fig.6).

Fig. 6 Mechanisms involved in autoantibodies against anti-TNF-α agents production
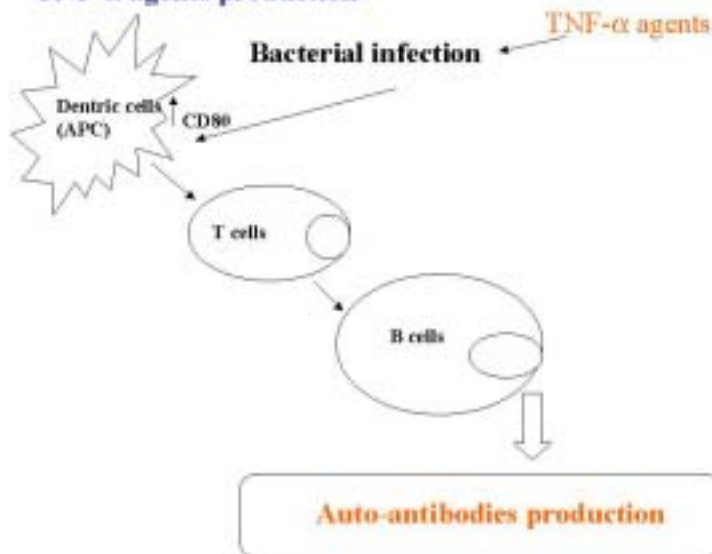
4.5.2. Antinuclear and anti-double stranded DNA antibodies induced by anti-TNF-α agents

What is the exact mechanism inducing ANA and anti-dsDNA development in patients treated with TNF-blocking agents (see Fig.7)?

The *in vivo* neutralisation of TNF-α in RA patients has a profound stimulatory effect on humoral immunity to DNA and other nuclear antigens, which may be due to various possible mechanisms, including the interruption of apoptosis and the down-regulation of T helper (Th) cell activity [67].

The induction of autoimmunity to nuclear antigens may reflect the inhibition of cytotoxic cells that have the potential to suppress autoreactive B cells, a mechanism that has been proposed to explain the presence of SLE-like autoimmunity in chronic graft-versus-host disease. Infliximab and adalimumab bind to the TNF-α on cell surfaces, and may lead to

apoptotic cell death and the release of nucleosome autoantigens that induce anti-ds DNA antibodies in a subpopulation of genetically susceptible patients [92,93]. It is also possible that TNF-α directly modulates the immunogenicity of DNA by means of its effects on serum amyloid P and complement factors C1q and C4b, which bind chromatin DNA in apoptotic bodies and account for the clearance of DNA; for example, the absence of these mediators in mice leads to the development of antinuclear autoimmunity and lupus-like disease [94]. It is interesting in this context that the combined inactivation of TNF-α and IL-6 prevents the induction of acute phase proteins such as serum amyloid P (SAP), thus indicating a pathway whereby reduced TNF-α may favour the development of lupus. As nucleosomes co-cluster in apoptotic bodies with other lupus auto-antigens during apoptosis, it is temping to speculate that the persistence of circulating apoptotic cells triggers autoantibody production in SLE, a hypothesis that is consistent with the findings that a series of post-translational protein modifications and a substantial increase in the production of reactive oxygen species take place during apoptosis, and these are known to increase the immunogenicity of apoptotic cell-containing autoantigens [95]. Viral infections may contribute to the immunogenicity of nucleosomes and trigger an autoimmune response: i.e. nucleosomes and virus can co-cluster in apoptotic bodies and induce an autoimmune response that spreads from the viral antigen to the co-clustered nucleosome.

Fig. 7 Antibodies anti-nuclear and anti-double stranded DNA induced by TNF-alpha blocking agents: possible mechanisms
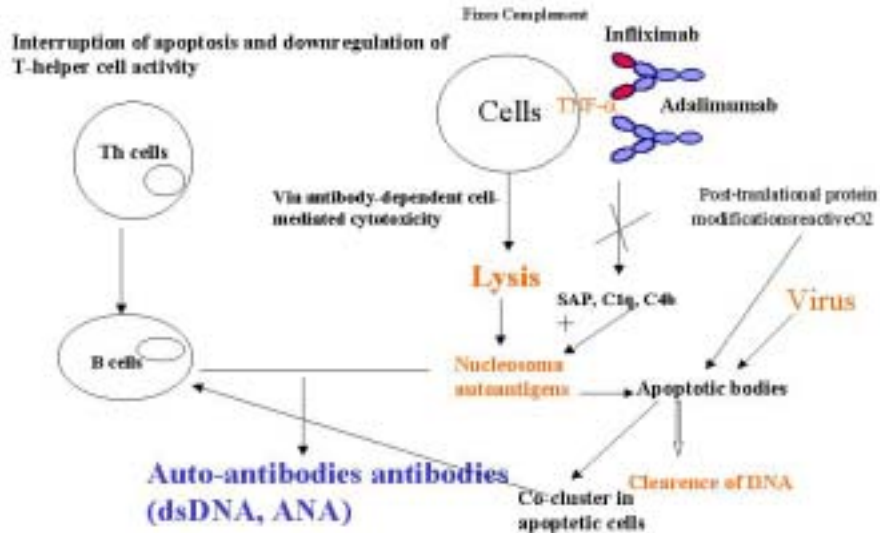
4.5.3. Anti-phospholipid autoantibodies induced by anti-TNF-α treatment

One possible explanation for the induction of aCL positivity in patients treated with TNF-α blocking agents is that the down-regulation of TNF-α leads to the up-regulation of IL-10, which in turn activates autoreactive B cells and thus induces autoantibody production [96]. Alternatively, lower TNF-α levels may lead to a generalised increase in Th2 activity which, albeit through other cytokine pathways, may lead to B cell activation and autoantibody formation [96]. Ferraccioli *et al*. [12] have shown that the appearance of these autoantibodies correlates with bacterial urinary or upper respiratory tract infections, and that antibiotic treatment restores normal aCL antibody levels. One possible explanation as to why the infection can lead to the appearance of aCL can be found in bacterial DNA, which is enriched in unmethylated CpG motifs that can activate CD86 expression, and the synthesis of IL-6 by B cells and IFN-γ by NKT and Th 1 cells

[97,98]. These motifs, which are expressed by both *Staphylococcus* and *E coli* DNA, may well activate B cells once TNF-α is blocked by the biological agent (see Fig.8) [12].

Most of the anti-dsDNA and aCL antibodies detected during anti-TNF-α treatment are of the IgM isotype [2]. Natural autoreactive autoantibodies may suppress autoimmunity by inducing B cell tolerance and, thus, the negative selection of autoreactive B cells. The larger pool of autoantibodies observed during anti-TNF-α treatment may be due to higher autoreactive IgM production, but could also be an induced population that can further switch IgG and give rise to a well-known pathogenic effect [99]. The high frequency of IgM may also be a result of TNF blockade, as it has been demonstrated in a murine model of collagen-induced arthritis that monoclonal anti-TNF-α antibodies reduce isotype switching to IgG in the local draining lymph node.

Fig. 8 Anti-phospholipid autoantibodies induced by anti-TNF- α treatment
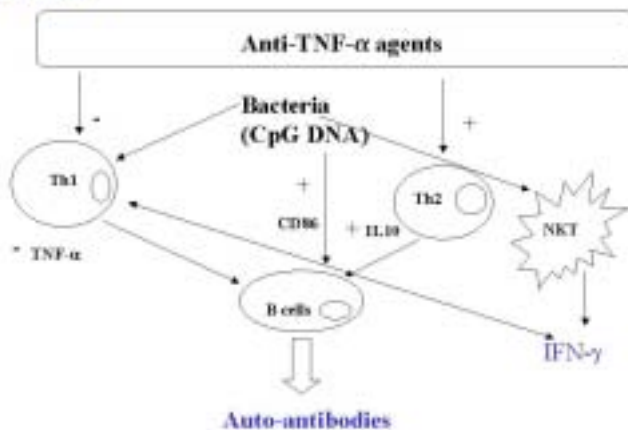
4.5.4. Rheumatoid arthritis-related autoantibodies (such as RF and anti-cyclic citrullinated peptides) and anti-TNF-α agents

Over the last few decades, a number of autoantibody systems have been described as being associated with RA [100-103,24]. RF, the oldest and most widely known of these autoantibodies, is directed to the Fc part of IgG molecules and can be detected in up to 80% of RA patients, but it is also found in various other diseases and healthy controls.

The antibodies against citrulline-containing epitopes (anti-CCP) have the greatest clinical potential in RA [100]. Citrulline is a non-standard acid insofar as it is not incorporated into proteins during protein synthesis, but it can be generated via the post-translational modification of arginine residues by peptidylargimine deimanase enzymes. The conversion of arginine into citrulline involves the replacement of an amine group by an oxygen atom in the side chain of the amino acid, and is associated with the loss of a positive charge (at neutral pH). Although this conversion leads to a relatively small chemical alteration in the protein involved, the reactivity of the autoantibodies that react with citrulline-containing epitopes seems to be critically dependent on the presence of a citrulline residue [100].

Anti-CCP antibodies have shown a high degree of specificity (96-98%) and reasonable sensitivity for the diagnosis of RA [100-103]. The fact that they can often be detected years before the manifestation of the disease suggests that the initial trigger for the development of RA may occur long before the appearance of RA symptoms [102]. On the basis of these data, Visser *et al*. [104] developed a set of diagnostic criteria for early arthritis that can immediately discriminate self-limiting, persistent non-erosive and erosive arthritis. It is also known that a greater prevalence of anti-CCP antibodies is

associated with the development of severe radiological damage, and that high serum RF levels independently predict worsening damage [103].

The mechanisms by which anti-TNF-α agents may decrease the generation of autoantibodies such as anti-CCP and RF are not understood, and any attempted can only be speculative (see Fig.9). However, it has been known that TNF-α blocking agents can down-regulate the production of various inflammatory cytokines and mediators, and that these anti-inflammatory effects may reduce autoantibody generation, particularly in the synovial compartment. It has also been shown that citrullination is a post-translation modification of proteins in the apoptotic process, and that citrullinated fibrin is one of the major citrullinated proteins in rheumatoid synovia, and thus represents a major antigenic target of anti-filaggrin antibodies [105]. Another possibility (but one that needs further study) is that the changes in serum anti-CCP levels during anti-TNF-α treatment are related to modulations in apoptotic cell death [64].

Fig. 9 Rheumatoid Factor and anti-cyclic citrullinated peptides relationship with anti-TNF-α agents
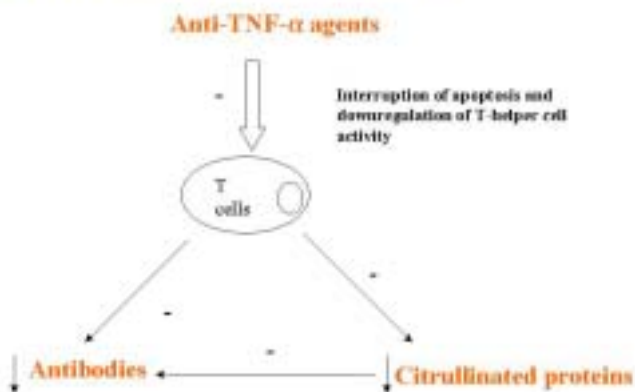
5. HYPOTHESIS

The introduction of TNF-α inhibitors has been a major advance in the treatment of patients with inflammatory arthritis and bowel disease. However, clinical trials have shown that TNF-α blocking agents lead to the development of autoantibodies, such as ANAs, anti-DNA and aPL: infliximab has led to the development of ANAs in 63.8% of RA patients and 49.1% of CD patients, and to anti-dsDNA antibodies in 13% of RA patients and 21.5% of CD patients, and adalimumab has led to the appearance of ANAs and anti-dsDNA autoantibodies in respectively 12.9% and 5.3% of RA patients [29,32]. However, despite the development of ANAs and anti-dsDNA antibodies in a fairly high proportion of patients during anti-TNFα therapy, the incidence of lupus-like syndrome is low: one case has been described during adalimumab trials, and some cases induced by infliximab and etanercept have been reported, but it is difficult to determine whether they were actually related to the anti-TNF-α agents [1-10, 33-35]. Many post-marketing studies have evaluated the frequency of these autoantibodies and their correlation with the efficacy and duration of treatment, but the data are very controversial. Some authors have found a correlation between the appearance of aCL and a worse clinical outcome and/or bacterial infection in RA patients treated with infliximab or etanercept, and Vermeire *et al.* [4] found more skin-related adverse symptoms in CD patients who developed autoantibodies, and also a clearly association between ANAs and female gender [11,12,4].

On the basis of these data, the hypothesis of the present thesis is that the clinical use of anti-TNF-α agents in patients with autoimmune diseases is associated with the development of a variety of autoantibodies due to an imbalance of the immune system.

6. OBJECTIVES

6.1. Main objectives

To determine: 1) the frequency and correlation of autoantibody development at standardised timepoints in refractory/inflammatory and fistulising CD patients in a routine clinical setting; 2) the relationships between the presence of autoantibodies, adverse clinical events and responses to anti-TNF-α therapy; and 3) whether non organ-specific autoantibodies are induced by adalimumab, as has been reported in the case of other TNF-α blocking agents

6.2. Objectives of first paper:

Adalimumab clinical efficacy is associated with rheumatoid factor and anti-cyclic citrullinated peptide antibody titre reduction: a one-year prospective study.

Arthritis Res Ther. *In press*

To evaluate prospectively: 1) the clinical efficacy of adalimumab; 2) whether the prevalence and titres of RA-associated autoantibodies such as RF and anti-CCP correlate with treatment effect; and 3) whether non organ-specific autoantibodies are induced by adalimumab as reported in the case of other TNF-α blocking agents.

6.3. Objectives of second paper:

Autoantibody profile during short-term infliximab treatment for Crohn's disease: a prospective cohort study.

Aliment Pharmacol Ther 2005;22:453-61.

1. To determine the frequency and correlation of autoantibody development at standardised points in refractory/inflammatory and fistulising CD patients in a routine clinical setting;

2. To relate the findings to disease status before the start of infliximab treatment;

3. To investigate the relationships between the presence of autoantibodies, adverse clinical events and responses to infliximab.

7. ORIGINAL PAPERS

ADALIMUMAB CLINICAL EFFICACY IS ASSOCIATED WITH IN

RHEUMATOID FACTOR AND ANTI-CYCLIC CITRULLINATED PEPTIDE

ANTIBODY TITRE REDUCTION: A ONE YEAR PROSPECTIVE STUDY

Atzeni F, Sarzi-Puttini P, Dell' Acqua D, de Portu S, Cecchini G, Cruini C, Carrabba M,

Meroni PL.

Adalimumab clinical efficacy is associated with rheumatoid factor and anticyclic citrullinated peptide antibody titre reduction: a one year prospective study.

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ABSTRACT

Studies on autoantibody production in patients treated with tumor necrosis factor (TNF)-α inhibitors reported contradictory results. We investigated in a prospective study the efficacy of a treatment with the human monoclonal anti-TNF-α antibody (adalimumab) in rheumatoid arthritis (RA) patients and we evaluated the relationship between treatment efficacy and incidence and titres of disease-associated and non organ-specific autoantibodies. Fifty seven RA patients not responsive to methotrexate and treated with adalimumab were enrolled. Antinuclear, anti-double stranded (ds)DNA, anti-nuclear extractable antigens (ENA), anti-cardiolipin (aCL), anti-beta 2 glycoprotein (β2GPI) antibodies, rheumatoid factor (RF) and anti-cyclic citrullinated peptide (CCP) antibodies were investigated at baseline and after 6 and 12 months of follow-up. Comparable parameters were evaluated in further 55 patients treated with methotrexate only. Treatment with adalimumab induced a significant reduction of RF and anti-CCP serum levels, and the decrease in antibody titres correlated with the clinical response to the therapy. A significant induction of ANA and IgG/IgM anti-dsDNA autoantibodies was also found in 28% and 14.6% patients respectively, while aCL and anti-β2GPI antibodies were not detected in a significant manner. No association between ANA, anti-dsDNA, aCL and anti-β2GPI antibodies and clinical manifestations was found. Clinical efficacy of adalimumab is associated with the reduction of RF and anti-CCP serum levels that was detected after 24 weeks and remaining stable till the 48th week of treatment. Antinuclear and anti-dsDNA, but not anti-phospholipid autoantibodies, can be induced by adalimumab but at lower extent in comparison to studies with other anti-TNF blocking agents.

INTRODUCTION

Clinical trials in rheumatoid arthritis (RA) have demonstrated that tumor necrosis factor α (TNF-α) blocking agents are highly beneficial for most patients refractory to classic treatment with disease-modifying anti-rheumatic drugs [1-4]. However, a significant proportion of patients is still relatively resistant to such a therapy [5]. No reliable markers predictive for the clinical response have been identified, although a recent report suggests that rheumatoid factor (RF) and anti-cyclic citrullinated peptide (anti-CCP) antibody titre reduction might be a useful adjunct in assessing the efficacy of treatment [6]. Reduction of IgM-RF titres was initially described by Charles et al. in a small series of patients receiving infliximab [7], but then inconsistent findings were reported [8-11]. Recently, two papers showed a decrease in RF and anti-CCP antibody titres in RA patients treated with infliximab [6,8]. In both studies the decrease paralleled with the disease activity score improvement; but one group reported a return to baseline titre levels by prolonging the follow-up to 54 and 78 weeks [8]. On the other hand, autoantibodies against non organ-specific auto-antigens have been reported during treatment with TNF-α blocking agents. Thus, antinuclear antibodies (ANA) and anti-double-stranded DNA (anti-dsDNA) antibodies have been respectively described in up to 86% and 57% of RA patients treated with the TNF-α blocking agent infliximab [3,7,12-16]. Lower percentages were reported in patients treated with etanercept

[17]. Interestingly, these autoantibodies were only anecdotally associated with clinical manifestations suggestive for a drug induced systemic lupus erythematosus (SLE) [17]. Regarding anti-dsDNA antibodies, the occurrence of low affinity antibodies of the IgM or IgA isotype was thought to explain the lack of such an association in contrast with the widely accepted relationship between high affinity antidsDNA IgG antibodies and SLE [13]. Although anti-nuclear and anti-dsDNA antibodies have been reported at higher prevalence in patients treated with infliximab than with etanercept and in spite of the lack of any flare in a patient with previous infliximab-induced SLE when etanercept therapy was started, the occurrence of these autoantibodies has been considered a drug class related side effect [17,18].

Finally, anti-phospholipid antibodies - mainly detectable by the anti-cardiolipin assay (aCL) - were also reported in RA patients receiving TNF-α blockers. In some cases their appearance was related to concomitant infectious processes [19], but again contrasting results were reported and no correlation with the clinical manifestations specific for the anti-phospholipid syndrome was clearly found [8,9,16]. However, a paper suggested that they might be predictive of a poor clinical outcome [20].

Adalimumab, a fully human anti-TNF-α monoclonal antibody was recently approved for the treatment of both moderate and severe RA [4,21,22]. The present 1-year study was planned to evaluate in a prospective manner: i) the clinical efficacy of adalimumab, ii) whether the prevalence and titres of RA associated autoantibodies such as RF and anti-CCP antibodies correlate with treatment effect, and iii) whether non organ-specific autoantibodies are induced by adalimumab as reported for other TNF-α blocking agents.

MATERIALS AND METHODS

Patient sera.

Fifty-seven patients (53 women and 4 men; mean age at baseline 56 years [range 28-83]) with refractory RA, were included in the study. The patients were selected according to the inclusion criteria of the Adalimumab Research in Active RA (ReAct), an open-label multicenter, multinational phase IIIb study conducted primarily in Europe. In the ReAct study, patients were assigned to receive single self-injections of adalimumab subcutaneously at 40 mg every other week in addition to their pre-existing but inadequate therapies [22]. All patients fulfilled the 1987 American College of Rheumatology (ACR) classification criteria for RA [23] and were treated with methotrexate (mean dosage 10 mg/week [range 7.5-20 mg]) and adalimumab (40 mg every other week as a single dose by sc injection). Additional 55 RA patients treated with methotrexate only were followed up and evaluated with comparable parameters at 6-months' intervals.

Written informed consent was obtained from all patients and the study was approved by the Research and Ethics Committee of the L Sacco University Hospital in Milan.

Demographic and clinical data are presented in Table 1. During the study, 42 patients of the adalimumab group received concomitant corticosteroids (7,5mg/day), 48 non steroidal antiinflammatory drugs (NSAIDs), and/or analgesics and 6 other drugs. Patients were followed clinically by the same physician during this period at regular intervals and in particular when they were receiving adalimumab. Clinical assessment included number of tender and swollen joints, duration of morning stiffness,

erythrocyte sedimentation rate (ESR), and C-reactive protein (CRP) (Table 2). Clinical features suggestive of infections or autoimmune disorders were also recorded. ESR and CRP and significant concomitant clinical features suggestive of infections or autoimmune disorders were recorded accurately (Table 2). The DA28 criteria [24,25] were applied to assess clinical efficacy. Eighteen patients discontinued adalimumab treatment before the end of the study, between 3 and 12 months, because of adverse events, treatment inefficacy or severe infectious disease.

Blood was drawn between 08:00 and 09:00 in the morning when the patients visited the outpatient clinic on day 0 (screening evaluation), and after 6 and 12 months of treatment. The blood was immediately centrifuged and the serum was stored at – 80°C

Detection of rheumatoid factor and anti-cyclic citrullinated peptide antibodies.

Tests for IgM RF and anti-CCP antibodies were performed at baseline and after 6 and 12 months of adalimumab treatment. IgM RF was measured by immunonephelometry using the quantitative N Latex RF system (Dade Behring, Marburg, Germany). Rheumatoid factor titres higher than 15 IU/ml were considered positive. Anti-CCP antibodies were tested using a second generation commercially available enzymelinked immunosorbent assay (ELISA) kit (Menarini Diagnostics, Florence, Italy) as described [26]. Briefly, 100 μl anti-CCP standards (0, 2, 8, 30 and 100 U/ml), controls and patient samples (1:100 in PBS) were distributed into the appropriate wells. The microtitre plates were coated with highly purified synthetic cyclic peptides

containing modified arginine residues. After incubation for 60 min, the wells were washed three times with 200 µl wash buffer (borate buffer, 0.8% sodium azide).

The microplates were then incubated for 30 min at room temperature with alkaline phosphatelabelled murine monoclonal antibody to human IgG and washed again three times. A chromogenic substrate solution (Mg2+ phenolphthalein monophosphate buffered solution) was added to each well. After 30 min the reaction was stopped using sodium hydroxide–EDTA–carbonate buffer. The absorbance was read at 550 nm. Serum samples were evaluated in triplicate, and the upper normal limit (15 arbitrary units [AU] /ml) was assumed according to the manufacturer's recommendations. In order to follow the changes in antibody levels during therapy, all serum samples displaying a high concentration (> 100 AU/ml) were evaluated after a further $10 \times$ dilution and then corrected for this additional dilution factor. To avoid any plate-to-plate variation of anti-CCP measurements, plates from the same batch (batch n. 470094) have been used; inter- and intra-assay variability was < 9%.

Detection of antinuclear antibodies.

Anti-nuclear antibodies were performed at baseline and after 6 and 12 months of adalimumab treatment, by indirect immunofluorescence technique (IIF) using HEp2 cells as described [27]. Titres > 1:160 were considered positive. Sera positive for ANA by IIF were further analysed for the presence of anti-extractable nuclear antigens (ENA) by the Addressable Laser Bead Immunoassays (Menarini Diagnostics, Italy) according to the manufacturer's instructions [28].

Tests for anti-dsDNA IgG autoantibodies were performed at baseline and after 6 and 12 months of adalimumab treatment by EIA (Pharmacia Diagnostics, Friburg,

Germany); positive samples were also evaluated by Farr assay and by IIF using C. Luciliae (CLIFT) as described [29]. Anti-dsDNA antibodies of the IgM isotype have been also detected by CLIFT with a specific antihuman μ chain antiserum (MP Biomedicals, Aurora, USA).

Detection of anti-phospholipid antibodies.

Anti-phospholipid antibodies (aPL) were detected as anti-cardiolipin (aCL) and as anti-beta 2 glycoprotein I (β2GPI) by ELISA as described [30]; values were expressed as IgG/IgM aPL Units (GPL/MPL respectively and considered positive when >10 GPL or MPL) for aCL and as OD values for anti-β2GPI antibodies (results higher than the 95th percentile of 50 normal healthy controls were considered positive: >0.130 for IgG and >0.280 for IgM respectively) [30]. Anti-cardiolipin and anti-β2GPI autoantibodies were evaluated at baseline and after 6 and 12 months of adalimumab treatment. The sera of the control RA patient group were analyzed twice with a 1-year interval.

Statistics

Statistical analysis (95% and 99% confidence interval) was performed with the $\chi2$ test for when applicable and with Fisher's exact test in other conditions (when the expected value under null hypothesis was < 5) . Wilcoxon's test was applied in comparisons of continuous variables. Correlations were expressed using Spearman's rank correlation coefficient. Probability (*p*) values less than 0.05 were considered statistically significant. Data were analyzed with SPSS statistical software 10.00 for Windows (SPSS, Inc, Chicago, IL, USA). Statistical analysis was calculated according to last observation carried forward (LOCF)

RESULTS

Response to therapy.

ACR20 response was achieved by 88% of patients at 24 weeks, and by 80% at 48 weeks. ACR50 and ACR 70 percentages were 51, 54 and 14, 26 at 24 and 48 weeks respectively. Table 2 reports the decrease of DAS 28 values, the tender and swollen joint counts and the ESR and CRP values during the study. Patients' group treated with methotrexate alone displayed a stable disease activity during the study with no significant changes in all the clinical assessment parameters (data not shown).

Modification of anti-CCP antibody and RF titres during adalimumab treatment.

At baseline, 46 out of the 57 RA patients (80.7%) were positive for anti-CCP antibodies, and 43 out of 57 (75%) were positive for RF. A strong correlation between anti-CCP and RF at baseline was observed ($\chi 2$, $p < 0.001$). Though no patients who were positive for anti-CCP or RF at baseline became negative after anti-TNF-α treatment, both RF serum titres and anti-CCP antibodies decreased significantly at week 24 ($p < 0.01$ and $p < 0.01$, respectively) and 48 ($p < 0.01$ and $p < 0.01$, respectively) (Table 2). When we grouped the patients on the basis of their clinical response (ACR improvement criteria) to adalimumab, a significant decrease in anti-CCP antibodies and RF serum titres was observed in those who were clinically improved according to ACR 20 ACR 50 and ACR 70 criteria at week 48 (Table 3, 4). The Spearman 's correlation coefficient of the improvement in the DAS-28 with the reduction of RF titre at week 48 was 0.316 ($p = 0.018$), and for the anti-CCP antibodies titer was 0.33 ($p = 0.013$). No significant change in RF and anti-CCP titres was observed in patients treated with methotrexate alone (data not shown).

Occurrence of antinuclear antibodies in patients treated with adalimumab.

At baseline, 4 out of 57 (7%) adalimumab-treated RA patients, and 5 out of 55 (9%) methotrexate-treated RA patients tested positive for ANA (Table 5). After 12 months of therapy, the induction of ANA was observed in 16 out of 57 (28%) adalimumab-treated RA patients, and in 8 out of 55 (14.5%) with methotrexate only (Table 5). The difference in ANA positivity before and after the end of the follow up was statistically significant for the adalimumab-treated group only ($p < 0.01$). All the induced ANAs were still positive at the end of the study, including 6 out of 18 patients who discontinued the treatment. Furthermore, ANA titres increased up to twofold in 4 out of the 16 sera of adalimumab-treated RA patients positive at baseline, being the titres at the baseline lower than at the end of the follow up but without any statistically significant difference. No patient was positive for IgG or IgM anti-dsDNA autoantibodies either in the adalimumab-treated and in the methotrexate-treated group at baseline. By solid-phase ELISA, the presence of IgG anti-dsDNA autoantibodies was observed in 2 out of 57 (3.5%) adalimumab-treated RA patients after 6 months of treatment and in 4 out of 57 (7%) after 12 month treatment (Table 5). All the positive samples displayed low antibody titres (< 25 AU/ml, normal values = 17 AU/ml, mean values + 5 SD of 100 normal blood donors, age and sex matched). The positive samples displayed low titres (< 1/80) at the CLIFT assay and were negative at the Farr assay (data not shown). Immunoglobulin G anti-dsDNA autoantibodies were associated with the positivity for ANA, and remained positive until the end of the study, including 3 out of 4 positive patients who discontinued the treatment.

Five out of 57 (5.1%) and 7 out of 57 (14.6%) patients receiving adalimumab were positive for IgM anti-dsDNA antibodies after 24 and 48 weeks of treatment respectively. The occurrence of IgM anti-dsDNA antibodies was associated with the presence of IgG anti-dsDNA only in 2 patients at the 24th week and in 4 patients at the 48th week after treatment. None of the RA patients treated with methotrexate only displayed IgG or IgM anti-dsDNA antibodies during the follow up . Regarding anti-ENA antibodies, three patients were positive for anti-Ro (52 and 60 kDA) before adalimumab and two additional patients became positive during treatment. Four patients were positive for anti-Ro (52 and 60 kDA) antibodies before methotrexate treatment and an additional one became positive for anti-Ro at the end of the follow up (Table 5).

The formation of anti-nuclear and anti-dsDNA autoantibodies was not associated to any clinical event.

Occurrence of anti-phospholipid antibodies in RA patients treated with adalimumab.

No RA patients were positive for aCL or for anti-β2GPI autoantibodies at baseline. At the end of the study, aCL were detected at low titres (< 20 GPL or MPL units) in two patients in both groups; anti-β2GPI autoantibodies were found in one patient in the adalimumab-treated group at low titres (IgG 0.201 and IgM 0.312 O.D. values respectively) and in none of the patients treated with methotrexate only. All aCL and anti-β2GPI autoantibodies were detected in patients positive for ANA. No correlation was found between aPL and the clinical status (including lupus-like symptoms or thrombosis) or the occurrence of side effects (including infections).

DISCUSSION

Anti-TNF-α agents, such as infliximab and etanercept, have been reported to be beneficial for RA patients not responsive to the conventional treatment [1-3,5]. Our study confirms that adalimumab, a new fully human anti-TNF-α monoclonal antibody, is also effective in improving the clinical scores in RA patients. Reduction of RF and anti-CCP antibody titres has been recently correlated with clinical improvement after infliximab therapy in RA patients [6-8]. It has been suggested that TNF-α blocking might display an inhibitory effect on the production of antibodies closely related with RA disease activity [6]. Actually, besides their diagnostic value, high RF serum levels were shown to be an independent predictor for deteriorating radiological damage, and a greater prevalence of anti-CCP antibodies was found in patients who develop severe radiological damage [31-36]. However, while RF titre reduction was also reported by other groups after infliximab and etanercept therapy [7,8,10,11,37-39] contrasting results were found regarding anti-CCP antibody levels [7,8,10,11,37-39]. Such a discrepancy might be, at least in part, related to the different periods of follow-up and to the modalities to measure the antibody levels in the different studies. In fact, most of the enrolled patients displayed an aggressive form of the disease with high anti-CCP antibody titres; but, not all the studies carried out serial serum sample dilutions and tried to reduce the batch to batch variability in performing the solid phase assays for anti-CCP detection. This could have made the laboratory tests not sensitive enough to detect variations in the antibody titres during treatment.

Our results show a significant decrease in anti-CCP antibodies and RF titres both after 6 and 12 months of adalimumab therapy. Thus, serial evaluation of these antibodies

appears to be useful in monitoring the clinical course of RA patients undergoing therapy with adalimumab as previously suggested for infliximab [6]. It is possible that anti-CCP- positive RA patients might display a more active disease associated with a higher response to therapy in comparison to patients negative for anti-CCP antibodies. An additional study with a larger series of anti-CCP negative RA patients would be necessary to evaluate such a hypothesis, because of the too small number of anti-CCP negative patients in the present study.

On the other hand, results of studies on the association between conventional RA treatment response and decrease in RF and/or anti-CCP autoantibodies have been inconsistent. A decrease in serum RF levels has been reported in association with successful treatment with methotrexate and gold salts [40]. A more recent study confirmed the association between RF titre decrease and treatment response; in contrast, shorter disease duration but not a specific treatment was associated with a decline in anti-CCP levels [39]. We did not find any variation in RF and anti-CCP antibody serum levels in the group of RA patients treated with methotrexate alone. However, it must be pointed out that these patients did not display the same clinical characteristics of the RA patients selected for the adalimumab treatment. Moreover, they were already in treatment with methotrexate when included and displayed a stable clinical course of the disease. Thus, it is likely that a possible antibody titre reduction could have already taken place with no additional effect of treatment being detectable. The mechanisms by which TNF-α blocking agents could lead to a decrease in titres of autoantibodies closely related to RA is still matter of speculation. Either the down-regulation of pro-inflammatory processes and/or the modulation of apoptosis have

been suggested to play a role in autoantibody production or in protein citrullination that eventually might trigger the B cell responses [17,41,42]. On the other hand we confirmed the development of ANA and anti-dsDNA autoantibodies in our adalimumab-treated patients. While the prevalence of IgG anti-dsDNA was comparable to that recently reported by Keystone et al., the number of ANA positive patients was little bit larger at the end of the treatment [43]. However, both autoantibodies occurred at lower frequency than those reported in series of patients treated with infliximab or etanercept [17]. As previously reported in patients treated with different anti-TNFα blocking agents an increased frequency of anti-dsDNA antibodies of the IgM isotypes have been also found in our patients treated with adalimubab [7]. Clinical monitoring of the subgroup of patients positive for ANA and anti-dsDNA autoantibodies did not show any manifestation potentially related to a full-blown lupus disease. Accordingly, IgG anti-dsDNA autoantibodies appeared to be at low titres and to display low affinity, as demonstrated by their negativity at the Farr assay. We did not find aPL at baseline in our RA patients. Anti-cardiolipin autoantibodies were induced in 3,5% (2 out of 57) and 1,8% (1 out of 55) of our RA patients, treated respectively with adalimumab or methotrexate only while only one patient in the adalimumab group became positive for IgG and IgM anti-β2GPI antibodies. Moreover, the titres were all low and no clinical manifestations potentially related to the anti-phospholipid syndrome were recorded. Previous studies reported higher aPL frequencies both at baseline [44,45] and after anti-TNF-α therapy [8,9,16,19]. The difference with our results is likely related to the specificity of the assays we used as demonstrated in a recent multicenter study [30].

Although at lower frequency than that reported with other TNF-α blocking agents, antinuclear antibodies as well as aPL were clearly associated with adalimumab treatment. It has been suggested that a complex series of events related to TNF-α blockage might play a role in favouring the appearance of these autoantibodies. A dysregulation of apoptosis seems to be the most likely mechanism. Apoptotic cells do actually expose nuclear antigens on their surface and apoptotic blebs were reported to expose anionic PL (mainly phosphatidylserine) that in turn are able to bind circulating β2GPI; both phenomena might act as persistent immunogenic stimuli which could end into an antibody response against the self molecules [46,47]. In line with such a hypothesis is the recent demonstration that plasma levels of plasma nucleosomes were higher after infliximab treatment [48]. However, dysregulation and/or the inhibition of cytotoxic T lymphocyte response normally suppressing autoreactive B-cells might be involved [17,49].

Conclusion

In conclusion, our results confirm the clinical efficacy of adalimumab, a new fully human anti-TNF-α monoclonal antibody, in improving the clinical score in active RA patients not responsive to the conventional treatment. Such an effect is associated with the reduction of serum levels of RF and anti-CCP antibody levels that was detected after 24 weeks and remained stable until 48 weeks of treatment. Induction of ANA, low titre IgG/IgM anti-dsDNA but not aPL appears to be a drug class-related effect, since increased antibody levels were found during treatment; however no related clinical manifestations were recorded and antibody frequency was found in a lower extent than is own from studies with other TNF blocking agents.

Competing interests

The author(s) declare that they have no competing interests.

Authors' contributions

FA, MC participated in the design of the study and in the clinical evaluation of the patients.

DDA, GC and CC carried out the immunoassays. PSP and PM participated in the design and coordination of the study and helped to draft the manuscript. SD participated in the study and performed the statistical analysis. All authors read and approved the final manuscript.

References

1. Bathon JM, Martin RW, Fleischmann RM, Tesser JR, Schiff MH, Keystone EC, Genovese C, Wasko MC, Moreland LW, Weaver AW, *et al.*: **A comparison of etanercept and methotrexate in patients with early rheumatoid arthritis**. *N Engl J Med* 2000, **343:**1586-1593.

2. Elliott MJ, Maini RN, Feldmann M, Kalden JR, Antoni C, Smolen JS, Leeb B, Breedveld FC, Macfarlane JD, Bijl H: **Randomised double-blind comparison of chimeric monoclonal antibody to tumour necrosis factor alpha (cA2) versus placebo in rheumatoid arthritis**. *Lancet* 1994, **344:**1105-1110.

3. Lipsky PE, Van der Heijde DM, St Clair EW, Furst DE, Breedveld FC, Kalden JR, Smolen JS, Weisman M, Emery P, Feldmann M, *et al.*: **Infliximab and methotrexate in the treatment of rheumatoid arthritis. Anti-Tumor Necrosis Factor Trial in Rheumatoid Arthritis with Concomitant Therapy Study Group**. *N Engl J Med* 2000, **343:**1594-1602.

4. Moreland L: **Adalimumab in rheumatoid arthritis.** *Curr Rheumatol Rep.* 2004;**6:**333-334.

5. St Clair EW: **Infliximab treatment for rheumatic disease: clinical and radiological efficacy**. *Ann Rheum Dis* 2002, (suppl.II) **61:**ii67-ii69.

6. Alessandri C, Bombardieri M, Papa N, Cinquini M, Magrini L, Tincani A, Valesini G: **Decrease of anti-cyclic citrullinated peptide antibodies and rheumatoid factor following anti-TNFa therapy (infliximab) in**

rheumatoid arthritis is associated with clinical improvement. *Ann Rheum Dis* 2004, **63:** 1218-1221

7. Charles PJ, Smeenk RJ, De Jong J, Feldmann M, Maini RN**: Assessment of antibodies to double-stranded DNA induced in rheumatoid arthritis patients following treatment with Infliximab, a monoclonal antibody to tumor necrosis factor-α: findings in openlabel and randomised placebo-controlled trials.** *Arthritis Rheum* 2000; **43:** 2383-2390.

8. Bobbio-Pallavicini F, Alpini C, Caporali R, Avalle S, Bugatti S, Montecucco C: **Autoantibody profile in rheumatoid arthritis during long-term infliximab treatment.** *Arthritis Res Ther* 2004; **6:** 264-272.

9. Eriksson C, Engstrand S, Sundqvist KG, Rantapaa-Dahlqvist S: **Autoantibody formation in patients with rheumatoid arthritis treated with anti-TNFα.** *Ann Rheum Dis* 2005, **64:** 403-407.

10. De Rycke L, Verhelst X, Kruithof E, Van den Bosh F, Hoffman IEA, Veys EM, De Keyser F: **Rheumatoid factor, but not anti-cyclic citrullinated peptide antibodies, is modulated by infliximab treatment in rheumatoid arthritis**. *Ann Rheum Dis* 2005, **64:** 299-302.

11. Caramaschi P, Biasi D, Tonolli E, Pieropan S, Martinelli N, Carletto A, Volpe A, Bambara LM: **Antibodies against cyclic citrullinated peptides in patients affected by rheumatoid arthritis before and after infliximab treatment.** *Rheumatol Int.* 2005, Feb 23 [Epub ahead of print]

12. Antivalle M, Marazza MG, Randisi G, Sarzi-Puttini P, Dell'Acqua D, Carrabba M: **Longterm treatment of rheumatoid arthritis with infliximab: efficacy,**

side effects and autoantibodies induction [abstract]. *Arthritis Rheum* 2002, **46:**s534.

13. De Rycke L, Kruithof E, Van Damme N, Hoffman IE, Van den Bossche N, Van den Bosch F, Veys EM, De Keyser F: **Antinuclear antibodies following infliximab treatment in patients with rheumatoid arthritis or spondylarthropathy.** *Arthritis Rheum* 2003; **48:** 1015-23.

14. Louis M, Rauch J, Armstrong M & Fitzcharles MA: **Induction of autoantibodies during prolonged treatment with infliximab.** *J Rheumatol* 2003, **30:** 2557-2562.

15. Allanore Y, Sellam J, Batteux F, Job Deslander C, Weill B, Kahan A: **Induction of autoantibodies in refractory rheumatoid arthritis treated by infliximab**. *Clin Exp Rheum* 2004, **22:** 756-758.

16. Ferraro-Peyret C, Coury F, Tebib JG, Bienvenu J, Fabien N: **Infliximab therapy in rheumatoid arthritis and ankylosing spondylitis-induced specific antinuclear and antiphospholipid autoantibodies without autoimmune clinical manifestations: a two year prospective study.** *Arthritis Res Ther* 2004, **6:** R535-R543.

17. Ziolkowska M, Maslinski W: **Laboratory changes on anti-tumor necrosis factor treatment in rheumatoid arthritis.** *Curr Opin Rheumatol* 2003, **15:** 267-273.

18. Debandt M, Vittecoq O, Descamps V, Le Loet X, Meyer O: **Anti-TNFα-induced systemic lupus erythematosus.** *Clin Rheumatol* 2003, **22:**56-61.

19. Ferraccioli G, Mecchia F, Di Poi E, Fabris M: **Anticardiolipin antibodies in rheumatoid patients treated with etanercept or conventional comnination therapy: direct and indirect evidence for a possible association with infections.** *Ann Rheum Dis* 2002, **61**: 358-361.

20. Jonsdottir T, Forslid J, van Vollenhoven A, Harju A, Brannenmark S, Klareskog L: **Treatment with anti-TNFa antagonists in patients with rheumatoid arthritis induces anticardiolipin antibodies (ACLA): ACLA predict worse clinical outcome with infliximab and more frequent treatment-limiting infusion reactions**. *Ann Rheum Dis* 2004, **63**:1075-1078.

21. Weinblatt ME, Keystone EC, Furst DE, Moreland LW, Weisman MH, Birbara CA, Teoh LA, Fischkoff SA, Chartash EK: **Adalimumab, a fully human anti-tumor necrosis factor alpha monoclonal antibody, for the treatment of rheumatoid arthritis in patients taking concomitant methotrexate: the ARMADA trial**. *Arthritis Rheum.* 2003 ;**48:**35-45.

22. Burmester GR, Monteagudo Saez I, Malaise M, Canas da Silva J, Webber DG, Kupper H: **Efficacy and safety of Adalimumab (HUMIRA) in European Clinical Practice: The ReAct trial.** *Ann Rheum Dis* **2004** [abstract], s63.

23. Arnett FC, Edworthy SM, Bloch DA, McShane DJ, Fries JF, Cooper NS, Healey LA, Kaplan SR, Liang MH, Luthra HS: **The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis.** *Arthritis Rheum* 1988, **31:**315-324.

24. van der Heijde DM, van´t Hof M, van Riel PL, van de Putte LB: **Development of a disease activity score based on judgment in clinical practice by rheumatologists.** *J Rheumatol* 1993; **20:** 579-581.

25. Felson DT, Anderson JJ, Boers M, Bombardier C, Furst D, Goldsmith C, Katz LM, Lightfoot R Jr, Paulus H, Strand V: **American College of Rheumatology. Preliminary definition of improvement in rheumatoid arthritis.** *Arthritis Rheum* 1995, **38:**727-735.

26. Avcin T, Cimaz R, Falcini F, Zulian F, Martini G, Simonini G, Porenta-Besic V, Cecchini G, Borghi MO, Meroni PL: **Prevalence and clinical significance of anti-cyclic citrullinated peptide antibodies in juvenile idiopathic arthritis**. *Ann Rheum Dis*. 2002; **61:**608-611.

27. Bayer PM, Bauerfeind S, Bienvenu J, Fabien N, Frei PC, Gilburd B, Heide KG, Hoier-Madsen M, Meroni PL, Monier JC, Monneret G, Panzeri P, Shoenfeld Y, Spertini F, Wiik A: **Multicenter evaluation study on a new HEp2 ANA screening enzyme immune assay.** *J Autoimmun.* 1999 ;**13:**89-93.

28. Fritzler MJ. **New technologies in the detection of autoantibodies: evaluation of addressable laser bead immunoassays (ALBIA).** In "From animal models to human genetics: research on the induction and pathogenicity of autoantibodies" - Autoantigens, Autoantibodies, Autoimmunity; vol. 4. Conrad K, Bachmann MP, Chan EKL, Fritzler MJ, Humbel RL, Sack U, Shoenfeld Y Eds. Pabst Science Publishers, Berlin, Germany. 2004, 449-462.

29. Riboldi P, Gerosa M, Moroni G, Radice A, Sinico A, Tincani A, Meroni PL: **Anti-DNA antibodies: a diagnostic and prognostic tool for systemic lupus erythematosus?** *Autoimmunity*, 2005; **38:** 39-45.

30. Tincani A, Allegri F, Sanmarco M, Cinquini M, Taglietti M, Balestrieri G, Koike T, Ichikawa K, Meroni P, Boffa MC: **Anticardiolipin antibody assay: a methodological analysis for a better consensus in routine determinations - a cooperative project of the European Antiphospholipid Forum.** *Thromb Haemost.* 2001 ;**86:**575-583.

31. Bukhari M, lunt M, Harrison BJ, Scott DG, Symmons DP, Silman AJ: **Rheumatoid factor is the major predictor of increasing severity of radiographic erosions in rheumatoid arthritis: results from the Norfalk Arthritis Register Study, a large inception cohort**. *Arthritis Rheum* 2002; **46:** 906-912

32. Schellekens GA, Visser H, de Jong BA, van den Hoogen FH, Hazes JM, Breedveld FC, van Venrooij WJ : **The diagnostic properties of rheumatoid arthritis antibodies recognizing a cyclic citrullinated peptide.** *Arthritis Rheum* 2000; **43:** 155-163.

33. Kroot EJ, de Jong BA, van Leeuwen MA, Swinkels H, van den Hoogen FH, van't Hof M, van de Putte LB, van Rijswijk MH, van Venrooij WJ, van Riel PL: **The prognostic value of anti-cyclic citrullinated peptide antibodies in patients with recent-onset rheumatoid arthritis**. *Arthritis Rheum* 2000; **43:** 1831-1835.

34. Meyer O, Labarre C, Dougados M, Goupilee P, Cantagrel A, Dubois A, Nicaise-Roland P, Sibilia J, Combe B: **Anti-citrullinated protein/peptide antibody**

assay in early rheumatoid arthritis for predicting five year radiographic damage. *Ann Rheum Dis* 2003; **62**: 120-126.

35. Bas S, Genevay S, Metyer O, Gabay C: **Anti-cyclic citrullinated peptide antibodies, IgM and IgA rheumatoid factors in the diagnosis and prognosis of rheumatoid arthritis.** *Rheumatology* 2003; **42:** 677-680

36. van Venrooij WJ, Hazes JM, Visser H: **Anticitrullinated protein/peptide antibody and its role in the diagnosis and prognosis of early rheumatoid arthritis.** *Neth J Med* 2002; **60:** 383-388

37. Chen HA, Lin KC, Chen CH, Liao HT, Wang HP, Chang HN, Tsai CY, Chou CT. **The effect of etanercept on anti-cyclic citrullinated peptide antibodies and rheumatoid factor in patients with rheumatoid arthritis.** *Ann Rheum Dis* 2005 Jun 23; [Epub ahead of print]

38. Yazdani-Biuki B, Standlmainer E, Mulabecirovic A, Brezinschek R, Tilz G, Demel U, Mueller T, Brickmann K, Graninger WB, Brezinschek HP. **Blockade of tumor necrosis factor a significantly alters the serum levels of IgG- and IgA-rheumatoid factor in patients with rheumatoid arthritis.** *Ann Rheum Dis*. 2005; 64: 1224-1226.

39. Mikuls TR, O'Dell JR, Stoner JA, Parrish LA, Arend WP, Norris JM, Holers VM: **Association of rheumatoid arthritis treatment response and disease duration with declines in serum levels of IgM rheumatoid factor and anti-cyclic citrullinated peptide antibody.** *Arthritis Rheum* 2004; 50: 3776-2872.

40. Furst DE: **Rational use of disease-modifying antirheumatic drugs.** *Drugs.* 1990;**39:**19-37

41. Inagaki M, Takahara H, Nishi Y, Sugawara K, Sato C: **Ca 2+-dependent deimination induced disassembly of intermediate filaments involves specific modification of the amino-terminal head domain.** *J Biol Chem* 1989; **264:** 18119-18127.

42. Lugering A, Schmidt M, Lugering N, Pauels HG, Domaschke W, Kucharzik T: **Infliximab induces apoptosis in monocytes from patients with chronic active Crohn's disease by using a caspase-dependent pathway.** *Gastroenterology* 2001; **121**: 1145-1157.

43. Keystone E, Haraoui B: **Adalimumab therapy in rheumatoid arthritis.** *Rheum Dis Clin N Am* 2004; **30:** 349-364.

44. Keane A, Woods R, Dowding V, Roden D, Barry C: **Anticardiolipin antibodies in rheumatoid arthritis.** *Br J Rheumatol* 1987; **26:** 346-50.

45. Wolf P, Gretler J, Aglas F, Auer-Grumbach P, Rainer: **Anticardiolipin antibodies in rheumatoid arthritis: their relation to rheumatoid nodules and cutaneous vascular manifestations.** *Br J Dermatol* 1994; **131**: 48-51.

46. Casciola-Rosen L, Rosen A, Petri M, Schlissel M: **Surface blebs on apoptotic cells are sites of enhanced procoagulant activity: implications for coagulation events and antigenic spread in systemic lupus erythematosus.** *PNAS* 1996; **93**: 1624-1629.

47. Price BE, Rauch J, Shia MA, Walsh MT, Lieberthal W, Gilligan HM, O'Laughlin T, Koh JS, Levine JS: **Anti-phospholipid autoantibodies bind to**

apoptotic, but not viable, thymocytes in a beta 2-glycoprotein I-dependent manner. *J Immunol* 1996; **157**: 2201-2208.

48. D'Auria F, Rovere-Querini P, Giazzon M, Ajello P, Baldissera E, Manfredi AA, Sabbadini MG**: Accumulation of plasma nucleosomes upon treatment with anti-tumour necrosis factor-alpha antibodies.** *J Intern Med.* 2004 ;**255**:409-418.

49. Via CS, Shustov A, Rus V, Lang T, Nguyen P, Finkelman FD: **In vivo neutralization of TNF-alpha promotes humoral autoimmunity by preventing the induction of CTL.** *J Immunol* 2001, **167**:6821-6826.

Table 1. Clinical and demographic characteristics of the patients

	RA patient group	RA control group
Number of patients	57	55
Mean age, years (range)	56 (28–83)	63 (30–83)
Sex (F/M)	53/4	45/10
Disease duration, years (range)	8 (1–27)	6 (1–25)
Adalimumab treatment	57	0
Concomitant medications		
NSAIDs	48	34
Corticosteroids	42	30
Methotrexate	57	55
Other	6	0

Table 2.   **Mean clinical characteristic of patients with RA at baseline and after 24 and 48 weeks of adalimumab treatment**

Variable	Week 0	Week 24	P value	Week 48	P value
DAS 28	5.4 (1.3)	3.6 (1.2)	<0.01	2.7 (0.9)	<0.01
Tender Joint count	12.4 (4.7)	5.1 (3.5)	<0.01	4.9 (3.5)	< 0.01
Swollen Joint count	10.4 (3.8)	3.2 (3.4)	<0.01	3.12 (3.4)	< 0.01
ESR (mm/h)	35 (17)	26 (16)	<0.01	24 (15)	<0.01
C-reactive protein (mg/dl)	42(22.7)	21 (15.2)	<0.01	15 (14.8)	<0.01
Anti-CCP (AU) * (46)	116.9 (43.6)	100.5 (46.5)	<0.01	78.5 (43.9)	< 0.01
RF (UI)	121.7 (120.6)	81 (90)	<0.01	70.2 (82.7)	< 0.01

* Only patients who were anti-CCP positive at baseline were included in the evaluation.

Table 3.   **Decrease of RF titres in patients treated with adalimumab: correlation with the**

clinical response to treatment (at 24 and 48 weeks)

ACR response week 24	Week 0	Week 24	P value	ACR response week 48	Week 0	Week 48	P value
< 20% (n= 6)	*130.5 (97.9)	116.5 (88.6)	NS	< 20% (n=3)	89 (73)	60.3 (49.2)	ND°
ACR 20 (n=22)	155.3 (147.5)	109 (123)	< 0.0001	ACR 20 (n=23)	164.5 (141.3)	105.7 (112.6)	< 0.0001
ACR 50 (n=21)	94.1 (60.9)	57.3 (36.2)	< 0.0001	ACR 50 (n=16)	96.4 (73.2)	51.6 (41)	0.0001
ACR 70 (n=8)	94.8 (164.2)	40.4 (54)	NS	ACR 70 (n=15)	89.5 (123.4)	37.5 (40.7)	0.018

* mean (±S.D)

° ND: not done

Table 4. **Decrease of anti-CCP titres in patients treated with adalimumab: correlation with the clinical response to treatment (at 24 and 48 weeks) (only patients with anti CCP positive at baseline were included in the evaluation- n pts = 46)**

ACR response week 24	Week 0	Week 24	P value	ACR response week 48	Week 0	Week 48	P value
<20 % (n=6)	*118.4 (34.9)	111.8 (48.8)	NS	<20 % (n=3)	107 (14.1)	68.4 (23.3)	ND°
ACR 20 (n=21)	111.5 (45.9)	104.3 (48.5)	NS	ACR 20 (n=21)	121.8 (48.2)	88.6 (52.6)	0.001
ACR 50 (n=16)	121.9 (45.1)	93.9 (46.4)	0.001	ACR 50 (n=13)	119.6 (45.8)	73.5 (36.2)	0.001
ACR 70 (n=3)	126.8 (51.9)	85.9 (43.5)	NS	ACR 70 (n=9)	105.3 (37.7)	65.3 (35.5)	0.003

* mean (±S.D)

° ND: not done

Table 5.   **Antinuclear antibody detection during adalimumab treatment**

	Week	ANA* N.of positive sera (%)	Anti-dsDNA * N.of positive sera (%)	Anti-ENA * N.of positive sera (%)
Adalimumab (n = 57)	0	4 (7%)	0 (0%)	3 (5,2%)
	24	9 (16%)	2 (3,5%)	0
	48	12 (21%)	4 (7 %)	2 (3,5%)
Total		16 (28%)°	4 (7 %)°°	5 (9%)
P value		<0.01	<0.05	NS
Control RA (n = 55)	0	5 (9%)	0	4 (7 %)
	24	2 (3,5%)	0	0
	48	3 (5,2%)	0	1 (1,7%)
Total		8 (14.5%)°	0 (0%)°°	5 (8,7%)
P value		NS		NS

*ANA: antinuclear autoantibodies; anti-dsDNA: anti-double-stranded DNA autoantibodies; anti-ENA:

anti-nuclear extractable antigens.

RESULTS AND COMMENTS:

This prospective study investigated the efficacy of adalimumab, a human monoclonal anti-TNF-α antibody, in the treatment of RA, and its relationship with the incidence and titres of RF, anti-CCP and non organ-specific autoantibodies.

Fifty-seven RA patients (53 women and four men) who satisfied the 1987 American College of Rheumatology (ACR) criteria for RA were treated with methotrexate (mean dose 10 mg/week) and adalimumab (40 mg every other week in a single dose s.c. injection); a further 55 RA patients were treated with MTX alone and evaluated using comparable parameters at 6-month intervals [106].

At baseline, ANAs were tested by means of indirect immunofluorescence (IIF) using Hep-2 cells, anti-extractable nuclear antigens (ENAs) by means of addressable laser bead immunoassays, and anti-DNA by means of EIA; the positive samples were also evaluated using Farr's assay and IIF with *C. luciliae* (CLIFT). Anti-phospholipid antibodies were detected as anti-cardiolipin (aCL) and anti-beta 2 glycoprotein I (β2GPI) antibodies by means of an enzyme-linked immunosorbent assay (ELISA); IgM RF by means of immunonephelometry using the quantitative N Latex RF system, and anti-CCP by means of a second-generation commercially available ELISA kit [107-110]. Clinical response was evaluated on the basis of the American College of Rheumatology (ACR) and European League Against Rheumatism (EULAR) response criteria for RA.

An ACR20 response was achieved by 88% of the patients after 24 weeks, and by 80% after 48 weeks; the ACR50 and ACR70 percentages were 51% and 54% after 24 weeks, and 14% and 26% after 48 weeks [111-112]. There was a close correlation between baseline serum anti-CCP and RF levels ($\chi 2$, $p<0.001$). Though no patients who were

positive for anti-CCP or RF at baseline became negative after anti-TNF-α treatment, both RF serum titres and anti-CCP antibodies decreased significantly at week 24 (p<0.01 and p<0.01, respectively) and 48 (p<0.01 and p<0.01, respectively). When we grouped the patients on the basis of their clinical response (ACR improvement criteria) to adalimumab, a significant decrease in anti-CCP antibodies and RF serum titres was observed in those who were clinically improved according to ACR 20 ACR 50 and ACR 70 criteria at week 48. Spearman's correlation coefficient between the improvement in DAS-28 and the reductions in RF and anti-CCP titres at week 48 were respectively 0.316 (p=0.018) and 0.33 (p=0.013). A significant induction of ANA and IgG/IgM anti-dsDNA autoantibodies was found in respectively 28% and 14.6% of the patients, whereas the number of detected aCL and anti-β2GPI antibodies was not significant. There was no association between ANA, anti-dsDNA, aCL or anti-β2GPI antibodies and clinical manifestations.

It was concluded that the clinical efficacy of adalimumab is associated with reductions in serum RF and anti-CCP levels that are detectable after 24 weeks and remain stable until the 48th week of treatment. Antinuclear and anti-dsDNA (but not anti-aPL) antibodies can be induced by adalimumab, but to a lesser extent than that reported in the case of other anti-TNF blocking agents.

7.2. AUTOANTIBODY PROFILE DURING SHORT-TERM INFLIXIMAB TREATMENT FOR CROHN'S DISEASE: A PROSPECTIVE COHORT STUDY

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Autoantibody profile during short-term infliximab treatment for Crohn's disease: a prospective cohort study

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SUMMARY

Background: The potential clinical implications of autoimmunity during treatment with infliximab are unclear.
Aim: To determine the frequency and correlation of autoantibody formation in patients with Crohn's disease treated with infliximab in a routine clinical setting.
Methods: Sixty-three patients with refractory/inflammatory (31) and/or fistulising Crohn's disease (32), received an infliximab infusion at a dose 5 mg/kg in weeks 0, 2 and 6, and were evaluated for the development of antinuclear, anti-double-stranded DNA, anti-Sm, anti-RNP, anti-SSA, anti-SSB and anti-histone antibodies. The correlates with pharmacological treatments, the response to infliximab and adverse events were evaluated.
Results: Antinuclear antibodies were found in five of the 63 patients (8%) at baseline and in 26 (42%) after

10 weeks ($P < 0.001$). Of the 26 antinuclear antibody-positive patients who were further subtyped, nine of 63 (17%) had double-stranded DNA ($P = 0.003$), and 1.5% were extractable nuclear antigen (ENA) and antihistone-positive. Five patients were initially positive for anticardiolipin antibodies and two more patients became positive during infliximab treatment. New autoantibody formation was more frequent in the patients with inflammatory/refractory disease than in those with fistulising disease (17 vs. 7; $P = 0.02$). One patient developed drug-induced lupus without major organ damage.
Conclusions: Autoantibody formation occurs in 42% of patients (8% of these patients were positive before infliximab treatment) with Crohn's disease receiving induction treatment with infliximab, but the clinical significance of this remains to be determined.

INTRODUCTION

Crohn's disease (CD) is a chronic inflammatory disease of the gastrointestinal tract whose variable clinical course is characterized by segmental, transmural inflammation and granulomatous changes, the origin of which is unknown.

Laboratory and clinical research suggests that tumour necrosis factor (TNF)-α acts as a pivotal inflammatory mediator, and that its specific inhibition interrupts the major mechanisms of mucosal inflammation in CD.[1] Infliximab (Remicade, Centocor, Inc., Malvern, PA, USA) is a chimaeric IgG$_1$ monoclonal antibody to TNFα and it represents a significant advance in the treatment of CD patients. Controlled clinical trials have demonstrated its efficacy in rapidly inducing and maintaining remission in patients with moderate/severe refractory CD, healing endoscopic lesions and in the short-term and long-term treatment of draining perianal (PA) fistulae.[2–6] Moreover, audit data from North America and Europe have shown that its efficacy in clinical practice are comparable with that observed in clinical trials.[7–13]

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The concern for safety when CD patients are treated with infliximab is particularly important. The development of antinuclear (ANAs) and antidouble-stranded DNA (dsDNA) antibodies has been described in CD and rheumatoid arthritis (RA) trials. In particular, according to the reported safety data, 63.8% of RA patients and 49.1% of CD patients developed newly positive ANAs during infliximab treatment, and respectively 13% and 21.5%, respectively, developed newly positive anti-dsDNA antibodies.[13] Also there have been rare reports of lupus-like syndrome of the beginning infliximab treatment for CD.[14–16] Two important papers outlined previously the risk of immunogenicity induced by infliximab treatment in CD patients. Baert *et al.*[17] described that the development of antibodies against infliximab leads to infusion reactions and a reduced duration of response to treatment. Concomitant immunosuppressive therapy reduces the magnitude of the immunogenic response and therefore it is important to associate methotrexate (MTX) and infliximab in order to reduce the risk of the appearance of anti-idiotypic autoantibodies. Vermeire *et al.*[18] described a cumulative ANA incidence at 24 months in 71 of 125 (56.8%). Almost half of these patients developed ANAs after the first infusion, and >75% became ANA-positive after fewer than three infusions. However, only two patients (both anti-histone and dsDNA-positive) developed drug-induced lupus without major organ damage, and one developed autoimmune haemolytic anaemia.[17]

Given the limited available data concerning the potential clinical implications of autoimmunity during treatment with infliximab, we carried out this prospective cohort study of patients treated with infliximab for refractory/inflammatory and fistulising CD in a routine clinical setting with the aim of determining the frequency and correlation of autoantibody development at standardized time points. The findings were related to disease status before the start of infliximab treatment, and the relationship between the presence of autoantibodies, adverse clinical events and infliximab responses were investigated.

MATERIALS AND METHODS

The study was conducted at L. Sacco University Hospital (Milan, Italy) by members of the Department of Gastroenterology, the Rheumatology Unit, and a tertiary care medical centre for the treatment of CD.

Patient selection

The study population was a previously described[13] cohort of 63 patients with a firm diagnosis of CD attending our Gastrointestinal Unit, who were treated with infliximab for refractory/inflammatory and/or fistulising CD between June 1999 and February 2002. Concurrently, 19 ankylosing spondylitis (AS) patients who started infliximab (5 mg/kg; infusions at weeks 0, 2, 6, 14, 22 and 30) gave blood samples at baseline and at week 30 as controls for autoantibody production. Demographic, clinical and laboratory data at baseline are presented in Table 1.

Refractory/inflammatory disease was defined as a Crohn's Disease Activity Index (CDAI)[19] of 150–400 at the time of infusion, an inability to reduce the steroid dose or a lack of response to at least two courses of corticosteroids in the previous 6 months; fistulising CD was defined as the presence of single or multiple draining abdominal or PA fistulae for at least 3 months.[5]

The criteria excluding infliximab treatment were hepatic disease, renal dysfunction, clinically significant lung disease, systemic infection, pregnancy or a desire to become pregnant, a wish to father a child during the study, and a history of cancer or tuberculosis. Before treatment, the patients were screened for tuberculosis by means of a tuberculin skin test and chest X-ray, and those showing signs of previous tuberculosis were prescribed prophylactic therapy with isoniazide. Patients with symptomatic intestinal stenosis and/or intra-abdominal abscesses were also excluded, as were those needing emergency or elective surgery during the study.

Scheduled visits

The patients were prospectively registered on the day of their initial infusion, when data were collected concerning their demographic characteristics, the extent and duration of CD, extraintestinal manifestations, previous CD-related surgery, previous and concomitant medical treatment(s), and smoking habits.

All of the patients received a 2-h infusion of infliximab 5 mg/kg (and were subsequently observed for a further 2 h) in weeks 0, 2 and 6 according to a titration schedule recommended by Centocor, Inc.;[20] after the first infusion, the clinical assessment was repeated in weeks 2, 6 and 10. Serum samples for detection of autoantibodies were collected, and stored at −70 °C,

Table 1. Demographic and baseline clinical and laboratory characteristics of the patients

	Inflammatory CD (n = 31)	Fistulising CD (n = 32)	Ankylosing spondylitis (n = 19)
Females, n (%)	21 (67.7)	11 (34.4)	9 (47)
Age (years)			
Median (range)	30 (14–69)	35 (20–69)	29 (23–64)
Mean (s.d.)	31.2–10.3	39.2–13.2	34–11.7
Duration of CD (months)			
Median (range)	48 (1–204)	122.5 (12–216)	ND
Mean (s.d.)	49.8–43.2	110.2–65.0	
Previous surgery, n (%)	7 (22.6)	18 (56.3)	ND
Intestinal CD location, n (%)			
Small bowel only	3 (9.7)	1 (3.1)	ND
Colon only	6 (19.4)	6 (18.8)	
Small bowel and colon	21 (67.7)	25 (78.1)	
Stomach and colon	1 (3.2)	0	
Concomitant medications, n (%)			
Aminosalicylates	7 (22.6)	7 (21.9)	
Sulfasalazine	2 (6.5)	6 (18.8)	6 (31.5)
Corticosteroids	19 (61.3)	8 (25.0)	
AZA/MP	7 (22.6)	8 (25.0)	6 (31.5)
Antibiotics	0	7 (21.9)	7 (36.8)
Methotrexate, n (%)			
None	4 (12.9)	5 (15.6)	
CDAI			
Median (range)	240 (155–400)	180 (70–340)	ND
Mean (s.d.)	238.2–61.5	171.7–60.5	
Autoantibodies at baseline			
ANA (≥1:80)	3	2	1
Anti-dsDNA IgG and IgM	0	0	0
Anti-ENA	1	0	0
aCL IgG and IgM	1	1	1
CRP (hg/mL)			
Median (range)	2.7 (0.1–30)	1.3 (0.1–14.4)	2.8 (0.3–21)
Mean (s.d.)	5.2–6.7	2.7–3.1	4.1–4.4
ESR (mm/h)			
Median (range)	32.0 (12–91)	32.0 (1–112)	28 (15–98)
Mean (s.d.)	39.4–24.8	39.3–29.1	37–23.5

CD, Crohn's disease; AZA, azathioprine; MP, mercaptopurine; CDAI, Crohn's Disease Activity Index; ANA, antinuclear antibody; dsDNA, double-stranded DNA; IgG, immunoglobulin G; ENA, extractable nuclear antigen; aCL, anticardiolipin; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate.

just before the first infliximab infusion and at 2, 6 and 10 weeks of therapy. Serological investigations were carried out, blindly, at the end of the study in all serum samples taken at the different time points.

In the case of the patients with refractory CD, 'clinical remission' was defined as a CDAI of ≤ 150 at each scheduled visit, and a 'clinical response' as a reduction from baseline of ≥70.

In the case of the patients with PA or enterocutaneous (EC) fistulae, response was classified into three categories: a 'complete response' was defined as the closure of any draining fistulae at week 10, with closure being defined as no draining despite gentle finger compression; a 'partial response' was defined as a reduction of 50% or more from baseline in the number of fistulae at week 10; all of the other outcomes were defined as a non-response.

The patients on steroids were followed up in order to check whether their dose could be tapered or had to be increased. The patients receiving azathioprine, mercaptopurine (MP) (6-mercaptopurine), sulfasalazine or

mesalazine (mesalamine) were required to maintain the same dose during the study.

Autoantibody detection

The ANAs were tested by a standard indirect immuno-fluorescence (IIF) technique as previously described, using a BX 51 Olympus fluorescence microscope (Olympus Optical Co., Hamburg, Germany) at 40× power. Serum was first diluted 1:80 in phosphate-buffered saline (PBS) and overlaid onto fixed Hep2 cell slides (Immuno Concept, Sacramento, CA, USA) in a moist chamber for 30 min at room temperature. The positive samples (titre = 1:80) were then evaluated at increasing dilutions in PBS up to 1:640.

Anti-double-stranded DNA antibodies

Anti-dsDNA antibodies were determined by IIF. IIF was performed at 1:10 serum dilution in PBS using *Crithidia luciliae* as substrate (Inova, San Diego, CA, USA) and antihuman immunoglobulin G (IgG; γ-chain specific) as fluorescence conjugate (Delta Biologicals, Miami, FL, USA).

Autoantibodies to extractable nuclear antigens

Autoantibodies to extractable nuclear antigens (ENA; both screening and individual antigen profile) were evaluated using an enzyme-linked immunosorbent assay (ELISA). The specimens were tested on the individual antigen ELISA regardless of their results on the initial screening ELISA, because both assays were set up at the same time. Specificity for each of the main ENA antigens [Sm, RNP, SSA(Ro), and SSB(La), SCL-70, Jo-1] was determined. The test procedures were performed according to the directions supplied in the manufacturers' package inserts (Orgentec Diagnostika, Mainz, Germany).

Anticardiolipin, anti-β2-glycoprotein antibodies and antihistone antibodies

Commercially available ELISA kits (Orgentec Diagnostika) were used to detect IgG anticardiolipin (aCL) and IgM aCL, by means of a peroxidase conjugate solution of either polyclonal rabbit antihuman IgG (heavy and light chains) or polyclonal rabbit antihuman IgM (heavy and light chains) according to the manufacturer's instruc-

tions. The absorbance was read at 450 nm. The upper normal limits were 10 U/mL for IgG aCL and 7 U/mL for IgM aCL. Anti-β2-glycoprotein I (β2-GPI) antibodies and antihistone antibodies was analysed by ELISA (Orgentec Diagnostika [Mainz, Germany] and Bio-Rad [Redmond, WA, USA], respectively).

Safety evaluation

The adverse events occurring during the course of the study were recorded, including infusion reactions and all of the subsequent adverse events potentially related to infliximab. An 'infusion reaction' was defined as any adverse event occurring during the infusion or the 2-h postinfusion observation period, and a 'severe infusion reaction' as an infusion complicated by a significant change in vital signs or the development of chest pain, wheeze, dyspnoea, vomiting, abdominal pain or rash.

Data analysis

The data were statistically analysed using the *t*-test for quantitative variables and the chi-square or Fisher's exact test for qualitative variables. The descriptive statistics included mean and median values, and ranges. The data used for the efficacy analysis were expressed on both an intention-to-treat (ITT) and per-protocol basis. A *P*-value of <0.05 was considered statistically significant. We also made simple and multiple logistic regression analyses in order to assess whether the ANAs were associated with any of the demographic or clinical variables.

RESULTS

Patient characteristics

The study population consisted of 31 men and 32 women (median age 33 years; range: 14–69); the indication for treatment was inflammatory/refractory disease in 31 and fistulising disease in 32. The demographic, clinical and laboratory characteristics of the patients are shown in Table 1.

Clinical response

Refractory Crohn's disease. An ITT analysis of the 2-week data showed that 14 patients (45.2%) were

Table 2. Clinical response and clinical remission in inflammatory/refractory CD

	Intention-to-treat evaluation (weeks)			Per-protocol evaluation (weeks)		
	2	6	10	2	6	10
Clinical response (CDAI decrease ≥70)	14/31 (45.2%)	25/31 (64.5%)	20/31 (80.6%)	14/29 (48.3%)	20/29 (71.4%)	25/27 (92.6%)
Clinical remission	10/31 (31.3%)	17/31 (54.8%)	22/31 (71.9%)	10/29 (34.5%)	17/28 (60.7%)	22/27 (81.5%)

CD, Crohn's disease; CDAI, Crohn's Disease Activity Index.

clinical responders, with a median reduction in the CDAI of 111 in comparison with baseline, and 10 (31.3%) were in clinical remission. Six weeks after the first infusion, 20 patients (64.5%) achieved a reduction of ≥70 in the CDAI, and 17 (54.8%) were in clinical remission. Four weeks after the third infusion (week 10), 25 patients (80.6%) were clinical responders, and 22 (71%) were in clinical remission (Table 2). Two patients received only one of the scheduled infusions, and two received only two; if these are excluded from the week 10 analysis, the response and remission rates were respectively 92.6% and 81.5% (Table 2).

Fistulising Crohn's disease. After 10 weeks, 15 of the 32 patients with fistulising CD (46.9%) were complete responders, eight (25%) partial responders and nine (28.1%) non-responders. Four patients who did not complete the scheduled infusions showed complete (three cases) or partial fistula closures (one patient).

Safety. The adverse effects recorded at the times of infusion and 4 weeks after the last infusion affected 10 patients (15.9%).

Seven of these discontinued treatment: two because of severe infusion reactions to the first or second infusion, three because of the development of liver, PA or intra-abdominal abscesses after the second infusion, and two because of pneumonia or intestinal occlusion after the first infusion. The pneumonia developed in a male patient who had a peripheral white blood cell count of 16 000/mL, a chest X-ray revealed an area of infiltration involving less than a full segment in the right lung, and *Streptococcus pneumoniae* was isolated in sputum; treatment with oral amoxicillin/clavulanic acid 1 g b.d. for 10 days led to a complete recovery. The case of intestinal occlusion occurred in an asymptomatic female patient whose mild intestinal stenosis without obstructive manifestations at baseline worsened after the first infusion and required ileal resection.

The other three patients experienced mild infusion reactions in two cases, and an episode of nausea in one; none of them required treatment discontinuation.

Four of these 10 patients (40%) experiencing adverse events were receiving concomitant immunosuppressive therapy, along with 11 of the 53 patients (20.7%) who did not experience any adverse effects. Two patients discontinued the treatment after the first or second infusion for reasons unrelated to adverse events.

Autoantibody frequencies, titres and correlates. The ANAs were found in five of the 63 patients (8%) at baseline, and in 26 (42%) after 10 weeks. The five patients who were ANA-positive at baseline remained so at all of the subsequent time points, but only two had titres of >1:80; two patients became ANA-positive after only one infusion and 19 after receiving induction treatment with infliximab (Table 3). The ANA fluorescence pattern after infliximab treatment was homogenous in 75% of the cases. Twenty of the 26 ANA-positive patients had ANA titres of >1:80 at week 10.

None of the patients was IIF-positive for anti-dsDNA antibodies at baseline, but nine (17%) were positive after 10 weeks ($P = 0.003$).

The frequency of the other autoantibodies did not significantly change during the study (Table 3). None of the patients was positive for ENA antibodies at baseline, and only one (1.5%) became positive for anti-SSA(Ro) and antihistone during treatment.

Anticardiolipin antibodies (ACA), mostly of IgM isotype, were found in five of the 63 patients (8%) at baseline, and in seven (12%) after 10 weeks. The five patients who were aCL-positive at baseline remained so at all of the subsequent time points.

Only one patient developed a lupus-like syndrome with arthralgia affecting the small joints, non-itching crops of purple skin lesions and a clear butterfly facial rash, but without any major organ damage.[16] This patient was ANA-negative before infliximab treatment but, after the

Table 3. Autoantibody profile before and after treatment with infliximab (63 CD patients) and 19 AS patients

Number of positive sera	Baseline (CD patients)	Baseline (AS patients)	Week 2 (CD patients)	Week 6 (CD patients)	Week 10 (CD patients)	Week 30 (AS patients)	Baseline vs. week 10 (CD patients)	Baseline vs. week 30 (AS patients)
ANA (≥1:80)	5	1	7	7	26*	6	$P < 0.001$	$P = 0.045$
Anti-dsDNA (≥10)	0	0	2	6	9	2	$P = 0.003$	N.S.
Anti-ENA	0	0	1	1	1	1	N.S.	N.S.
Anticardiolipin IgM	5	1	5	6	7	2	N.S.	N.S.
Anticardiolipin IgG	2	1	1	2	2	2	N.S.	N.S.
Anti-β2-GPI IgM	0	0	0	0	0	0	N.S.	N.S.
Anti-β2-GPI IgG	0	0	0	0	0	0	N.S.	N.S.
Antihistone	0	0	0	2	3	2	N.S.	N.S.

* 20 with a titre of >1:80.

N.S., non-significant; ANA, antinuclear antibodies; AS, ankylosing spondylitis; dsDNA, double-stranded DNA antibodies; ENA, extractable nuclear antigen antibodies; β2-GPI, β2-glycoprotein I antibodies; IgM, immunoglobulin M; CD, Crohn's disease.

first infusion, laboratory investigations found positivity for ANA (an IIF 1:640 speckled pattern), anti-dsDNA (1:80 by IIF) and anti-ENA (by ELISA); the antihistone antibodies tested by ELISA, and lupus anticoagulant (LAC) and anticardiolipin antibodies (aCL) were negative. A diagnosis of infliximab-induced lupus was made, and the treatment was stopped. However, 3 months after the withdrawal of infliximab, and despite treatment with prednisone 25 mg/day, the patient still showed clinical and laboratory symptoms of systemic lupus erythematosus (SLE), and was therefore treated with prednisone 20 mg/day for SLE and oral mesalazine 2.4 mg/day for CD. Six months later, the SLE-related symptoms had disappeared and anti-dsDNA levels had returned to normal. No correlation between the presence of antibodies and other adverse events, such as infusion reactions, was found.

Simple logistic regression identified only one variable associated with the presence of ANA: new autoantibody formation was more frequent in the patients with inflammatory/refractory disease than in those with fistulising CD (17 vs. 7; $P = 0.02$).

No other treatment or disease characteristic discriminated the patients who developed antibodies from those who did not. In particular, no correlation was found between the development of autoantibodies and underlying disease activity, concomitant medication or the clinical response to infliximab.

DISCUSSION

The introduction of TNF blockade has been a breakthrough in the management of severe refractory or fistulising CD.[1, 6] Infliximab has also been found to be effective in other immune-mediated diseases, such as RA and, recently, psoriasis and spondyloarthropathy (AS).[21, 22]

However, two major problems associated with this treatment are immunogenicity and autoimmunity; as the induction of ANAs and anti-dsDNA antibodies during treatment with anti-TNFα agents has been highlighted by clinical trials and postmarketing surveillance.[23–27] Charles et al.[25] described the induction of anti-dsDNA antibodies in 7% of RA patients treated with infliximab (with or without MTX), and noted that their isotype differed from IgG, and De Rycke et al.[24] confirmed the high prevalence of ANAs and anti-dsDNA antibodies in RA patients treated with infliximab. Increased ANA titres and the induction of anti-dsDNA antibodies are more pronounced in AS than in RA,[26] but ANAs may be more likely in cases of untreated RA that in those of untreated AS, and the absence of associated MTX therapy in AS may contribute to the observed difference.

We found that 26 (42%) of our 63 infliximab-treated CD patients developed new autoantibodies: ANAs were present in only five patients (8%) at baseline and in 26 (42%) at 10 weeks. Our findings are very similar to those reported by Vermeire et al.;[18] after treatment seronegativisation of ANA was observed in only 5% of patients. The shorter existence of antibodies in RA patients than in those with CD may be explained by the use of MTX in RA patients, but there are no data to prove this; however, it is interesting that Boehm et al.[28] found that MTX therapy can lead to a decrease in circulating autoantibodies in patients with cutaneous lupus.

The aCL and anti-β2-GPI may be associated with CD.[29] aCL was found in one study, in half of the patients with CD who had active disease.[29] Two later studies, each

comparing more than 100 IBD patients and healthy controls, showed no correlation between aCL and anti-β2-GPI cofactor levels, disease activity and the number of thromboembolic events.[30, 31] In a recent paper, frequencies of both IgM and IgG aCL positivity increase in patients treated with TNF-α antagonists for 3 months or longer. Increasing age, a greater number of prior disease modifying antirheumatic drugs (DMARDs) and a greater disease activity at baseline are predictors for the development of aCL. In our cohort of patients, five were aCL-positive at baseline and two more patients developed these autoantibodies during infliximab treatment but with no apparent clinical effect.[32]

The exact mechanism by which infliximab treatment induces ANAs is still unknown. Infliximab is an IgG$_1$ antibody that binds TNF-α on the cell surface, fixes complement, and induces the lysis of TNFα-producing cells by means of antibody-dependent cell-mediated cytotoxicity. Vermeire et al.[18] speculated that this may release intracellular particles whose subsequent exposure to the immune system drives a sustained ANA response, and so mechanisms other than antibody-dependent cell-mediated cytotoxicity may play a role. It has been shown in mice that a lack of serum amyloid P (the murine analogue of acute-phase C-reactive protein) decreases the clearance of chromatin.[33] The profound downregulation of C-reactive protein in humans treated with infliximab may lead to a similar decrease in nuclear clearance and prolonged immune system exposure of excessive amounts of intracellular material, which could potentially induce and maintain an ANA response as a result of repeated stimulation.[34–36]

The ELISA and IIF did not detect anti-dsDNA antibodies in any of our patients before therapy, but in nine (17%) after infliximab. As expected, they were associated with clinical manifestations of SLE in only one case.[16] The diagnostic and prognostic value of anti-dsDNA antibodies remains controversial because lupus-associated anti-dsDNA antibodies are classically of the IgG isotype, whereas IgM or IgA anti-dsDNA antibodies may also occur in other diseases (mixed connective tissue disease, primary Sjogren's syndrome, scleroderma, RA, AS, chronic active hepatitis, primary biliary cirrhosis). None of our patients was anti-ENA-positive at baseline, and only one (1.5%) developed anti-ENA and antihistone antibodies during treatment. This occurred without any clinical manifestations.

We found that new autoantibody formation was clearly more frequent in patients with inflammatory/ refractory disease than in those with fistulising CD (17 vs. 7; $P = 0.02$), despite the majority of these patients being in concomitant treatment with steroids and/or immunosuppressive drugs. It is very difficult to establish what may have caused this difference. Although this finding may depend by chance, hypothetically, several other factors may be involved, such as different patterns of cytokines in the two subsets of disease. The 10-week evaluation of the 31 patients with refractory/inflammatory CD (4 weeks after the end of the third infusion) showed that 25 (80.6%) were clinical responders, 22 (71%) were in clinical remission and 14 (74%) had discontinued steroid treatment; at the same time point, 15 of the 32 patients with fistulising CD (46.9%) were complete responders, eight (25%) partial responders and nine (28.1%) non-responders. Our data provide further evidence that infliximab is effective in inducing the rapid remission of CD, and that ANA formation is not related to the clinical response. In a controlled trial, Baert et al.[17] found that the presence of autoantibodies was a risk factor for the development of adverse effects, but our results clearly show that there was no correlation between these antibodies and adverse events.

We did not measure anti-TNF-α antibodies, but the low incidence of infusion reactions suggest that they were probably rare.[17] Infliximab therapy can lead to the formation of anti-infliximab antibodies, whose presence has been associated with infusion reactions in 6.9–19% of patients. Infusion reactions are important immunological events induced by the presence of substantial serum concentrations of such antibodies, after which infliximab rapidly disappears from the serum and becomes undetectable within 4 weeks.[37, 38] Once an infusion reaction has occurred, the duration of the response to subsequent infusions decreases but, although there was a clear relationship between the duration of response and infliximab concentrations, it was no stronger than the correlation with antibody concentrations.

In conclusion, ANA formation is frequently seen in CD patients receiving induction treatment with infliximab, especially in those with inflammatory/refractory disease. Most patients develop autoantibodies early after the first infusion, and most remain ANA-positive (only a minority become seronegative); however, only one of our patients developed a lupus-like syndrome with clinical symptoms of polyarthritis, a clear butterfly facial rash and anti-dsDNA antibodies, and without any

major organ damage.[16] The clinical significance of autoantibody formation therefore remains to be determined.

ACKNOWLEDGEMENT

REFERENCES

1 Blam ME, Stein RB, Lichtenstein GR. Integrating anti-tumor necrosis factor therapy in inflammatory bowel disease: current and future perspectives. Am J Gastroenterol 2001; 96: 1977–97.

2 Targan SR, Hanauer SB, van Deventer SJH, et al. A short-term study of chimeric monoclonal antibody cA2 to tumor necrosis factor α for Crohn's disease. N Engl J Med 1997; 337: 1029–35.

3 Rutgeerts P, D'Haens G, Targan S, et al. Efficacy and safety or retreatment with anti-tumor necrosis factor antibody (infliximab) to maintain remission in Crohn's disease. Gastroenterology 1999; 117: 761–9.

4 Hanauer SB, Feagan BG, Lichtenstein GR, et al. Maintenance infliximab for Crohn's disease: the ACCENT I randomised trial. Lancet 2002; 359: 1541–9.

5 D'Haens G, van Deventer S, van Hogezand R. Endoscopic and histological healing with infliximab anti-tumor necrosis factor antibodies in Crohn's disease: a European multicenter trial. Gastroenterology 1999; 116: 1029–34.

6 Present DH, Rutgeerts P, Targan S, et al. Infliximab for the treatment of fistulas in patients with Crohn's disease. N Engl J Med 1999; 340: 1398–405.

7 Sands BE, Anderson FH, Bernstein CN, et al. Infliximab maintenance therapy for fistulizing Crohn's disease. N Engl J Med 2004; 350: 876–85.

8 Cohen RD, Tsang JF, Hanauer SB. Infliximab in Crohn's disease: first anniversary clinical experience. Am J Gastroenterol 2000; 95: 3469–77.

9 Farrell RJ, Shah SA, Lodhavia PJ, et al. Clinical experience with infliximab therapy in 100 patients with Crohn's disease. Am J Gastroenterol 2000; 95: 3490–7.

10 Ricart E, Panaccione R, Loftus EV, Tremaine WJ, Sandborn WJ. Infliximab for Crohn's disease in clinical practice at the Mayo Clinic: the first 100 patients. Am J Gastroenterol 2001; 96: 722–9.

11 Arnott IDR, Mcdonald D, Williams A, Ghosh S. Clinical use of infliximab in Crohn's disease: the Edinburgh experience. Aliment Pharmacol Ther 2001; 15: 1639–46.

12 Hommes DW, van de Heisteeg BH, van der Spek M, Bartelsman JFWM, van Deventer SJH. Infliximab treatment for Crohn's disease: one-year experience in a Dutch Academic Hospital. Inflamm Bowel Dis 2002; 8: 81–6.

13 Ardizzone S, Colombo E, Maconi G, et al. Infliximab in the treatment of Crohn's disease: the Milan experience. Dig Liver Dis 2002; 34: 411–8.

14 Centocor, Inc. Remicade (Infliximab). Data on File. Malvern, PA, USA: Centocor, Inc.

15 Klapman JB, Ene-Stroescu D, Becker MA, Hanauer SB. A lupus-like syndrome associated with infliximab therapy. Inflamm Bowel Dis 2003; 9: 176–8.

16 Sarzi-Puttini P, Ardizzone S, Manzionna G, et al. Infliximab-induced lupus in Crohn's disease: a case report. Dig Liver Dis 2003; 35: 814–7.

17 Baert F, Noman M, Vermeire S, et al. Influence of immunogenicity on the long-term efficacy of infliximab in Crohn's disease. N Engl J Med 2003; 348: 601–8.

18 Vermeire S, Noman M, Van Assche G, et al. Autoimmunity associated with anti-tumor necrosis factor alpha treatment in Crohn's disease: a prospective cohort study. Gastroenterology 2003; 125: 32–9.

19 Best WR, Becktel JM, Singleton JW, Kern F Jr. Development of a Crohn's disease activity index: National Cooperative Crohn's Disease Study. Gastroenterology 1976; 70: 439–44.

20 Centocor, Inc. Remicade (Infliximab). Package Insert. Malvern, PA, USA: Centocor, Inc., 1998.

21 Elliott MJ, Maini RN, Feldmann M, et al. Randomised double-blind comparison of chimeric monoclonal antibody to tumour necrosis factor alpha (cA2) vs. placebo in rheumatoid arthritis. Lancet 1994; 344: 1105–10.

22 Brandt J, Haibel H, Cornely D, et al. Successful treatment of active ankylosing spondylitis with the anti-tumor necrosis factor alpha monoclonal antibody infliximab. Arthritis Rheum 2000; 43: 1346–52.

23 Gottlieb AB, Masud S, Ramamurthi R, et al. Pharmacodynamic and pharmacokinetic response to anti-tumor necrosis factor-alpha monoclonal antibody (infliximab) treatment of moderate to severe psoriasis vulgaris. J Am Acad Dermatol 2003; 48: 68–75.

24 De Rycke L, Kruithof E, Van Damme N, et al. Antinuclear antibodies following infliximab treatment in patients with rheumatoid arthritis or spondylarthropathy. Arthritis Rheum 2003; 48: 1015–23.

25 Charles PJ, Smeenk RJT, de Jong J, Feldmann M, Maini RN. Assessment of autoantibodies to double-stranded DNA induced in rheumatoid arthritis patients following treatment with infliximab, a monoclonal antibody to tumor necrosis factor α: findings in open-label and randomized placebo-controlled trials. Arthritis Rheum 2000; 43: 2383–90.

26 Ferrero CF, Coury F, Tebib JG, Bienvenu J, Fabien N. Infliximab therapy in rheumatoid arthritis and ankylosing spondylitis-induced specific antinuclear and antiphospholipid autoantibodies without autoimmune clinical manifestations: a two-year prospective study. Arthritis Res Ther 2004; 6: 535–43.

27 Bobbio-Pallavicini F, Alpini C, Caporali R, et al. Autoantibody profile in rheumatoid arthritis during long-term infliximab treatment. Arthritis Res Ther 2004; 6: 264–72.

28 Boehm IB, Boehm GA, Bauer R. Management of cutaneous lupus erythematosus with low-dose methotrexate: indication for modulation of inflammatory mechanisms. Rheumatol Int 1998; 18: 59–62.

29 Vianna JL, D'Cruz DP, Khamashta MA, Asherson RA, Hughes GR. Anticardiolipin antibodies in a patient with Crohn's disease and thrombosis. Clin Exp Rheumatol 1992; 10: 165–8.

30 Chamouard P, Grunebaum L, Wiesel ML, *et al.* Prevalence and significance of anticardiolipin antibodies in Crohn's disease. Dig Dis Sci 1994; 39: 1501–4.

31 Koutroubakis IE, Petinaki E, Anagnostopoulou E, *et al.* Anti-cardiolipin and anti-beta2-glycoprotein I antibodies in patients with inflammatory bowel disease. Dig Dis Sci 1998; 43: 2507–12.

32 Jonsdottir T, Forslid J, van Vollenhoven A, *et al.* Treatment with tumour necrosis factor alpha antagonists in patients with rheumatoid arthritis induces anticardiolipin antibodies. Ann Rheum Dis 2004; 63: 1075–8.

33 Blickerstaff MC, Botto M, Hutchinson WL, *et al.* Serum amyloid P component controls chromatin degradation and prevents antinuclear auto-immunity. Nat Med 1999; 5: 694–7.

34 Casiano CA, Tan EM. Recent development in the understanding of antinuclear autoantibodies. Int Arch Allergy Immunol 1996; 111: 308–13.

35 Lugering A, Schmidt M, Lugering N, *et al.* Infliximab induces apoptosis in monocytes from patients with chronic active Crohn's disease by using a caspase-dependent pathway. Gastroenterology 2001; 121: 1145–57.

36 Bell DA, Morrison B. The spontaneous apoptotic cell death of normal human lymphocytes in vitro: the release of, and immunoproliferative response to, nucleosomes in vitro. Clin Immunol Immunopathol 1991; 60: 13–26.

37 Antoni C, Braun J. Side effects of anti-TNF therapy: current knowledge. Clin Exp Rheumatol 2002; 20: 152–7.

38 Day R. Adverse reactions to TNF-alpha inhibitors in rheumatoid arthritis. Lancet 2002; 359: 540–1.

RESULTS AND COMMENTS:

This study investigated the development of non organ-specific autoantibodies (ANA, anti-dsDNA, ENA, antiphospholipid antibodies) in a cohort of CD patients treated with infliximab (a chimeric monoclonal autoantibody), their frequency, and their correlations with disease status before the start of infliximab treatment, adverse events, and responses to infliximab at stardardised timepoints.

The study involved a cohort of 63 patients (31 men and 32 women) with a diagnosis of CD who were treated with infliximab for refractory/inflammatory disease (31 patients) or fistulising disease (32 patients): the former was defined as a Crohn's Disease Activity Index (CDAI) of 150-400 at the time of infusion, an inability to reduce the steroid dose, and a lack of response to at least two courses of corticosteroids in the previous six months; the latter was defined as the presence of a single or multiple draining abdominal or perianal (PA) fistulae for at least three months [20,113]. ANAs were tested by means of indirect immunofluorescence (IIF), anti-dsDNA antibodies by means of IIF with *C. luciliae* (CLIFT), and anti-aPL antibodies (detected as aCL and anti-β2GPI) by means of enzyme-linked immunosorbent assay (ELISA). All of the patients received a 2-hour infusion of infliximab 5 mg/kg in week 0, 2 and 6 according to a titration schedule recommended by Centocor Inc. [20].

The 10-week clinical response and remission rates in the patients with inflammatory/refractory disease were respectively 92.6% and 81.5%; of the 32 patients with fistulising CD, 15 (46.9%) were complete responders, eight (25%) partial responders, and nine (28.1%) non-responders. ANAs were found in five of the 63 patients (8%) at baseline, and in 26 (42%) after 10 weeks. None of the patients was CLIFT-

positive for anti-dsDNA antibodies at baseline, but nine (17%) were positive after 10 weeks (P=0.0003). aCL antibodies (mainly of the IgM isotype) were found in five of the 63 patients (8%) at baseline, and in seven (12%) after 10 weeks. Only one patient developed lupus-like syndrome, with prevalent skin involvement and without any major organ damage. The most relevant (and totally new) finding was that new autoantibody formation was more frequent in the patients with inflammatory/refractory disease than in those with fistulisng CD (17 *vs* 7; P=0.02).

In conclusion, ANA formation is frequent in CD patients receiving induction treatment with infliximab, especially in those with inflammatory/refractory disease, although the reason for the difference is very difficult to establish Although many of the CD patients developed autoantibodies, only one developed lupus-like syndrome.

8. DISCUSSION

The development of neutralising and non-neutralising autoantibodies in patients treated with anti-TNF-α agents has been reported in clinical trials, but the reported frequencies of ANAs, anti-dsDNA and aPL antibodies, as well as their correlations with disease status and adverse events, are conflicting [29,1-12]. Further studies in this field will demonstrate whether autoantibodies can be used as markers of resistance to biological agents, and what is necessary to do in clinical practice [12].

Our studies have concentrated on the efficacy of adalimumab and infliximab in two major inflammatory diseases: RA and CD; the frequency of autoantibody production; and whether autoantibodies are induced by adalimumab as has been reported in the case of other TNF-α blocking agents. We have also investigated the relationship of the autoantibodies induced by adalimumab and infliximab with disease status and adverse events.

In particular, we have evaluated the development of autoantibodies in RA, the prevalence and titres of RA-associated autoantibodies (such as RF and anti-CCP antibodies), and the correlations between them and anti-TNF-α treatment. RA patients treated with adalimumab and achieving a clinical improvement on the basis of the ACR20 and ACR50 criteria show a significant decrease in serum CCP antibody and RF titres: after 48 weeks of treatment, Spearman's correlation coefficients between the improvement in DAS-28 and the reduction in RF and anti-CCP titres were respectively 0.318 (p=0.018) and 0.33 (p=0.013). This demonstrates that these autoantibodies can be useful in monitoring the clinical course of RA patients undergoing therapy with adalimumab, as has been previously suggested in the case of infliximab [25,26,93].

The mechanism(s) by which TNFα blocking agents decrease the titres of RA-related autoantibodies is still a matter of speculation. However, it has been suggested that the down-regulation of pro-inflammatory processes and/or the modulation of apoptosis may play a role in autoantibody production or protein citrullination, and thus eventually trigger B cell responses [64]. Other groups have reported reduced RF titres after infliximab and etanercept treatment, but their findings in relationship to anti-CCP antibody levels are conflicting, possibly because of differences in the duration of follow-up and the methods used to measure autoantibody levels [3,25,26]. Most of the enrolled patients had aggressive disease with high anti-CCP antibody titres, but not all the studies carried out serial serum sample dilutions or tried to reduce batch variability when performing the solid assays. This may have meant that the laboratory tests were not sensitive enough to detect variations in antibody titres during treatment.

We have confirmed that, like other anti-TNF agents, adalimumab induces ANA and anti-dsDNA, but less frequently than reported in series of patients treated with infliximab. As previously reported in patients treated with different anti-TNF-α agents, our adalimumab-treated patients showed an increased frequency of anti-dsDNA antibodies of the IgM isotype, but those positive for ANA and anti-dsDNA antibodies did not experience any manifestation related to lupus-like syndrome[2]. aPL (as aCL) antibodies were found in only 3.5% of the patients treated with adalimumab, and the titres of IgG and IgM anti-β2GPI antibodies were low and did not lead to any clinical manifestations. Previous studies have reported higher aPL frequencies both at baseline and after anti-TNF-α therapy, but the differences in comparison with our results is probably due to the specificity of the assays we used as demonstrated in a recent multicentre study [11, 110].

On the other hand, 42% of 63 CD patients treated with infliximab developed ANAs. The higher rate of ANA positivity in CD patients than in those with RA may be explained by the use of methotrexate (MTX) in the latter. Boehm *et al*. [31] found that MTX therapy can decrease circulating autoantibodies in patients with cutaneous lupus (aCL antibodies – mainly of the IgM isotype – were found in five patients at baseline and seven after infliximab treatment, but had no apparent clinical effect). Our aCL results were similar to those found in RA patients, and it is very interesting to note that new autoantibody formation was clearly more frequent in patients with inflammatory/refractory disease than in those with fistulising CD (17 *vs* 7; P=0.02), despite the fact that the majority of these patients were receiving concomitant treatment with steroids and/or immunosuppressive drugs.

The results of these studies confirm that anti-TNF-α agents are effective in patients with chronic inflammatory diseases such as RA and CD.

We have found that the frequency of ANAs and anti-dsDNA antibodies is higher in CD patients than in those with RA, which may be explained on the basis of: 1) the structure of the monoclonal drugs; 2) the concomitant use of MTX in RA patients; and 3) the duration of follow-up.

Like infliximab and etanercept, adalimumab induces autoantibody production, but less frequently than infliximab. Adalimumab is efficacious, and this effect is correlated with decreases in RF and anti-CCP titres.

A long follow-up is necessary to evaluate whether the pathogenic role of the autoantibodies induced by biological agents changes, and whether patients become resistant to treatment.

There are still a number of unanswered questions.

1.   Can autoantibodies be used as markers of resistance to biological agents?

2.   What is the role (if any) played by aPL antibodies in patients treated with biological agents?

9. CONCLUSIONS

9.1. CONCLUSIONS OF THE FIRST STUDY

1. The clinical effect of adalimumab is associated with reduction in the serum levels of RF and anti-CCP antibodies in responsive patients.

2. The induction of ANAs and low-titre IgG/IgM anti-dsDNA (but not aPL) antibodies seems to be a drug class-related effect as increased antibody levels were found during treatment; however no related clinical manifestations were recorded and the frequency of antibody detection was less than that observed in the case of other TNF-α blocking agents.

3. Adalimumab, a new fully human anti-TNF-α monoclonal antibody, improves clinical scores in active RA patients unresponsive to conventional treatment.

9.2. CONCLUSIONS OF THE SECOND STUDY

1. ANA formation is frequently seen in CD patients receiving induction treatment with infliximab, especially in those with inflammatory/refractory disease.

2. Most patients develop autoantibodies soon after the first infusion, and most remain ANA positive

3. However, only one patient developed a lupus-like syndrome, with clinical symptoms of polyarthritis, a clear butterfly facial rush and anti-dsDNA antibodies, and without any major organ damage.

9.3. FINAL CONCLUSIONS

TNF-α agents improve clinical scores in active RA and CD patients unresponsive to conventional treatment. However, most patients develop autoantibodies, but only occasionally related to clinical manifestations.

10. REFERENCES

1.   Ferraro-Peyret C, Coury F, Tebib JG, Bienvenu J, Fabien N. Infliximab therapy in rheumatoid arthritis and ankylosing spondylitis-induced specific antinuclear and antiphospholipid autoantibodies without autoimmune clinical manifestations: a two-year prospective study. Arthritis Res Ther 2004; 6: 535–43.

2.   Charles PJ, Smeenk RJ, De Jong J, Feldmann M, Maini RN. Assessment of antibodies to double-stranded DNA induced in rheumatoid arthritis patients following treatment with Infliximab, a monoclonal antibody to tumor necrosis factor-α: findings in open-label and randomised placebo-controlled trials. Arthritis Rheum 2000; 43: 2383-90.

3.   Bobbio-Pallavicini F, Alpini C, Caporali R, Avalle S, Bugatti S, Montecucco C. Autoantibody profile in rheumatoid arthritis during long-term infliximab treatment. Arthritis Res Ther 2004; 6: 264–72.

4.   Vermeire S, Noman M, Van Assche G, Baert F, Van Steen K, Esters N, *et al.* Autoimmunity associated with anti-tumor necrosis factor alpha treatment in Crohn's disease: a prospective cohort study. Gastroenterology 2003; 125: 32–9.

5.   Louis M, Rauch J, Armstrong M et al. Louis M, Rauch J, Armstrong M, Fitzcharles MA. Induction of autoantibodies during prolonged treatment with infliximab. J Rheumatol 2005;32:760-1.

6.   Antivalle M, Marazza MG, Randisi G, Sarzi-Puttini P, Dell'Acqua D, Carrabba M. Longterm treatment of rheumatoid arthritis with infliximab: efficacy, side effects and autoantibodies induction. Arthritis Rheum 2002; 46: [abstract] s534.

7.   De Rycke L, Kruithof E, Van Damme N, Hoffman IE, Van den Bossche N, Van den Bosch F, *et al.* Antinuclear antibodies following infliximab treatment in

patients with rheumatoid arthritis or spondylarthropathy. Arthritis Rheum 2003; 48: 1015-23.

8.   Allanore Y, Sellam J, Batteux F, Job Deslandre C, Weill B, Kahan A. Induction of autoantibodies in refractory rheumatoid arthritis treated by infliximab. Clin Exp Rheumatol 2004;22:756-8.

9.   Ziolkowska M, Maslinski W. Laboratory changes on anti-tumor necrosis factor treatment in rheumatoid arthritis. Curr Opin Rheumatol 2003; 15: 267-73.

10.   Sarzi-Puttini P, **Atzeni F.** Autoimmunity induced by adalimumab during rheumatoid arthritis treatment. Autoimmunity reviews 2004; [abstract] s27.

11.   Jonsdottir T, Forslid J, van Vollenhoven A, Jonsdottir T, Forslid J, van Vollenhoven A, *et al.* Treatment with tumour necrosis factor alpha antagonists in patients with rheumatoid arthritis induces anticardiolipin antibodies. Ann Rheum Dis 2004; 63: 1075–8.

12.   Ferraccioli G, Mecchia F, Di Poi E, Fabris M. Anticardiolipin antibodies in rheumatoid patients treated with etanercept or conventional combination therapy: direct and indirect evidence for a possible association with infections. Ann Rheum Dis 2002; 61: 358-61.

13.   Taylor PC, Williams RO, Feldmann M. Tumour necrosis factor alpha as a therapeutic target for immune-mediated inflammatory diseases. Curr Opin Biotechnol 2004:557-63.

14.   Wane CF. Network communications: lymphotoxins, LIGHT, and TNF. Annu Rev Immunol 2005;23:787-819.

15. Bathon JM, Martin RW, Fleischmann RM, Tesser JR, Schiff MH, Keystone EC, *et al.* A comparison of etanercept and methotrexate in patients with early rheumatoid arthritis. N Engl J Med 2000; 343:1586- 93.

16. Elliott MJ, Maini RN, Feldmann M, Elliott MJ, Maini RN, Feldmann M, Kalden JR, Antoni C, Smolen JS, *et al.* Randomised double-blind comparison of chimeric monoclonal antibody to tumour necrosis factor alpha (cA2) versus placebo in rheumatoid arthritis. Lancet 1994; 344:1105-10.

17. Lipsky PE, van der Heijde DM, St Clair EW, Furst DE, Breedveld FC, Kalden JR, *et al.* Infliximab and methotrexate in the treatment of rheumatoid arthritis. Anti-Tumor Necrosis Factor Trial in Rheumatoid Arthritis with Concomitant Therapy Study Group. N Engl J Med 2000; 343:1594-1602.

18. Moreland L. Adalimumab in rheumatoid arthritis. Curr Rheumatol Rep 2004; 6:333-4.

19. St Clair EW. Infliximab treatment for rheumatic disease: clinical and radiological efficacy. Ann Rheum Dis 2002; 61:ii67-ii69.

20. Ardizzone S, Colombo E, Maconi G, Ardizzone S, Colombo E, Maconi G, Bollani S, Manzionna G, Petrone MC, *et al.* Infliximab in the treatment of Crohn's disease: the Milan experience. Dig Liver Dis 2002; 34: 411–8.

21. Sarzi-Puttini P, **Atzeni F**. Il quadro clinico dell'artrite reumatoide. Reumatismo 2004; 3;22-5.

22. Sarzi-Puttini P, D'Ingianna E, Fumagalli M, Sarzi-Puttini P, D'Ingianna E, Fumagalli M, Scarpellini M, Fiorini T, Cherie-Ligniere EL, *et al.* An open, randomized comparison study of cyclosporine A, cyclosporine A + methotrexate

and cyclosporine A + hydroxychloroquine in the treatment of early severe rheumatoid arthritis. Rheumatol Int 2005;25:15-22.

23. **Atzeni F**, Filippini D, Sarzi-Puttini P, Cazzola M. La terapia farmacologica dell'artrite reumatoide: farmaci per la terapia di fondo DMARDs (Disease-Modifying Antirheumatic Drugs). In Riabilitazione nell'artrite reumatoide. Ed M Cazzola, P Sarzi-Puttini 2004; pp. 113-26.

24. Vander Cruyssen B, Peene I, Cantaert T, Hoffman IE, De Rycke L, Veys EM, *et al.* Anti-citrullinated protein/peptide antibodies (ACPA) in rheumatoid arthritis: Specificity and relation with rheumatoid factor. Autoimmun Rev 2005;4:468-74.

25. Alessandri C, Bombardieri M, Papa N, Cinquini M, Magrini L, Tincani A, *et al.* Decrease of anti-cyclic citrullinated peptide antibodies and rheumatoid factor following anti-TNFa therapy (infliximab) in rheumatoid arthritis is associated with clinical improvement. Ann Rheum Dis 2004; 63: 1218-21.

26. De Rycke L, Verhelst X, Kruithof E, Van den Bosch F, Hoffman IE, Veys EM, *et al.* Rheumatoid factor, but not anti-cyclic citrullinated peptide antibodies, is modulated by infliximab treatment in rheumatoid arthritis. Ann Rheum Dis 2005; 64: 299-302.

27. Weels RW, Blennerhassett MG.The increasing prevalence of Crohn's disease in industrialized societies: the price of progress? Can J Gastroenterol 2005;19:89-95.

28. Hanauer SB. Efficacy and safety of tumor necrosis factor antagonists in Crohn's disease: overview of randomized clinical studies. Rev Gastroenterol Disord 2004;4:s18-24.

29. Centocor, Inc. Remicade (Infliximab). Data on File. Malvern, PA, USA: *Centocor, Inc.*

30. Baert F, Noman M, Vermeire S, Van Assche G, D' Haens G, Carbonez A, *et al.* Influence of immunogenicity on the long-term efficacy of infliximab in Crohn's disease. N Engl J Med 2003; 348: 601–8.

31. Boehm IB, Boehm GA, Bauer R. Management of cutaneous lupus erythematosus with low-dose methotrexate: indication for modulation of inflammatory mechanisms. Rheumatol Int 1998; 18: 59–62.

32. Keystone E, Haraqui B. Adalimumab therapy in rheumatoid arthritis. Rheum. Dis Clin N Am 2004; 30: 349–64.

33. Klapman JB, Ene-Stroescu D, Becker MA, Hanauer SB. A lupus-like syndrome associated with infliximab therapy. Inflamm Bowel Dis 2003; 9: 176–8.

34. Cush JJ. Unusual toxicities with TNF inhibition: heart failure and drug-induced lupus. Clin Exp Rheum 2004; 22: S141-7.

35. Shakoor N, Michalska M, Harris CA, Block JA. Drug-induced systemic lupus erythematosus associated with etanercept therapy. Lancet 2002; 359: 579–80.

36. Black RA, Rauch CT, Kozlosky CJ, Peschon JJ, Slack JL, Wolfson MF, *et al.* A metalloproteinase disintegrin that releases tumour-necrosis factor-α from cells. Nature 1997;385: 729-33.

37. Nawroth PP, Bank I, Handley D, Cassimeris J, Chess L, Stern D. Tumor necrosis factor/cachectin interacts with endothelial cell receptors to induce release of interleukin 1. J Exp Med 1986;163:1363-75.

38. Butler DM, Maini RN, Feldmann M, Brennan FM. Modulation of pro-inflammatory cytokine release in rheumatoid synovial membrane cell cultures: comparison of monoclonal anti TNF-α antibody with interleukin I receptor antagonist. Eur Cytokine Netw 1995;6:225-30.

39. Chin JE, Winterrowd GE, Krzesicki RF, Sanders ME. Role of cytokines in inflammatory synovitis: the coordinate regulation of intercellular adhesion molecule 1 and HLA class I and class II antigens in rheumatoid synovial fibroblasts. Arthritis Rheum 1990;33 :1776-86.

40. Tilders FJ, DeRijk RH, Van Dam AM, Vincent VA, Schotanus K, Persoons JH. Activation of the hypothalamus-pituitary-adrenal axis by bacterial endotoxins: routes and intermediate signals. Psychoneuroendocrinology 1994;19:209-32.

41. Klippel JH, Dieppe PA. Rheumatology. *III Edition.*

42. Sarzi-Puttini P, Fiorini T, Panni B, Turiel M, Cazzola M, **Atzeni F**. Correlation of the score for subjective pain with physical disability, clinical and radiographic scores in recent onset rheumatoid arthritis. BMC Musculoskelet Disord. 2002;3:18.

43. Choy EHS, Panayi GS**.** Cytokine pathways and joint inflammation in rheumatoid arthritis. N Engl J Med 2001;344:907-16.

44. **Atzeni F**, Del Papa N, Sarzi-Puttini P, Bertolazzi F, Minonzio F, Capsoni F. CD69 expression on neutrophils from patients with rheumatoid arthritis. Clin Exp Rheumatol 2004;22:331-4.

45. Sarzi-Puttini P, **Atzeni F.** Il ruolo dell'IL1-Ra nell'evoluzione del danno erosivo nell'artrite reumatoide.   Progr Reumatol 2002; 3;36-41.

46. Traunmuller F. Etiology of Crohn's disease: Do certain food additives cause intestinal inflammation by molecular mimicry of mycobacterial lipids? Med Hypotheses 2005; 65:859-64.

47. Porcelli SA, Modlin RL. The CD1 system: antigen-presenting molecules for T cell recognition of lipids and glycolipids. Ann Rev Immunol 1999;17:297–329.

48. Bendelac A. CD1: presenting unusual antigens to unusual lymphocytes. Science 1995; 269:185–6.

49. Gumperz JE, Brenner M. CD1-specific T cells in microbial immunity. Curr Opin Immunol 2001; 13:471–8.

50. Brooks EG, Balk SP, Aupeix K, Colonna M, Strominger JL, Groh-Spies V. Human T-cell receptor (TCR) a/b+ CD4-CD8-T cells express oligoclonal TCRs, share junctional motifs across TCR Vb-gene families, and phenotypically resemble memory T cells. Proc Natl Acad Sci USA 1993; 90:11787–91.

51. Sieling PA, Chatterjee D, Porcelli SA, Prigozy TI, Mazzaccaro RJ, Soriano T, *et al*. CD1-restricted T cell recognition of microbial lipoglycan antigens. Science 1995; 269: 227–30.

52. Strober W, Ludviksson BR, Fuss IJ. The pathogenesis of mucosal inflammation in murine models of inflammatory bowel disease and Crohn disease. Ann Intern Med 1998;128:848–56.

53. Fuss IJ, Heller F, Boirivant M, Leon F, Yoshida M, Fichtner-Feigl S, *et al.* Nonclassical CD1d-restricted NK T cells that produce IL-13 characterize an atypical Th2 response in ulcerative colitis. J Clin Invest 2004;113:1490–7.

54. **Atzeni F,** Sarzi-Puttini P. I farmaci biologici in corso di artrite reumatoide. In Riabilitazione nell'artrite reumatoide. Ed M Cazzola, P Sarzi-Puttini 2004; pp.127-36.

55. Nash PT, Florin TH. Tumour necrosis factor inhibitors. Med J Aust; 183:205-8.

56. Haraoui B. Differentiating the efficacy of tumor necrosis factor inhibitors. J Rheumatol 2005; 74:3-7.

57. Moreland LW, Cohen SB, Baumgartner SW, Tindall EA, Bulpitt K, Martin R, *et al.* Long-term safety and efficacy of etanercept in patients with rheumatoid arthritis. J Rheumatol 2001; 28: 1238–44.

58. Moreland LW, Schiff MH, Baumgartner SW, Tindall EA, Fleischmann RM, Bulpitt KJ, *et al.* Etanercept therapy in rheumatoid arthritis. A randomized controlled trial. Ann Intern Med 1999; 130: 478–86.

59. Bathon JM, Martin RW, Fleischmann RM, Tesser JR, Schiff MH, Keystone EC, *et al.* A comparison of etanercept and methotrexate in patients with early rheumatoid arthritis.N Engl J Med 2000;343:1586-93.

60. Kietz DA, Pepmueller PH, Moore TL. Clinical response to etanercept in polyarticular course juvenile arthritis. J Rheumatol 2001; 28: 360–2.

61. Kavanaugh A, St Clair EW, McCune WJ, Braakman T, Lipsky P. Chimeric anti-tumor necrosis factor-alpha monoclonal antibody treatment of patients with rheumatoid arthritis receiving methotrexate therapy. J Rheumatol 2000; 27: 841–50.

62. Maini R, St Clair EW, Breedveld F, Furst D, Kalden J, Weisman M, *et al.* Infliximab versus placebo in rheumatoid arthritis patients receiving concomitant methotrexate: a randomised phase III trial. Lancet 1999; 354: 1932–9.

63. Maini R, St.Clair EW, Breedveld F, Furst D, Kalden J, Weisman M, et al. Infliximab (chimeric anti-tumour necrosis factor-alpha monoclonal antibody) versus placebo in rheumatoid arthritis patients receiving concomitant methotrexate: a randomized phase III trial. ATTRACT study group. Lancet 1999; 354: 1932-9.

64. Lugering A, Schmidt M, Lugering N, Pauels HG, Domschke W, Kucharzik T. Infliximab induces apoptosis in monocytes from patients with chronic active Crohn's disease by using a caspase-dependent pathway. Gastroenterology 2001; 121: 1145–57.

65. Present DH, Rutgeerts P, Targan S, Hanauer SB, Mayer L, van Hogezand RA, *et al.* Infliximab for the treatment of fistulas in patients with Crohn's disease. N Engl J Med 1999; 340: 1398–405.

66. Comerford LW, Bickston SJ. Treatment of luminal and 520 fistulizing Crohn's disease with infliximab. Gastroenterol Clin 521 North Am 2004; 33:387–406.

67. Calabrese LH. Molecular differences in anticytokine therapies. Clin Exp Rheum 2001; 21: 241-8.

68. Capsoni F, Sarzi-Puttini P, **Atzeni F**, Minonzio F, Bonara P, Doria A, *et al.* Effect of adalimumab on neutrophil function in patients with rheumatoid arthritis. Arthritis Res Ther 2005;7:250-5.

69. Straub RH, Harle P, **Atzeni F**, Weidler C, Cutolo M, Sarzi-Puttini P. Sex Hormone Concentrations in Patients with Rheumatoid Arthritis Are Not Normalized During 12 Weeks of Anti-Tumor Necrosis Factor Therapy. J Rheumatol 2005;32:1253-8.

70. Sarzi-Puttini P, **Atzeni F**, Scholmerich J, Cutolo M, Straub RH. Anti-TNF antibody therapy improves glucocorticoid-induced insulin-like growth factor-1 (IGF-1) resistance without influencing myoglobin and IGF-1 binding proteins 1 and 3. Ann Rheum Dis 2005; [Epub ahead of print].

71. Straub RH, Sarzi-Puttini P, **Atzeni F**, Buttgereit F, Scholmerich J, Cutolo M. Anti-TNF antibody therapy does not change serum levels of cortisol binding globulin in patients with rheumatoid arthritis but it increased androstenedione relative to cortisol. Ann Rheum Dis 2005;64:1353-6.

72. Weinblatt ME, Keystone EC, Furst DE, Moreland LW, Weisman MH, Birbara CA*, et al.* Adalimumab, a fully human anti-tumor necrosis factor alpha monoclonal antibody, for the treatment of rheumatoid arthritis in patients taking concomitant methotrexate: the ARMADA trial. Arthritis Rheum 2003;48: 35-45.

73. Sarzi-Puttini P, Dell'Acqua D, **Atzeni F**. Adalimumab, a new anti-TNF-α agent. Clin Exp Rheum 2004;22 [abstract] s13.

74. Barthel HR, Gille T, Halbsguth A, Kramer M. Successful treatment with adalimumab in infliximab-resistant Crohn's disease.J Gastroenterol Hepatol 2005;20:1464-5.

75.   Bombardieri S, Tzioufas AG, McKenna F, Michel BA, Webber DG, Kupper H. Efficacy evaluation of adalimumab (Humira) in patients with a single and multiple prior biologics in the ReAct Trial. Arthritis Rheum 2004; 50: [abstract] s365.

76.   Keystone EC. Safety of biologic therapies – an update. J Rheumatol 2005; 74: 8-12.

77.   Imperato AK, Bingham CO 3rd, Abramson SB. Overview of benefit/risk of biological agents. Clin Exp Rheumatol 2004;22: S108-4.

78.   US Food and Drug Administration: Arthritis Advisory Committee March 4, 2003. Update on the TNF-α Blocking Agents. Available at www.fda.gov./ohrms/dockets/ac/03/briefing/3930B1_01_B-TNF. Briefing.htm.Accessed November 11, 2003.

79.   Wallis RS, Broder M, Wong J, Beenhouwer D. Granulomatous infections due to tumor necrosis factor blockade: correction. Clin Infect Dis 2004;39:1254-5.

80.   Furst DE, Cush J, Kaufmann S, Siegel J, Kurth R. Preliminary guidelines for diagnosing and treating tuberculosis in patients with rheumatoid arthritis in immunosuppressive trials or being treated with biological agents. Ann Rheum Dis. 2002;61:ii62-3.

81.   Curtis J, Shatin D, Bawson NSB, Braun M, Moreland L, Allison J, et al. Impact of risk communication on tuberculin skin testing for infliximab and etanercept users. Arthritis Rheum 2003; 48: [abstract] s1584.

82. Ekstrom K, Hjalgrim H, Brandt L, Baecklund E, Klareskog L, Ekbom A, *et al.* Risk of malignant lymphomas in patients with rheumatoid arthritis and in their first-degree relatives. Arthritis Rheum 2003;48:963-70.

83. Van Vollenhoven RF. Benefits and risks of biological agents: Lymphomas. Clin Exp Rheumatol 2004; 22: s122-5.

84. Sarzi-Puttini P, **Atzeni F**, Shoenfeld Y, Ferraccioli G. TNF-alpha, rheumatoid arthritis, and heart failure: a rheumatological dilemma. Autoimmun Rev 2005;4:153-61.

85. Sarzi-Puttini P, **Atzeni F**, Doria A, Iaccarino L, Turiel M. Tumor necrosis factor-a, biologic agents and cardiovascular risk. Lupus 2005; 14, 780–4.

86. Wolfe F, Michaud K. Heart failure in rheumatoid arthritis: rates, predictors, and the effect of anti-tumor necrosis factor therapy. Am J Med 2004;116:305-11.

87. Wada H, Saito K, Kanda T, Kobayashi I, Fujii H, Fujigaki S, *et al.* Tumor necrosis factor-alpha (TNF-alpha) plays a protective role in acute viralmyocarditis in mice: A study using mice lacking TNF-alpha. Circulation 2001;103:743-9.

88. Mann DL. Tumor necrosis factor and viral myocarditis: the fine line between innate and inappropriate immune responses in the heart. Circulation 2001;103:626-9.

89. Mohan N, Edwards ET, Cupps TR, Oliverio PJ, Sandberg G, Crayton H, *et al.* Demyelination occurring during anti-tumor necrosis factor alpha therapy for inflammatory arthritides.Arthritis Rheum 2001;44:2862-9.

90. Anderson PJ. Tumor necrosis factor inhibitors: clinical implications of their different immunogenicity profiles. Semin Arthritis Rheum 2005;34:19-22.

91.   Janeway CA Jr, Medzhitov R. Innate immune recognition. Annu Rev Immunol 2002;20:197-216.

92.   Bell DA, Morrison B. The spontaneous apoptotic cell death of normal human lymphocytes in vitro: the release of, and immunoproliferative response to, nucleosomes in vitro. Clin Immunol Immunopathol 1991; 60: 13–26.

93.   Amoura Z, Koutouzov S, Piette JC. The role of nucleosomes in lupus. Curr Opin Rheumatol 2000; 12: 369–73.

94.   Bickerstaff MC, Botto M, Hutchinson WL, Herbert J, Tennent GA, Bybee A, *et al.* Serum amyloid P component controls chromatin degradation and prevents antinuclear auto-immunity. Nat Med 1999; 5: 694–7.

95.   Casiano CA, Tan EM. Recent development in the understanding of antinuclear autoantibodies. Int Arch Allergy Immunol 1996; 111: 308–13.

96.   Isomaki P, Punnonem J. Pro- and anti-inflammatory cytokines in rheumatoid arthritis. Ann Med 1997; 29:499-507.

97.   Bauer M, Heeg K, Wagner H, Lipford GB. DNA activates human immune cells through a CpG sequence-dependent manner. Immunology 1999; 97:699-705.

98.   Krieg AM. The role of CpG motifs in innate immunity. Curr Opin Immunol 2000;12:35-43.

99.   Campbell IK, O'Donnell K, Lawlor KE, Wicks IP. Severe inflammatory arthritis and lymphadenopathy in the absence of TNF. J Clin Invest 2001;107:1519-27.

100.   De Rycke L, Peene I, Hoffman IE, Kruithof E, Union A, Meheus L, *et al.* Rheumatoid factor and anticitrullinated protein antibodies in rheumatoid arthritis:

diagnostic value, associations with radiological progression rate, and extra-articular manifestations. Ann Rheum Dis 20041;63:1587-93.

101. Avcin T, Cimaz R, Falcini F, Zulian F, Martini G, Simonini G, *et al.* Prevalence and clinical significance of anti-cyclic citrullinated peptide antibodies in juvenile idiopathic arthritis. Ann Rheum Dis 2002; 61:608-11.

102. Kroot EJ, de Jong BA, van Leeuwen MA, Swinkels H, van den Hoogen FH, van't Hof M, *et al.* The prognostic value of anti-cyclic citrullinated peptide antibodies in patients with recent-onset rheumatoid arthritis. Arthritis Rheum 2000; 43: 1831-5.

103. Berglin E, Johansson T, Sundin U, Jidell E, Wadell G, Hallmans G, *et al.* Radiological outcome in rheumatoid arthritis is predicted by the presence of antibodies against cyclic citrullinated peptide before and at disease onset, and by IgA-rheumatoid factor at disease onset. Ann Rheum Dis 2005; [Epub ahead of print].

104. Visser H, le Cessie S, Vos K, Breedveld FC, Hazes JM. How to diagnose rheumatoid arthritis early: a prediction model for persistent (erosive) arthritis. Arthritis Rheum 2002; 46:357-65.

105. Inagaki M, Takahara H, Nishi Y, Sugawara K, Sato C. Ca 2+-dependent deimination induced disassembly of intermediate filaments involves specific modification of the amino-terminal head domain. J Biol Chem1989; 264: 18119-27.

106. Arnett FC, Edworthy SM, Bloch DA, McShane DJ, Fries JF, Cooper NS, *et al.* The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum 1988; 31:315-24.

107. Bayer PM, Bauerfeind S, Bienvenu J, Fabien N, Frei PC, Gilburd B, *et al.* Multicenter evaluation study on a new HEp2 ANA screening enzyme immune assay. J Autoimmun 1999;13:89-93.

108. Fritzler MJ. New technologies in the detection of autoantibodies: evaluation of addressable laser bead immunoassays (ALBIA). In "From animal models to human genetics: research on the induction and pathogenicity of autoantibodies" - Autoantigens, Autoantibodies, Autoimmunity; vol. 4. Eds. Conrad K, Bachmann MP, Chan EKL, Fritzler MJ, Humbel RL, Sack U, Shoenfeld Y. Pabst Science Publishers, Berlin, Germany 2004; 449-62.

109. Riboldi P, Gerosa M, Moroni G, Radice A, Allegri F, Sinico A, *et al.* Anti-DNA antibodies: a diagnostic and prognostic tool for systemic lupus erythematosus? Autoimmunity 2005; 38: 39-45.

110. Tincani A, Allegri F, Sanmarco M, Cinquini M, Taglietti M, Balestrieri G, *et al.* Anticardiolipin antibody assay: a methodological analysis for a better consensus in routine determinations - a cooperative project of the European Antiphospholipid Forum. Thromb Haemost 2001;86:575-83.

111. Felson DT, Anderson JJ, Boers M, Bombardier C, Furst D, Goldsmith C, *et al.* American College of Rheumatology. Preliminary definition of improvement in rheumatoid arthritis. Arthritis Rheum 1995; 38:727-35.

112.   van der Heijde DM, van 't Hof M, van Riel PL, van de Putte LB. Development of a disease activity   score based on   judgment   in clinical practice by rheumatologists. J Rheumatol 1993; 20: 579-81.

113.   Best WR, Becktel JM, Singleton JW, Kern F Jr. Development of a Crohn's disease activity index: National Cooperative Crohn's Disease Study. Gastroenterology 1976; 70: 439–44.