SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO
DIRECCIÓN DE NUEVAS CREACIONES

2020
Bogotá, D.C.,

Abogada
ALICIA LLOREDA RICAURTE

ASUNTO

Radicación	08 054 162
Trámite	011
Evento	378
Actuación	519
Oficio	
Folios	029

12495

Patente de Invención	☑		Patente de Modelo de Utilidad	☐
Vía Nacional	☐			
Vía PCT (Fase Nacional)	☑	Capítulo I ☑	Capítulo II	☐
No. Solicitud Internacional	PCT/EP2006/04429 0			
Fecha de Presentación Internacional	14/Noviembre/2006			

Como resultado del examen de fondo realizado, con fundamento en el Artículo 45 de la Decisión 486 de la Comisión de la Comunidad Andina, se comunica:

1. Documentos estudiados

Descripción:	Folios 126 a 389	28/Mayo/2008
Reivindicaciones:	Folios 390 a 404	28/Mayo/2008
	Folio 682	30/Diciembre/2010
Listado de secuencias:	Folios 405 a 427	28/Mayo/2008
Dibujos:	Folios 428 a 474	28/Mayo/2008
Oposiciones:	Folios 500 a 582	Procaps 06/Abril/2010
	Folios 583 a 603	Lafrancol 06/Abril/2010
	Folios 608 a 675	Lafrancol 02/Julio/2010
Respuesta del solicitante:	Folios 679 a 682	30/Diciembre/2010
Prioridad:	US 60 737 291	15/Noviembre/2005
	US 60 864 463	06/Noviembre/2006

2. Análisis de la prioridad

Se acepta la reivindicación de prioridad porque esta solicitud fue presentada dentro del plazo establecido (Art. 9 D 486) y su contenido técnico corresponde con el de la prioridad reivindicada.

3. Objeto de la invención

Título de la solicitud: "Método para tratar daños en las articulaciones".

El problema planteado en esta solicitud consiste en la necesidad de suministrar nuevas opciones de tratamiento, particularmente aquellas que puedan apuntar a diferentes aspectos de la artritis reumatoide y ofrezcan niveles similares o mejores de beneficio clínico.

Para resolver este problema técnico la solicitud en estudio proporciona el uso del rituximab en un método para tratar el daño en una articulación en un sujeto con artritis reumatoidea.

El objeto de la presente solicitud se relaciona con la clasificación internacional de patentes (CIP): A61K39/395; A61K31/00; A61P19/02 y C07K16/28.

4. Reivindicaciones relativas a un uso o a un segundo uso (Art 14 y 21 D 486)

El uso de las reivindicaciones 1 – 5 no es patentable, de acuerdo con el Art 14.

Las reivindicaciones 1 – 5, que se refieren al producto rituximab ya patentado no pueden ser objeto de nueva patente, por el simple hecho de atribuírsele un uso distinto al originalmente comprendido por la patente inicial, de acuerdo con el Art 21. Debido a que este se había divulgado previamente en D1 que enseña el producto rituximab usado para el tratamiento de linfoma de células B (Todo el documento).

Se aclara al solicitante que a pesar de que la materia de las reivindicaciones 1 a 5 no es patentable el estado de la técnica ya anticipaba el uso reclamado.

Ítem	Documento	Título	Fecha de Publicación
D1	WO9411026	Therapeutic application of chimeric and radiolabeled antibodies to human B lymphocyte restricted differentiation antigen for treatment of B cell lymphoma	26/Mayo/1994
D2	Rheumatology 1999; 38: 1150-1152.	Remission of inflammatory arthropathy in association with anti-CD20 therapy for non-Hodgkin's lymphoma	Noviembre/1999
D3	WO0222212	Combination therapy for treatment of autoimmune diseases using B cell depleting/immunoregulatory antibody combination	21/Marzo/2002
D4	Rheumatology (2001) 40 (2): 205-211.	Sustained improvement in rheumatoid arthritis following a protocol designed to deplete B lymphocytes.	Febrero/2001
D5	Scand J Rheumatol 2004;33:82–86.	Improvement of refractory rheumatoid arthritis after depletion of B cells	2004
D6	WO2005060999	Detection of CD20 in therapy of autoimmune diseases	07/Julio/2005

D7	Genentech press release	Phase III Study Shows Rituxan Significantly Improves Symptoms in Patients with Rheumatoid Arthritis Who Inadequately Responded to Anti-TNF Therapies	5/Abril/2005
D8	US2004202658	Therapy of autoimmune disease in a patient with an inadequate response to TNF-alpha inhibitor	14/Octubre/2004
D9	WO0067796	Treatment of autoimmune diseases with antagonists which bind to B cell surface markers	16/Noviembre/2000
D10	Arthritis and rheumatism. Vol. 46, no. 8.	Efficacy of selective B cell blockade in the treatment of rheumatoid arthritis: evidence for a pathogenic role of B cells	Agosto/2002
D11	WO2004056312	Immunoglobulin variants and uses thereof	08/Julio/2004
D12	WO2004091657	Therapy of autoimmune disease in a patient with an inadequate response to a TNF-alpha inhibitor	28/Octubre/2004

D1 http://worldwide.espacenet.com/publicationDetails/biblio?DB=EPODOC&II=0&ND=3&adjacent=true&locale=en_EP&FT=D&date=19940526&CC=WO&NR=9411026A2&KC=A2 Divulga el anticuerpo rituximab y su uso para el tratamiento de linfoma de células B (Todo el documento).

D2 Revela el uso de 700mg de Rituximab (MabThera) en infusiones semanales para tratar daño articulatorio (artropatía) en un paciente que sufre también del linfoma no Hodgkin. El paciente también tomó diclofenaco brevemente en el momento de la terapia de anticuerpos, pero no agente anti-reumáticos. El pie y las radiografías mostraron erosiones tempranas de la articulación metatarsofalangeal y un estiloide ulnar.

D3 http://worldwide.espacenet.com/publicationDetails/biblio?DB=EPODOC&II=0&ND=3&adjacent=true&locale=en_EP&FT=D&date=20020321&CC=WO&NR=0222212A2&KC=A2 revela (Ejemplo 1, reivindicación 34) tratamiento de pacientes con diagnóstico clínico de artritis reumatoidea (RA) con Rituximab (Rituxan). Los pacientes son además opcionalmente tratados con uno o más agentes empleados para tratar RA tal como salicilato; NSAIDs tal como indometacina, fenilbutazona, derivados del ácido fenilacético (ej. Ibuprofeno y fenoprofeno), ácidos acéticos, naftalenos (naproxeno), ácido pirroalcanóico, ácidos indolacéticos, ácido antranílico halogenado, piroxicam, zomepirac, diflunisal, cloroquina, sales doradas, penicilamina, o agentes inmunosupresivos tales como metotrexato o corticosteroides en dosis conocidas o dosis reducidas. Rituximab es administrado por vía intravenosa de conformidad con cualquiera de los siguientes horarios de dosis: (A) 50mg/m^2 iv día 1, 150mg/m^2 iv en los días 8, 15 y 22, (B) 150mg/m^2 iv día 1, 375mg/m^2 iv los días 8, 15 y 22, (C) 375mg/m^2 iv los días 1, 8, 15 y 22.

D4 Revela un tratamiento de cinco pacientes con RA no controlada adecuadamente con medicación previa (tabla 1). Rituximab fue administrado en cuatro infusiones iv en los días 2, 8, 15 y 22 de 300, 600, 600 y 600mg respectivamente. Prednisolona oral se adminitró también en los días 1-22 y 750mg de ciclofosfamida en los días 4 y

17 (Pág. 206, columna de la derecha, párrafo 2). Los pacientes fueron evaluados al comienzo del tratamiento, a los 3 meses y luego mensualmente. El tratamiento mejoró la condición de los pacientes (tabla 2) y todos los pacientes redujeron el consumo de analgésicos (Pág. 207, columna de la izquierda, párrafos 2-4).

D5 Revela un tratamiento de cinco pacientes con RA refractaria a terapipa convencional incluyendo bloqueadores TNF-alfa (TNFa) (Pág. 82, columna de la derecha último párrafo-pág. 83, columna de la izquierda párrafo 1 tabla1). El tratamiento con corticosteroides o NSAID fue permitido durante el estudio. Los pacientes recibieron 375mg/m^2 de Rituximab en cuatro infusiones iv semanalmente y metotrexato. Se permitieron glucocorticoides al inicio del estudio (Pág. 83, columna de la izquierda). Los pacientes fueron observados durante al menos 44 semanas. Los resultados demostraron ser efectivos y que el Rituximab es seguro y eficaz para pacientes con RA resistentes a una variedad de tratamientos incluyendo bloqueo TNF (Pág. 85, columna de la derecha, úñtimo párrafo).

D6 http://worldwide.espacenet.com/searchResults?DB=worldwide.espacenet.com&locale=en_EP&query=WO2005060999&ST=singleline&compact=false revela un tratamiento de RA con rituximab o 2H7 humanizado (Pág. 12, línea 19-Pág. 14, línea 14, SEQ ID No. 1 y 2) usando un régimen de dosis seleccionado de cuatro administraciones semanales de 375mg/m^2, dos administraciones de 100mg en los días 1 y 15 o 1g tres veces. Los pacientes también pueden recibir metotrexato (10-25mg/semana) junto con un corticosteroide (metilprednisolona). Se lleva a cabo la evaluación con respuesta ACR20 en la semana 24 incluyendo puntaje radiográfico Sharp (ejemplo 1, reivindicaciones 1-7).

D7 Revela el estudio REFLEX que comprende el tratamiento de pacientes con RA activa quienes han tenido una respuesta inadecuada o fueron intolerantes a un tratamiento previo con una o más terapias anti-TNF. Los pacientes recibieron Rituximab de 1000mg por vía intravenosa en los días 1 y 15, metotrexato y un curso de dos semanas de corticosteroides. La evaluación se llevo a cabo usando ACR20 en la semana 24.

D8 http://worldwide.espacenet.com/searchResults?DB=EPODOC&locale=en_EP&query=US2004202658&ST=singleline&compact=false revela (ejemplo 1) el tratamiento de pacientes con RA activa quienes han tenido una respuesta inadecuada a una terapia TNF (etanercept, infliximab y/o adalimumab) con un anticuerpo CD20. Se usa Rituximab o 2H7v16, 1000mg iv en los días 10 y 15 o 375mg/m^2 iv semanalmente por cuatro semanas. La terapia de metotrexato concomitante (25mg/semana) y el corticosteroide que comprende prednisolona.

D9 http://worldwide.espacenet.com/searchResults?DB=EPODOC&locale=en_EP&query=WO0067796&ST=singleline&compact=false divulga el tratamiento de pacientes con diagnóstico de artritis reumatoidea con Rituximab, los cuales opcionalmente también son tratados con metotrexato (ejemplos 1 y 2).

D10 Revela el tratamiento de pacientes que tienen RA activa y la respuesta inadecuada a la terapia inmunosupresiva incluyendo anti-TNFa (tabla 1). La terapia de rituximab comprendio infusiones intravenosas durante 4 semanas de 375mg/m^2

de Rituximab con dosis baja de esteroides o NSAIDs (tabla 1). La evaluación incluyó radiografías (Pág. 2032, columna izquierda, último párrafo).

D11 http://worldwide.espacenet.com/searchResults?DB=worldwide.espacenet.com&locale=en_EP&query=WO2004091657&ST=singleline&compact=false revela el anticuerpo humanizado 2H7.v16 (ejemplos 5 y 6) y su uso, dos infusiones iv en los días 1 y 15 para tratar artritis reumatoidea severa en combinación con metotrexato. Los pacientes recibieron 10-25mg de metotrexato semanalmente durante al menos 12 semanas previas al estudio, prednisolona oral y NSAIDs. El documento enseña el uso de dosis escaladas de 2H7.v16 de 10mg a 1g.

D12 Revela (ejemplo 1) terapia para pacientes con RA refractaria hasta terapia anti-TNF usando un anticuerpo anti-CD20 seleccionado de Rituximab o 2H7.16 humanizado. Los pacientes recibieron 100mg iv del anticuerpo anti-CD20 los días 1 y 15 o 375mg/m^2 iv semanalmente durante 4 semanas. También pueden recibir metotrexato (10-25mg/semana) y corticosteroides.

5. Análisis de las Oposiciones

Argumentaciones de Procaps S.A.

1. Dice el oponente que esta solicitud pretende obtener patente para un uso y un método de tratamiento los cuales no son patentables de acuerdo al Artículo 14 de la Decisión 486 (ya que según dicho Artículo solo serán patentables los productos o procedimientos), y de acuerdo al Artículo 20 (que establece que no serán patentables los métodos terapéuticos o quirúrgicos para el tratamiento humano o animal).
2. Adiciona el oponente que la invención no es patentable porque la materia reclamada no es nueva según lo revelado en el documento WO0222212, el cual revela el tratamiento de enfermedades autoinmunes con la combinación de un anticuerpo inmunomodulador, como por ejemplo anticuerpos anti-CD40L y al menos un anticuerpo de células B, como CD19, CD20, CD22, CD23 o CD37, en la que estos anticuerpos pueden ser administrados por separado o en combinación, y en ambos, durante periodos prolongados de tiempo.
3. Finalmente, el oponente establece que la presente solicitud carece de aplicación industrial ya que reclaman un método terapéutico y estos no son aplicables industrialmente porque no se obtiene un producto o proceso; y no es reproducible o repetible a escala industrial.

Argumentaciones de Laboratorio Franco Colombiano LAFRANCOL S.A.

1. Afirma el oponente que esta solicitud carece de novedad y nivel inventivo porque los compuestos que hacen parte de las combinaciones reclamadas son conocidos mundialmente, para lo cual hace referencia a los documentos de patente referenciados en "The Merck Index" para cada una de dichas moléculas tal como Rituximab (WO9411026 y US5763137), etanercept (WO9406476 y US5605690), Infliximab (WO9216553) y Adalimumab (WO9729131 y US6090382).
2. Adicionalmente, el solicitante establece que la solicitud pretende reivindicar un método de tratamiento, el cual no se considera invención, y que los métodos

de tratamiento quirúrgicos, terapéuticos, o de diagnósticos aplicables al cuerpo humano y los relativos a animales, y además la mezcla (combinaciones) de productos (moléculas) conocidos y su variación, bien sea en uso, forma, dimensión o de materia, no se consideran invenciones por cuanto no son susceptibles de aplicación industrial, luego no pueden acceder a beneficios patentarios.

3. Finalmente, afirma el opositor que existen publicaciones de carácter científico que con anterioridad revelan la combinación entre Rituximab y Metotrexate, tales como: "Improvement of refractory Rheumatoid Arthritis after depletion of B cells", "Phase III study shows Rituxan® significantly improves symptoms in patients with Rheumatoid Arthritis who inadequately responded to anti-TNF therapies; third randomized trial to evaluate efficacy and safety of rituxan in RA" y "Efficacy of selective B cell blockade in the treatment of Rheumatoid Arthritis: Evidence for a phathogenic role of B cells", y los documentos de patente WO200222212, WO2005060999, US2004202658, WO200067796 y WO2004056312.

Respuestas de la Solicitante

Respecto a la oposición presentada tanto por la sociedad LA FRANCOL S.A como por la sociedad PROCAPS S.A, la solicitante establece que las objeciones presentadas no son de ninguna manera aplicables al nuevo capítulo reivindicatorio presentado.

Respuesta del Examinador Técnico

El nuevo capítulo reivindicatorio reclama el uso del compuesto Rituximab en un método para tratar el daño en una articulación, donde el Rituximab puede ser usado solo o en combinación con metotrexato, lo cual no es patentable de acuerdo a los Artículos 14 y 21 de la Decisión 486.

Adicionalmente, los documentos presentados en las Oposiciones, y que son los mismos relacionados en el reporte de búsqueda internacional, anticipan el objeto de la presente solicitud, ya que dichos documentos divulgan tanto al compuesto Rituximab, como su uso en el tratamiento de Artritis Reumatoidea, ya sea solo o en combinación con metotrexate, por lo tanto se considera que las oposiciones presentadas por las sociedades PROCAPS S.A y Laboratorio Franco Colombiano LAFRANCOL S.A se encuentran fundadas.

En cumplimiento del artículo 45 de la Decisión 486 de la Comisión de la Comunidad Andina, se le indica que debe dar respuesta dentro del término de sesenta (60) días contados a partir de la fecha de notificación de la presente comunicación. En caso de no dar respuesta al presente requerimiento, o si a pesar de la respuesta subsistieran los impedimentos para la concesión, se denegará la patente.

Se invita al solicitante se sirva indicar, si así lo considera, su renuncia al término faltante para dar respuesta y presentarla por escrito.

Notifíquese el presente oficio conforme a lo dispuesto en el numeral 5.2, literal e), del capítulo quinto del título primero de la Circular Única No. 10 de 2001.

JESUS FERNEL GARCÍA GARCÍA
Director de Nuevas Creaciones (E)

El anterior requerimiento fue notificado por fijación en lista, de fecha 18 JUL. 2012

Realizado por: Juanita Catalina Rico.
Revisado por: Consuelo Leguizamón L.

Rheumatology 1999;38:1150–1152

Case Report

Remission of inflammatory arthropathy in association with anti-CD20 therapy for non-Hodgkin's lymphoma

A. Protheroe, J. C. W. Edwards[1], A. Simmons, K. Maclennan and P. Selby

ICRF Cancer Medicine Research Unit, St James' University Hospital, Leeds and [1]Centre for Rheumatology, University College London, UK

Abstract

We describe a case involving a 53-yr-old male with a marginal zone B-cell lymphoma, associated with an IgM paraprotein and a rheumatoid factor-negative inflammatory polyarthropathy, treated with monoclonal anti-CD20 antibody. During the subsequent 12 weeks, evidence of synovitis reduced to a negligible level, despite no significant change in lymphoma bulk or paraprotein level. The relationship between the lymphoma and the arthropathy, and the likely mechanism of remission of the arthropathy, are discussed in the context of the potential value of anti-CD20 therapy in rheumatoid arthritis.

KEY WORDS: Anti-CD20, Lymphoma, Rheumatoid arthritis, B lymphocyte.

Case history

A 53-yr-old male presented in 1989 with lethargy, weight loss and lymphadenopathy. Investigations revealed marginal zone non-Hodgkin's lymphoma with an IgM paraprotein (37.9 g/l). Over the subsequent 9 yr, the disease followed an indolent course, treated in chronological order with: cyclophosphamide/epirubicin/vindesine/prednisolone; chlorambucil; methylprednisolone; interferon-α; plasmapheresis; fludarabine; chlorambucil; fludarabine methotrexate dexamethasone; plasmapheresis and anti-CD20 antibody.

In 1993, he first developed musculoskeletal symptoms in the form of shoulder pain, settling after a few weeks. Musculoskeletal symptoms gradually became progressive and more generalized. In 1995, he reported pain in the elbows, knees, wrists and ankles. Swelling was noted along ulnar and flexor aspects of both wrists, consistent with extensor carpi ulnaris and flexor tenosynovitis. Musculoskeletal symptoms responded temporarily (although partially) to chemotherapy, as did tumour bulk, and relapse appeared to predict progression of lymphoma. In July 1998, he was suffering widespread debilitating pain, particularly severe in the feet, necessitating the use of a wheelchair. The lymphoma was widespread, with bulky inguinal, iliac, para-aortic and axillary nodes, and a paraprotein level of 36.8 g/l.

For his progressive disease and associated intractable arthropathy, he received four 700 mg infusions of chimeric monoclonal anti-CD20 antibody (Mabthera, Roche Products) at weekly intervals. Approximately 3 weeks after receiving the antibody, he noticed improvement in joint pain and stiffness. Over the next few weeks, improvement continued such that 3 months later he was virtually symptom free and walking up to 5 miles a day. Figure 1 shows the patient's retrospective assessment of the time course of improvement in arthritic symptoms, obtained on 4 December 1998. This was the first complete resolution of his arthropathy, but CT scan showed no change in lymphoma bulk. The paraprotein level was 35.6 g/l. He remained essentially symptom free for 4 months, then pain and swelling recurred in the left midfoot, which settled 1 month later. The patient took diclofenac briefly at the time of monoclonal therapy, but no anti-rheumatic agents subsequently.

Musculoskeletal examination on 4 December 1998 was normal other than the left foot. Mild oedema was present over the midfoot dorsum. Passive pronation and supination of mid-tarsal joints provoked pain. The first to third metatarsophalangeal joints were mildly thickened and tender. Rheumatoid factor (RF) and autoantibody screen were negative, and plasma urate normal (0.33 mmol/l). Foot and hand radiographs showed early erosions of one metatarsophalangeal joint and one ulnar styloid. After a second brief relapse in

Submitted 28 January 1999; revised version accepted 28 April 1999.

Correspondence to: J. C. W. Edwards, UCL Centre for Rheumatology, 4th Floor, Arthur Stanley House, 40 Tottenham Street, London W1P 9PG, UK.

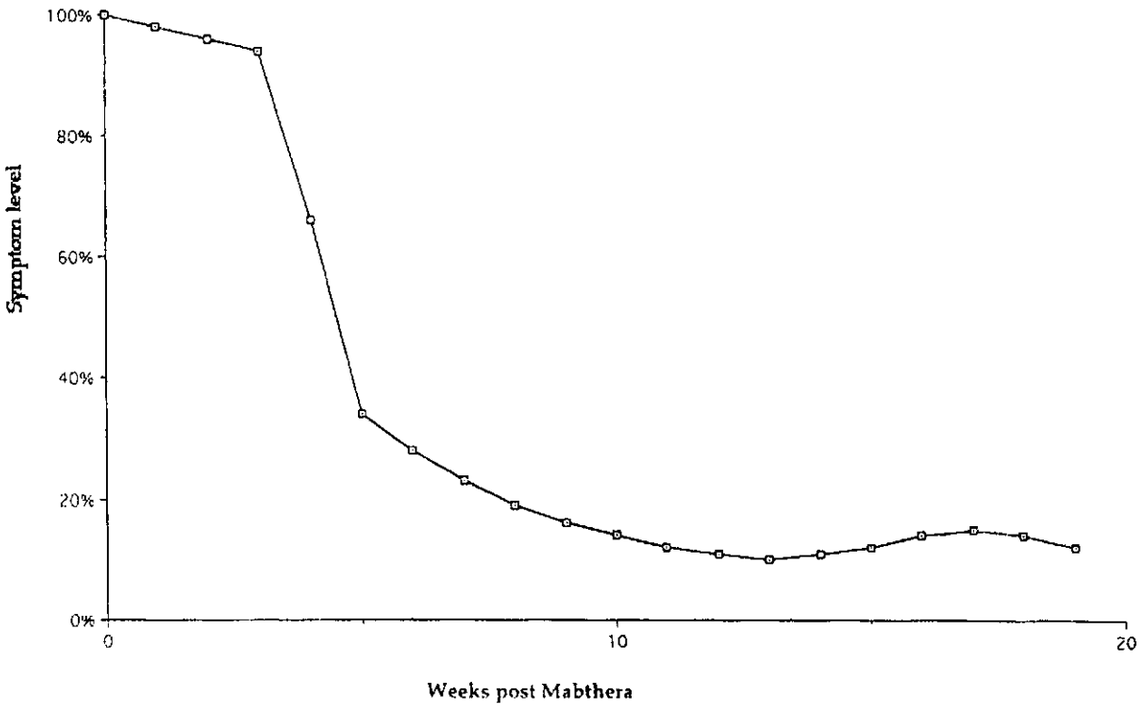

FIG. 1. Retrospective assessment by the patient of the course of regression of arthritic symptoms on a 0–100% scale.

foot pain, the patient remains essentially free of musculoskeletal symptoms in March 1999.

Discussion

The chimeric monoclonal anti-CD20 antibody C2B8 produces subtotal B-lymphocyte clearance both in cynomolgus monkeys and lymphoma patients [1, 2]. It is now licensed for use in B-cell lymphoma. Its use has also recently been considered for rheumatoid arthritis (RA) [3] and an open clinical trial is under way.

The onset of arthropathy following onset of lymphoma in this patient, and the initial correlation between rheumatic symptoms and tumour load, suggest that the arthropathy was a non-metastatic complication of the lymphoma. However, the remission following anti-CD20, independent of reduction in paraprotein, suggests that the arthropathy was not directly due to the paraprotein. It seems more likely to have been driven by a secondary autoimmune mechanism.

Remission of symptoms after anti-CD20 suggests that the arthropathy was immune complex mediated. By analogy with RA [3], it is likely that IgG- rather than IgM-based complexes were involved. A range of mechanisms can be envisaged, involving anti-idiotype responses, clonal relationships between tumour and immune complex-generating B cells and, conceivably, a triggering role for interferon-γ [4]. However, although the context is distinct, the response to anti-CD20 therapy provides encouragement for trials in RA.

The use of anti-CD20 in RA is based on a specific proposal: that the condition is driven by self-perpetuating autoantibody-secreting B cells [5], in most cases committed to IgG RF production. IgG RF oligomers have the specific potential to initiate inflammation in synovium and documented extra-synovial sites in RA by inducing tumour necrosis factor release from macrophages via the receptor FcγRIIIa [3]. IgG RFs also have the potential to feed back onto their parent B cells and perpetuate their own production, essentially because IgG RF is its own antigen.

This proposal led to the idea that long-term remission might be achieved by B-cell clearance. If sufficient autoantibody-committed B cells can be destroyed to break the cycle, then disease should be eradicated. Symptoms would be expected to decline much as observed in the case presented.

This is the first report of anti-CD20 producing a near complete resolution of an inflammatory arthropathy that we are aware of. Anti-CD20 therapy is now widely used in lymphoma. A few patients receiving this treatment will have coincidental RA or related autoimmune disorders. Documentation of the progress of such cases should be of great value in assessing the role of anti-CD20 therapy in such conditions.

References

1. Reff ME, Carner K, Chambers KS, Chinn PC, Leonard JE, Raab R *et al.* Depletion of B cells *in vivo* by a chimeric

mouse human monoclonal antibody to CD20. Blood 1994;83:435–45.
2. Maloney DG, Liles TM, Czerwinski DK, Waldichuk C, Rosenberg J, Grillo-Lopez A *et al.* Phase I clinical trial using escalating single-dose infusion of chimeric anti-CD20 monoclonal antibody (IDEC-C2B8) in patients with recurrent B-cell lymphoma. Blood 1994;84:2457–66.
3. Edwards JCW, Cambridge G. Rheumatoid arthritis: the predictable effect of small immune complexes in which antibody is also antigen. Br J Rheumatol 1998;37:126–30.
4. Vial T, Descotes J. Immune-mediated side-effects of cytokines in humans. Toxicology 1995;105:31–57.
5. Edwards JCW. Is rheumatoid factor relevant? In: Bird HA, Snaith ML, eds. Challenges in rheumatoid arthritis. Chapter 1. Blackwell Scientific, 1999, pp. 1–24.

Rheumatology 2001;40:205–211

Sustained improvement in rheumatoid arthritis following a protocol designed to deplete B lymphocytes

J. C. W. Edwards and G. Cambridge

University College London Centre for Rheumatology, London, UK

Abstract

Objectives. An open study of B-lymphocyte depletion was undertaken in rheumatoid arthritis (RA) patients to test the hypothesis that B lymphocytes may be essential to disease perpetuation.

Methods. Five patients with refractory RA were treated with a monoclonal anti-CD20 antibody, cyclophosphamide and prednisolone and followed for 12–17 months. Patient 2 received further treatments at 8 and 12 months and patient 4 at 11 months.

Results. At 26 weeks all patients satisfied the American College of Rheumatology ACR50 and patients 1–3 the ACR70 criteria of improvement, without further therapy. Patients 1, 3 and 5 achieved ACR70 at 1 yr and rheumatoid factor (RF) levels fell to normal. In patients 3 and 5, B lymphocytes returned without relapse. Patient 2 relapsed at 28 weeks and patient 4 at 38 weeks, coincident with the return of B lymphocytes in the presence of raised RF levels. Both achieved ACR70 on retreatment. Adverse events were limited to respiratory episodes (two patients) and marginal thrombocytopenia (one patient).

Conclusions. These findings are consistent with the concept that RA is critically dependent on B lymphocytes and suggest that B-lymphocyte depletion may be a safe and effective therapy.

KEY WORDS: B lymphocyte, Anti-CD20, Rituximab, Autoimmunity, Rheumatoid arthritis.

There is a consensus that the pathogenesis of rheumatoid arthritis (RA) includes roles for the T lymphocyte (interaction with antigen and major histocompatibility class II molecules), the macrophage (secretion of pro-inflammatory cytokines) and the synovial fibroblast (cartilage damage) [1–3]. The role of B lymphocytes is less well established, despite the most consistent immunological finding being production of autoantibodies directed against the Fc domain of IgG (rheumatoid factor, RF) [4, 5]. Histologically, the most specific features of rheumatoid synovium (also bone marrow) are follicles containing B lymphocytes and heavy infiltration with plasma cells [6].

Interest in the role of B lymphocytes has been rekindled by new information on the genetics, roles in cell signalling and crystal structure of RF [7–9]. Carson and co-workers [8] have argued that the mechanisms governing the survival of RF-committed B lymphocytes may differ from those for other autoreactive B lymphocytes. RF-committed B lymphocytes may obtain 'bypass' help from T lymphocytes recognizing foreign antigens [10]. This potentially resolves the paradox of a T-dependent anti-IgG response in the apparent absence of relevant autoreactive T lymphocytes.

Submitted 12 June 2000; revised version accepted 2 October 2000.

Correspondence to: J. C. W. Edwards, Rheumatology, Arthur Stanley House, 40–50 Tottenham Street, London W1P 9PG, UK.

In 1998, we extended these ideas to construct a pathogenic hypothesis [5, 11, 12], which formed the rationale for the present study. The hypothesis comprises two key postulates: (i) that B lymphocyte clones, committed to certain species of IgG RF, may perpetuate their own existence, and that of other RF-specific clones, both through 'bypass' T-cell help and by providing themselves with their own antigen complexed with the survival-promoting complement fragment C3d,g [12, 13]; and (ii) that subsets of IgG1 RF can be predicted to induce both articular and extra-articular features of RA by generating tumour necrosis factor α (TNF-α) through interaction with the IgG Fc receptor $Fc\gamma RIIIa$ [14–18]. Synovial and bone marrow involvement may be compounded by the readiness with which stromal cells from these tissues support the survival of B lymphocytes and plasma cells [19, 20].

This hypothesis led to the prediction that subtotal B-lymphocyte depletion might remove sufficient autoreactive B lymphocytes to induce the collapse of a pathogenic autoantibody-generating cycle, leading to long-term remission. An important caveat was that, in addition to B-lymphocyte depletion, it might be necessary to remove the autoantibodies implicated in driving their own production, in order to break the cycle.

B lymphocytes might also play a more general role in autoimmune disease, as antigen-presenting cells. B-lymphocyte depletion had already been considered

as a therapeutic strategy for autoimmune disease, but the prospect of requiring long-term B lymphopenia was discouraging. However, the idea of a 'vicious cycle', with the possibility that a short period of B lymphopenia might produce sustained improvement, appeared to justify a phase 1 clinical trial.

Clinical investigation of this concept was made possible by the availability of a specific B-lymphocyte-depleting agent of low toxicity. Rituximab (Mabthera: Roche Products, Welwyn, UK) is the clinical formulation of a murine IgG1 monoclonal anti-CD20 antibody [21, 22]. An open study of B-lymphocyte depletion in subjects with refractory RA was undertaken. The primary outcome measure was defined as the achievement of complete remission, as indicated by absence of clinical, biochemical or serological evidence of immunological inflammatory activity 6 months after a single course of treatment.

The chosen B-lymphocyte-depleting protocol combined rituximab with corticosteroid and cyclophosphamide in a single 3-week course. The following factors contributed to this decision: (i) there is anecdotal evidence, from patients coincidentally receiving both low- and high-dose cytotoxic regimens, that RA is about as difficult to cure as non-Hodgkin lymphoma and that monotherapy with a pan-lymphocyte-depleting anti-CD52 antibody has not been effective [23]; and (ii) our hypothesis [12] indicates that a threshold of B-lymphocyte depletion would need to be reached. The protocol was, therefore, based on the type of combination therapy used in B-cell lymphoma. However, doses of prednisolone and cyclophosphamide were limited to those considered unlikely to increase morbidity significantly, within ranges not found to produce prolonged clinical effects on their own [24–26]. A single short course was used on the basis that, if a vicious cycle were broken, continued therapy would be unnecessary.

Methods

Subjects

Five subjects, detailed in Table 1, satisfying the American College of Rheumatology (ACR) criteria for classical RA, were selected on the basis of severe inflammatory disease that had not been adequately controlled despite trials of at least five disease-modifying anti-rheumatic drugs (DMARDs) as single agents (Table 1). DMARDs (excluding steroids; see below) were discontinued from day 0. All patients used diclofenac and/or coproxamol as needed.

Treatment protocol

The study was approved by the local hospital ethics committee. All patients received the following treatment. (i) Monoclonal chimaeric anti-CD20 antibody, rituximab (Mabthera) as four i.v. infusions (over 3 h) on days 2, 8, 15 and 22, of 300, 600, 600 and 600 mg respectively. Patient 5 omitted dose 4 because of an episode of fever and pleuritic pain. (ii) Oral prednisolone 60 mg on days 1–22, reducing in the three older subjects (perceived to be at higher risk of toxicity) to 30 mg on days 11–22 and then withdrawn over 3 weeks in subjects not previously taking steroids and, in the other cases, to 5 mg daily over 6 weeks. (iii) Cyclophosphamide as i.v. infusions on days 4 and 17 of 750 mg.

Assessment

Patients were assessed at recruitment, immediately prior to treatment, fortnightly for 3 months and then monthly after treatment for duration of early morning stiffness in minutes, pain on a 100-mm visual analogue scale, number of swollen joints (out of 28 joints), number of tender joints (out of 28 joints), physician's and patient's global scores on a 100-mm scale, health status by a health assessment questionnaire, erythrocyte sedimentation rate (ESR), serum C-reactive protein concentration (CRP), full blood count, renal and liver function tests, serum immunoglobulins, RF by latex fixation (positive or negative) and the titre of rheumatoid arthritis particle agglutination (RAPA), and antinuclear antibody (ANA) titre. Circulating B ($CD19^+$ by flow cytometry) and total lymphocytes were measured before, immediately after and monthly from 3 months after therapy until normal. B-lymphocyte data were incomplete for logistic reasons but the period of B-lymphocyte repopulation was covered in all cases.

Two levels of response were defined: (i) an improvement score of ACR(n), where n is the minimum percentage improvement in five measures, as defined by the ACR [27], and (ii) a state of complete remission.

Table 1 Details of patients at entry to the study

Patient	Age (yr)	Gender	Duration of RA (yr)	Erosive	Functional class	RAPA (ever −ve)	ANA (ever +ve)	Extra-articular features	DMARD failure[b]	DMARD steroid at entry[b]
1	57	F	25	–	III	Yes	No	Anaemia[a]	GASMDP	P 7.5 mg day
2	42	F	21	–	III	Yes	Yes	Anaemia[a]	GASMD	A 100 mg day
3	69	F	40	–	III	Yes	Yes	–	GASMD	G 50 mg month
4	50	F	11	–	III	Yes	Yes	Anaemia[a]	GASMP	P 10 mg day
5	59	F	17	–	III	Yes	Yes	Subcutaneous nodules	GASMHD	M 12.5 mg week

[a]Haemoglobin < 12 g dl.
[b]G, gold; A, azathioprine; S, sulphasalazine; M, methotrexate; H, hydroxychloroquine; D, D-penicillamine; P, prednisolone.

Remission was defined as the absence of clinical or laboratory evidence of ongoing immunological activity. The ACR criteria for remission were not appropriate in the context of patients with disease of long duration, with pain and joint thickening due to irreversible joint damage. Remission was defined as early morning stiffness lasting < 10 min, pain score < 10 mm, fewer than four swollen joints (including knee effusions), fewer than two tender joints, ESR < 20 mm h, CRP < 10 mg l, RF negative by latex fixation and RAPA titre < 40.

Results

Clinical response

Clinical and laboratory indices with scales adjusted for comparability are shown in Fig. 1. Baseline, 6-month and 1-yr measurements, together with percentage improvement and ACR scores, are given in Table 2. All patients showed rapid improvement in synovitis. Anaemia resolved in all patients and nodules disappeared in patient 5. At 6 months, all patients achieved ACR50 and patients 1–3 achieved ACR70 without introduction of further therapy. The patients previously taking prednisolone had no difficulty in reducing their daily dose to 5 mg. All patients reduced their analgesic intake.

At 1 yr, patients 1 and 3 maintained improvement and patient 5 improved further, all achieving ACR70. ACR70 was maintained by patients 1 and 3 at the last assessment at 17 months. Patient 5 reports remaining well but declines further assessment visits. Despite a clinical impression of complete remission in patients 1–3 at 6 months and in patients 1, 3 and 5 at 1yr, the predefined criteria were not formally met because of residual pain and persisting, if reduced, RF levels.

Patient 2 developed a relapse of joint pain and swelling over a few days at week 28. Acute-phase measures rose rapidly. At 34 weeks she was retreated with a single 500-mg dose of rituximab and started on prednisolone 10 mg daily. The prednisolone was withdrawn over 3 months. ACR50 status was regained and was technically maintained at 52 weeks, but the CRP level had climbed again to 66.8 mg l and the patient was again retreated with two cycles of 500 mg rituximab and 750 mg cyclophosphamide, each under cover of 5 days of 60 mg prednisolone, and regained ACR70 at 17 months. Patient 4 relapsed from 38 weeks and was retreated at 49 weeks with two cycles of 500 mg rituximab and 750 mg cyclophosphamide, each under cover of 5 days of 60 mg prednisolone and achieved ACR70 at 17 months.

Immunology

B-lymphocyte counts fell to undetectable levels in all cases and remained below normal for at least 6 months (Fig. 1). Normal levels were regained in all but patient 1. In patients 2 and 4, the return of B lymphocytes coincided with clinical relapse on all three occasions. Total lymphocyte counts showed no consistent trend (Fig. 2). Levels of RF immunoglobulin (Ig) M fell in all patients (Fig. 3) at varying rates. RAPA titres for patients 1, 3 and 5 fell to 1:40, 1:40, 1:20, with a variably negative or equivocal latex test. RF levels were raised in patients 2 and 4 but not in patients 3 and 5, at the time of B-lymphocyte repopulation (and relapse). ANA titres fluctuated with no clear trend, mostly below a significant level of 1:20. Levels of Igs showed a modest fall, chiefly in the first 10 weeks. Baseline, 6-month and 1-yr levels (g l) were as follows: IgG, 11.4, 8.8 and 9.7 respectively; IgA, 2.4, 1.6, and 2.0; IgM, 1.6, 1.1 and 1.1.

Adverse events

No major adverse events attributable to the therapy were observed. No infusion-related toxicity was observed (in contrast to lymphoma patients). Patient 1 had a lower respiratory tract infection at week 27, which improved rapidly on antibiotic treatment. She had a history of asthma and a similar infection 10 yr before. Patient 2 developed transient thrombocytopenia, down to 110×10^3/mm^3, with minor bruising and also transient acne rosacea. Patient 5 had fever and chest pain on day 19, which settled on antibiotic treatment. She had similar episodes in the past year, with a differential diagnosis of infection or rheumatoid pleuritis.

Discussion

Irrespective of the mechanism by which they were achieved, the results obtained in this study suggest that the protocol used, or a modification thereof, may be of major benefit to subjects with RA. The original primary outcome measure was immunological rather than clinical and proved too stringent. Any statistical analysis of clinical benefit must, therefore, be *post hoc*. Nevertheless, despite small numbers of patients, a useful estimate of benefit can be made without the use of historical controls. A simple binomial analysis indicates that further, similar cases treated in the same way can be expected with 95% confidence to have a minimum chance of 47.8% of achieving ACR50 6 months after B-lymphocyte depletion. If the current status of subjects is taken as the end-point and the option of repeat treatment is included, the same percentage figures can be applied to ACR70 at 18 months. This worst-case estimate still compares well with other therapies [28, 29].

Use of historic controls is problematic, but ACR20/50/70 rates for placebo-treated subjects at 6 months are available from a range of DMARD studies. In a recent trial of etanercept [29], 11, 5 and 1% of 80 placebo-treated subjects achieved ACR20, 50 and 70 respectively at 6 months. Using these figures and a χ^2 analysis, the probability of the results of the present study being due to placebo response is estimated at $P < 0.001$ ($\chi^2 = 47.37$). Conceding a 10% placebo ACR50 rate, which has been observed in some trials, in the same size population the figure is still $P < 0.001$ ($\chi^2 = 22.89$).

Cyclophosphamide and steroid have a significant short-term effect on RA, and exclusion of the possibility

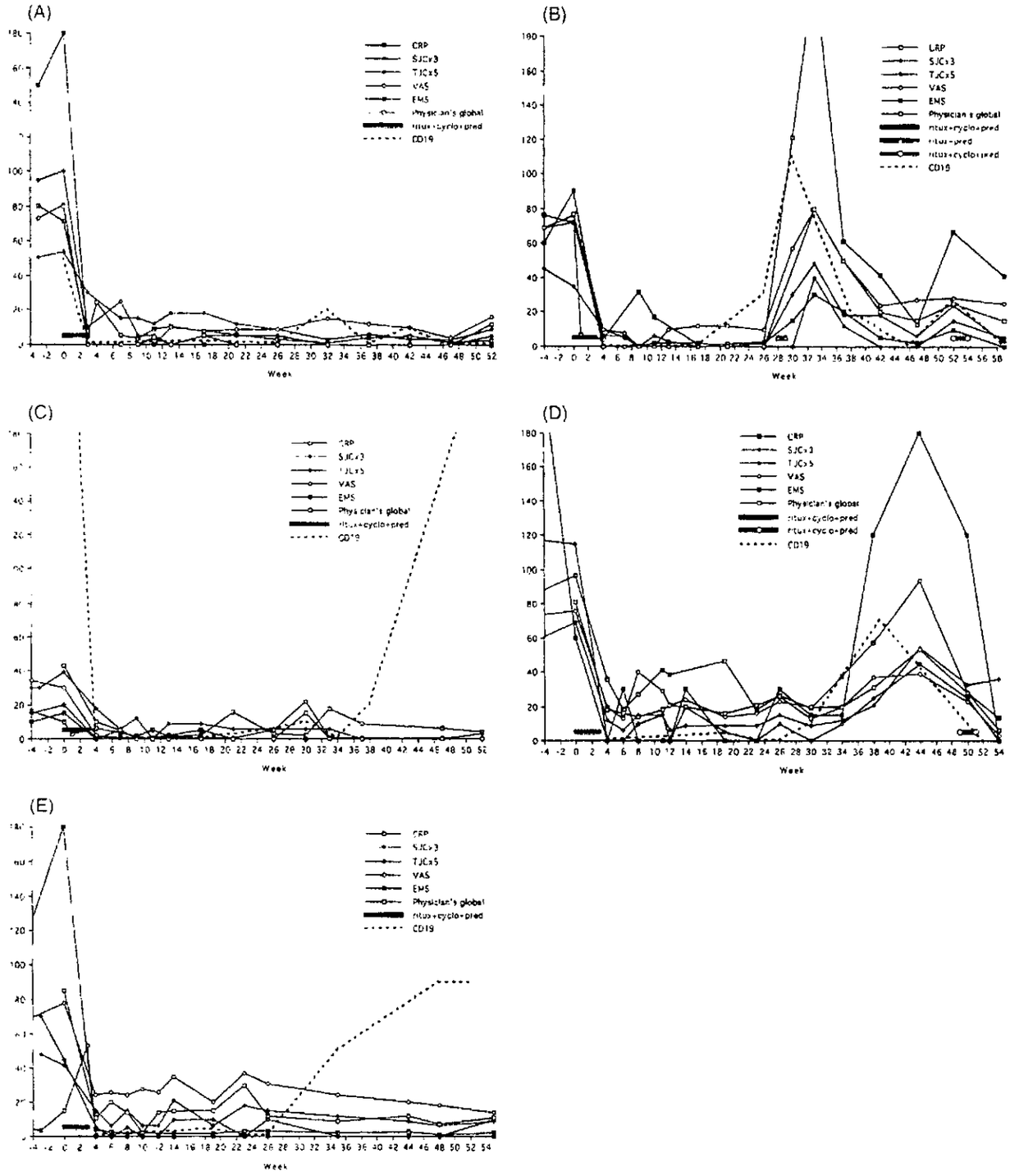

FIG. 1. Panels A–E show clinical and acute-phase indices and $CD19^-$ cell counts (per mm^3) for the first year following B-lymphocyte depletion in patients 1–5, respectively. Scales are adjusted to aid comparability.

that the results were due entirely to these drugs is an important issue. Data on the use of i.v. cyclophosphamide are sparse. However, recent studies confirm the accepted view that benefit 6 months after cyclophosphamide in this dose range is minimal [24–26], only one of eight subjects who received comparable or rather larger doses achieving ACR20 and none ACR50. On this basis, the probability of the present results being due to cyclophosphamide is estimated, by Fisher's exact test, as $P = 0.0008$. Analysis on the basis of any response

TABLE 2. Baseline, 6-month, 12-month and final outcome measures for patients 1–5 with percentage improvement and ACR category

Patient	Time point	Swollen joint count	Tender joint count	CRP (mg/l)	ESR (mm/h)	Pain VAS[a]	Physician's global assessment	Patient's global assessment	HAQ[b]	Early morning stiffness	ACR grade of improvement[c]
1	Baseline	18	20	71.4	65	81	80	82	1.75	1	
	26 weeks	3	1	3	10	9	0	7	0.75	0	
	% improved	83	95	96	85	89	100	91	57	100	70
	52 weeks	3	1	2.6	12	16	12	14	ND	0	
	% improved	83	95	96	82	80	85	83		100	70
	76 weeks	1	0	19.4	16	10	0	9	ND	0	
	% improved	94	100	73	75	88	100	89		100	70
2	Baseline	24	7	72	38	76	77	69	1.875	90	
	26 weeks	0	0	2.8	10	10	0	0	1.25	2	
	% improved	100	100	96	74	87	100	100	33	98	70
	52 weeks	8	3	66.8	29	28	25	28	ND	10	
	% improved	66	57	7	34	63	68	59		89	(50)
	76 weeks	2	0	3	11	20	0	0	ND	5	
	improved	92	100	96	71	74	100	100		94	(70)
3	Baseline	13	4	9.8	36	30	43	34	0.75	15	
	26 weeks	2	0	3.4	23	5	0	7	0.25	0	
	improved	85	100	65	36	83	100	79	66	100	70
	52 weeks	3	0	4.1	15	0	0	0	ND	0	
	% improved	92	100	58	58	100	100	100		100	70
	72 weeks	0	0	5.3	21	7	0	7	ND	0	
	improved	100	100	66	42	77	100	79		100	70
4	Baseline	23	23	96.8	92	76	81	72	2	60	
	26 weeks	5	2	26.7	58	23	26	22	–[d]	30	
	improved	78	91	72	37	70	68	69	–[d]	50	50
	52 weeks	11.5[e]	2.5	20.8	32	13	17	17	ND	60	
	% improved	50	89	79	65	83	79	76		0	(50)
	72 weeks	4	1	10.3	24	23	17	21	ND	30	
	% improved	83	96	89	74	70	79	71		50	(70)
5	Baseline	14	9	14.7	24	78	85	77	1	180	
	26 weeks	5	2	3.1	11	31	12	9	0.375	0	
	improved	64	78	79	54	60	86	88	63	100	50
	55 weeks	3	2	2.1	8	14	11	14	ND	0	
	% improved	79	78	86	67	82	87	82		100	70
	Declined further follow-up										

[a]Visual analogue score.
[b]Health assessment questionnaire
[c]ACR grades in parentheses are after retreatment.
[d]Abandoned because of language difficulty.
[e]Mean of 50- and 54-week data.

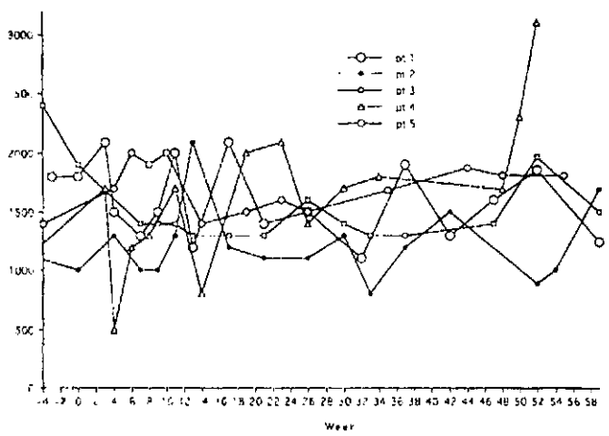

FIG. 2. Total lymphocyte counts (per mm^3) for patients 1–5 following B-lymphocyte depletion.

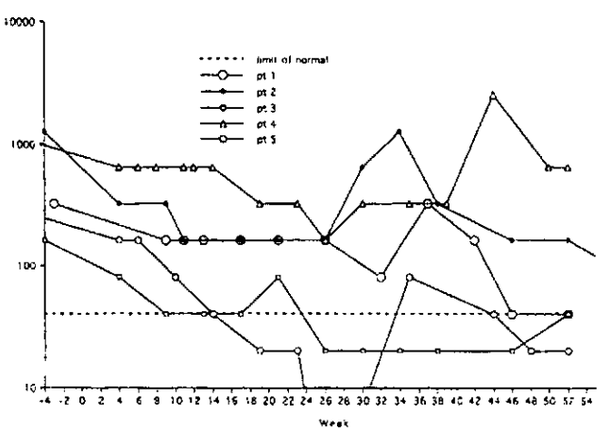

FIG. 3. RF RAPA titres for patients 1–5, following B-lymphocyte depletion.

at 6 months gives $P = 0.0047$. There remains a remote possibility that the present study has identified a protocol for steroid and cyclophosphamide with benefits way beyond all previous experience.

The further possibility, that steroid and cyclophosphamide contributed to the results, at least in part, through actions other than on B lymphocytes, seems very plausible. The original hypothesis did not predict that clinical benefit would follow immediately after B-lymphocyte depletion, but only when IgG RF levels had fallen significantly. The rapid improvement seen can be attributed to an anti-inflammatory 'kick start' from steroid and cyclophosphamide.

Cyclophosphamide and steroid may also contribute to B-lymphocyte death by either encouraging apoptosis or direct cytolysis. Rituximab monotherapy induces sustained remission in lymphoma less often than combination therapy. B lymphocytes in certain environments may be protected from biological killing by complement-inhibitory proteins, but may succumb if also exposed to cyclophosphamide.

Accepting all these possibilities, there remains a strong indication that B-lymphocyte depletion was a necessary component of the therapeutic action. Apart from the superior response to that reported with cyclophosphamide alone [24–26], when relapse occurred, on three occasions in two patients, it was in each case coincident with B-lymphocyte repopulation.

Although none of the patients fulfilled all predefined criteria at 6 months, the level and persistence of improvement in patients 1, 3 and 5 suggests that the immunological process had genuinely become inactive, at least at a point significantly upstream in the pathogenic pathway. The resistance of improvement to B-lymphocyte repopulation in patients 3 and 5 suggests that some sort of cycle can be broken if the conditions are favourable, as originally postulated. However, at least two possible forms of cycle need to be considered.

In the original hypothesis [12], disease perpetuation was ascribed to IgG RF-committed B-cell clones. It was argued that clones of this type should not return in the short term, following adequate depletion, unless continued IgG RF production provides sufficient stimulus to low-affinity RF clones to affinity-mature and or class-switch to replace the original clones. The relapse of the two subjects with persistently high IgM RF levels would fit with this, but the evidence is so far circumstantial. Moreover, IgM RF may be a poor surrogate for IgG RF. Existing IgG RF assays were considered to be unreliable for use on unfractionated serum.

The results are also consistent with the alternative possibility that RA is driven by autoreactive T lymphocytes, which depend on antigen presentation by B lymphocytes for maintaining their activation. This would imply a failure of T-cell tolerance, which ceases to generate disease, either temporarily or in the long term, following interruption of T–B lymphocyte interaction. It would accommodate interactions with RF-specific B lymphocytes, as presenters of complexed antigen, but with RF secretion being epiphenomenal.

Acknowledgements

The authors acknowledge financial support from the UCLH Directorate of Trauma, Orthopaedics and Rheumatology and Hoffman la Roche (grant in aid). We thank Dr N. Coppard, Professor D. Linch and Dr S. Devereux for discussions on protocol design, Dr R. Stevens and Professor K. MacRae for data interpretation and statistical analysis, and Dr M. Leandro for data processing.

References

1. Janossy G, Duke O, Poulter LW, Panayi G, Bofill M, Goldstein G. Rheumatoid arthritis: a disease of T lymphocyte-macrophage immunoregulation. Lancet 1981; ii:839–42.
2. Burmester GR, Stuhlmuller B, Keyszer G, Kinne RW. Mononuclear phagocytes and rheumatoid synovitis. Mastermind or workhorse in arthritis. Arthritis Rheum 1997;40:5–18.
3. Muller-Ladner U, Kriegsmann J, Gay RE, Gay S. Oncogenes in rheumatoid arthritis. Rheum Dis Clin North Am 1995;21:675–90.
4. Mannik M, Nardella FA. IgG rheumatoid factors and self association of these antibodies. Clin Rheum Dis 1985; 11:551–72.
5. Edwards JCW. Is rheumatoid factor relevant. In: Bird H, Snaith ML, eds. Rheumatoid arthritis, questions and uncertainties. Chapter 1. Oxford: Blackwell Scientific, 1999:3–23.
6. Gardner DL. Rheumatoid arthritis: cell and tissue pathology. In: Gardner DL, ed. Pathological basis of connective tissue diseases. London: Edward Arnold, 1992:444–526.
7. Thompson KM, Randen I, Borretzen M, Forre O, Natvig JB. Variable region gene usage of human monoclonal rheumatoid factors derived from healthy donors following immunization. Eur J Immunol 1994;24:165–7.
8. Kyburz D, Corr M, Brinson DC, Von-Damm A, Tighe H, Carson DA. Human rheumatoid factor production is dependent on CD40 signaling and autoantigen. J Immunol 1999;163:3116–22.
9. Sutton B, Corper A, Bonagura V, Taussig M. The structure and origin of rheumatoid factors. Immunol Today 2000; 21:177–82.
10. Roosnek E, Lanzavecchia A. Efficient and selective presentation of antigen–antibody complexes by rheumatoid factor B cells. J Exp Med 1991;173:487–9.
11. Edwards JCW, Cambridge G. Rheumatoid arthritis: the predictable effect of small immune complexes in which antibody is also antigen. Br J Rheumatol 1998;37:126–30.
12. Edwards JCW, Cambridge G, Abrahams VM. Do self-perpetuating B lymphocytes drive human autoimmune disease. Immunology 1999;97:1868–96.
13. Fearon DT, Carter RH. The CD19 CR2.TAPA-1 complex of B lymphocytes: linking natural to acquired immunity. Annu Rev Immunol 1995;13:127–49.
14. Winchester RJ, Agnello V, Kunkel HG. Gamma globulin complexes in synovial fluids of patients with rheumatoid arthritis. Clin Exp Immunol 1970;6:689–706.
15. Brown PB, Nardella FA, Mannik M. Human complement activation by self-associated IgG rheumatoid factors. Arthritis Rheum 1982;25:1101–7.

16. Edwards JCW, Sutton BJ, Grimmett J, Abrahams VM. Rheumatoid factor stereochemistry analysed by real space modelling [abstract]. Arthritis Rheum 1998;42(Suppl.):S84.
17. Abrahams VM, Cambridge G, Edwards JCW. Induction of tumour necrosis factor alpha production by human monocytes: a key role for Fc;RIIIa in rheumatoid arthritis. Arthritis Rheum 2000;43:608–16.
18. Bhatia A, Blades S, Cambridge G, Edwards JCW. Differential distribution of FcgRIIIa in normal human tissues. Immunology 1998;94:56–63.
19. Edwards JCW, Leigh RD, Cambridge G. Expression of molecules involved in B lymphocyte survival and differentiation by synovial fibroblasts. Clin Exp Immunol 1997;108:407–14.
20. Dechanet J, Merville P, Durand I, Banchereau J, Miossec P. The ability of synoviocytes to support terminal differentiation of activated B cells may explain plasma cell accumulation in rheumatoid synovium. J Clin Invest 1995;95:456–63.
21. Maloney DG, Grillo-Lopez AJ, Bodkin DJ. IDEC-C2B8: results of a phase I multiple-dose trial in patients with relapsed non-Hodgkin's lymphoma. J Clin Oncol 1997; 15:3266–74.
22. McLaughlin P, Grillo-Lopez AJ, Link BK *et al.* Rituximab chimeric anti-CD20 monoclonal antibody therapy for relapsed indolent lymphoma: half of patients respond to a four-dose treatment program. J Clin Oncol 1998;16:2825–33.
23. Isaacs JD, Watts RA, Hazleman BL *et al.* Humanised monoclonal antibody therapy for rheumatoid arthritis. Lancet 1992;340:748–52.
24. Burt RK, Georganas C, Schroeder J *et al.* Autologous hematopoietic stem cell transplantation in refractory rheumatoid arthritis. Arthritis Rheum 1999;42:2281–5.
25. Snowden JA, Biggs JC, Milliken ST, Fuller A, Brooks PM. A phase I/II dose escalation study of intensified cyclophosphamide and autologous blood stem cell rescue in severe, active rheumatoid arthritis. Arthritis Rheum 1999; 42:2286–92.
26. Breban M, Dougados M, Picard F *et al.* Intensified-dose cyclophosphamide and granulocyte-stimulating factor administration for haematopoietic stem cell mobilization in refractory rheumatoid arthritis. Arthritis Rheum 1999; 42:2275–80.
27. Felson DT, Anderson JJ, Boers M *et al.* American College of Rheumatology preliminary definition of improvement in rheumatoid arthritis. Arthritis Rheum 1995; 38:727–35.
28. Maini RN, Breedveld FC, Kalden JR *et al.* 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 1998;41:1552–63.
29. Moreland LW, Schiff MH, Baumgartner MD *et al.* Etanercept therapy in therapy for rheumatoid arthritis. Ann Intern Med 1999;130:478–86.

Scand J Rheumatol 2004;33:82–86

Improvement of refractory rheumatoid arthritis after depletion of B cells

C Kneitz, M Wilhelm, H-P Tony

Medizinische Poliklinik der Universitat Würzburg, Germany

Objective: B cells are involved in the pathogenesis of rheumatoid arthritis (RA). To evaluate the effect of therapeutic B-cell depletion for treatment of RA, an open label study has been performed using the B-cell depleting anti-CD20 antibody rituximab.

Methods: Five patients with refractory RA were treated weekly with four infusions of rituximab (375 mg/m^2) alone, or in combination with ongoing methotrexate (MTX). Patients were followed for at least 44 weeks and monitored for safety and tolerability of treatment.

Results: Treatment could be performed without serious side effects and resulted in peripheral B-cell depletion lasting between 36 weeks up to > 1 year. The follow-up revealed no significant treatment-associated side effects. At 22 weeks, 4/5 patients showed a significant improvement (> 1.2) of the Disease Activity Score (DAS28). The mean DAS28 of all patients declined from 6.2 to 4.1. At 44 weeks there was one drop-out, another patient still had a sustained response, and three patients showed slowly increasing disease activity (mean DAS28 of the remaining four patients: Week 0: 6.0: Week 22: 3.85: Week 44: 5.6). Despite relatively constant immunoglobulin levels, rheumatoid factor levels decreased parallel to disease activity.

Conclusion: In patients with refractory RA, B-cell depletion with rituximab is safe and well tolerated. A reduction of disease activity could be observed, which eventually deteriorated after B-cell repopulation. The findings give more evidence for B-cell targeted therapies in RA.

Rheumatoid arthritis (RA) is a chronic inflammatory disease, characterized by joint pain and swelling, pannus formation, and joint destruction. Pannus is characterized by hyperplastic synoviocytes and inflammatory cells that infiltrate the synovial membrane. Whereas the role of T cells in the immune response in RA has been investigated in detail, the role of B lymphocytes is not well defined (1, 2). Recent data suggest that intrinsic B-cell hyperreactivity may be an essential feature of the disease (3). B cells can undergo antigen-dependent differentiation in chronic synovitis (4). Furthermore, elimination of B cells by anti-CD20 antibodies disrupts T-cell activation and production of pro-inflammatory cytokines in a human synovium severe combined immunodeficiency (SCID)-mouse model (5). Depletion of B cells may therefore prove to be a reasonable immunoregulatory therapy.

Rituximab is a genetically engineered chimeric human/murine anti-CD20 monoclonal antibody, which provides effective B-cell depletion (6). The antibody is an IgG1 kappa immunoglobulin containing murine light- and heavy-chain variable region and human constant region sequences (6). The Fab domain (murine) of rituximab binds to the CD20 antigen on B cells, and the Fc domain (human) recruits immune effector functions to mediate B-cell lysis. Possible mechanisms of cell lysis include complement activation, antibody dependent cellular cytotoxicity, and induction of apoptosis (7, 8).

The use of rituximab is established for the therapy of B-cell lymphomas, with few side effects in patients with low tumour burden (7). Recent studies indicate limited but useful effects of rituximab therapy in patients with idiopathic thrombocytopenic purpura (ITP) (8), different types of vasculitis (9, 10) and systemic lupus erythematosus (SLE) (11, 12).

In 2001, Edwards published results from the first five RA patients treated with a combination of cyclophosphamide and rituximab, who showed significant improvement lasting > 1 year (13). Very recently, more positive data have become available using similar treatment protocols (14, 15). Here we provide data from an additional five RA patients refractory to conventional therapy, including tumor necrosis factor-α (TNF-α)-blockers, who showed a favourable response to rituximab therapy.

Christian Kneitz, Medizinische Poliklinik der Universität Würzburg, Klinikstrasse 6–8, 97070 Würzburg, Germany.
E-mail: c.kneitz@medizin.uni-wuerzburg.de

Received 24 April 2003
Accepted 20 October 2003

DOI: 10.1080/03009740310004379

Patients and methods

Patients met the American College of Rheumatology (ACR) criteria for RA (Table 1). They had been treated unsuccessfully with at least three disease-modifying antirheumatic drug (DMARD) regimen, including methotrexate (MTX). Patients 1, 3, 4, and 5 had also undergone failed TNF-α blocker treatment. Treatment with low-dose corticosteroids, or non-steroidal anti-inflammatory drugs (NSAIDs) was allowed during the study. Dosage of corticosteroids during the study period is indicated in Table 1. All patients gave informed consent before entering the study.

Treatment regimen

The protocol was approved by the ethics committee of the University of Würzburg, Germany. Rituximab (Mabthera, Hoffmann La-Roche, Grenzach-Whylen, Germany) at a dose of 375 mg/m^2 was administered intravenously once a week for a total of four infusions (on Days 1, 8, 15, and 22). The drug was reconstituted in normal saline to a concentration of 1–4 mg/mL. The initial infusion rate was 50 mg/h, with subsequent increase if no toxicity was seen. Premedication with 50 mg ranitidin and 0.67 mg clemastin was given to all patients. Blood pressure, heart rate, and vital signs were monitored during the infusion. Pre-existing therapy with MTX was continued at the same dosage if a partial response to MTX had been observed. Other DMARDs were stopped at least for 2 weeks before entering the protocol. Glucocorticoids up to 15 mg prednisolone were allowed at the beginning of the study.

The analysis of lymphocyte subsets by immunofluorescence staining was performed by incubating peripheral blood mononuclear cells (PBMC) with anti-CD19 and anti-CD3 antibodies [phycoerythrin (PE) or fluoroscein isothiocyanate (FITC)-labeled as indicated: all antibodies from Becton-Dickinson, Heidelberg, Germany]. Flow cytometric analysis was performed using a FACSCalibur (Becton Dickinson, San Jose, CA, USA). Frequencies of cell populations were calculated using CellQuest software. The absolute B-cell number was calculated per microlitre of blood, based on the frequencies of those cells among lymphocytes.

The primary efficacy end-point was improvement in the Disease Activity Score (DAS) 28 ≥ 1.2 at Week 22. Secondary outcome measures included swollen and tender joint counts, patient global assessment, erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) levels. Monitoring for adverse events was performed during the treatment period and at least every month during follow-up.

Results

Side effects

All patients completed the treatment schedule, which was well tolerated. No serious treatment-related side effects were observed during the whole study, in particular no severe infections. None of the patients developed antibodies against nuclear antigens.

Clinical response to therapy

All patients reached the primary efficacy point (Week 22) with a significant reduction in their DAS28 score, which was accompanied by a parallel fall in serum CRP levels (Table 1). Prednisolone could be tapered to 5 mg per day or less, except for

Table 1. Patient characteristics and outcome measures for Patients 1–5 at study entry and in the course of time after 22, 44, and 74 (one case) weeks.

Patient	Age (years)/ gender	Duration of RA (years)	MTX/ week (mg)	Time point	Prednisolone (mg/day)	SJC	Tender joint count	CRP (mg/dL)	ESR (mm/h)	Early morning stiffness	Pain VAS (query) (mm)	DAS28
1	63/m	9	25	Baseline	15	16	12	2.91	41	30	33	6.1
				22 weeks	12.5	7	3	1.32	19	0	25	4.1
				44 weeks	12.5	11	11	5.97	47	90	46	6.1
2	40/f	7	15	Baseline	5	20	22	1	23	0	50	6.8
				22 weeks	5	7	6	0.61	19	0	67	5.1
3	47/m	10	-	Baseline	5	6	2	5.23	35	60	35	4.5
				22 weeks	5	5	0	0.52	9	0	8	2.3
				44 weeks	5	6	0	0.89	8	n.d.	11	2.3
				74 weeks	5	11	5	3.6	21	120	5	4.4
4	61/f	13	-	Baseline	15	16	22	10.4	n.d. (57 Week 1)	180	83	7.7
				22 weeks	2.5	10	1	1.95	36	0	18	4.2
				44 weeks	2.5	19	22	5.95	56	210	53	7.4
5	62/f	30	20	Baseline	10	10	12	6.19	35	30	33	5.8
				22 weeks	2.5	12	5	1.08	28	15	19	4.8
				44 weeks	2.5	19	20	5.43	46	30	25	6.7

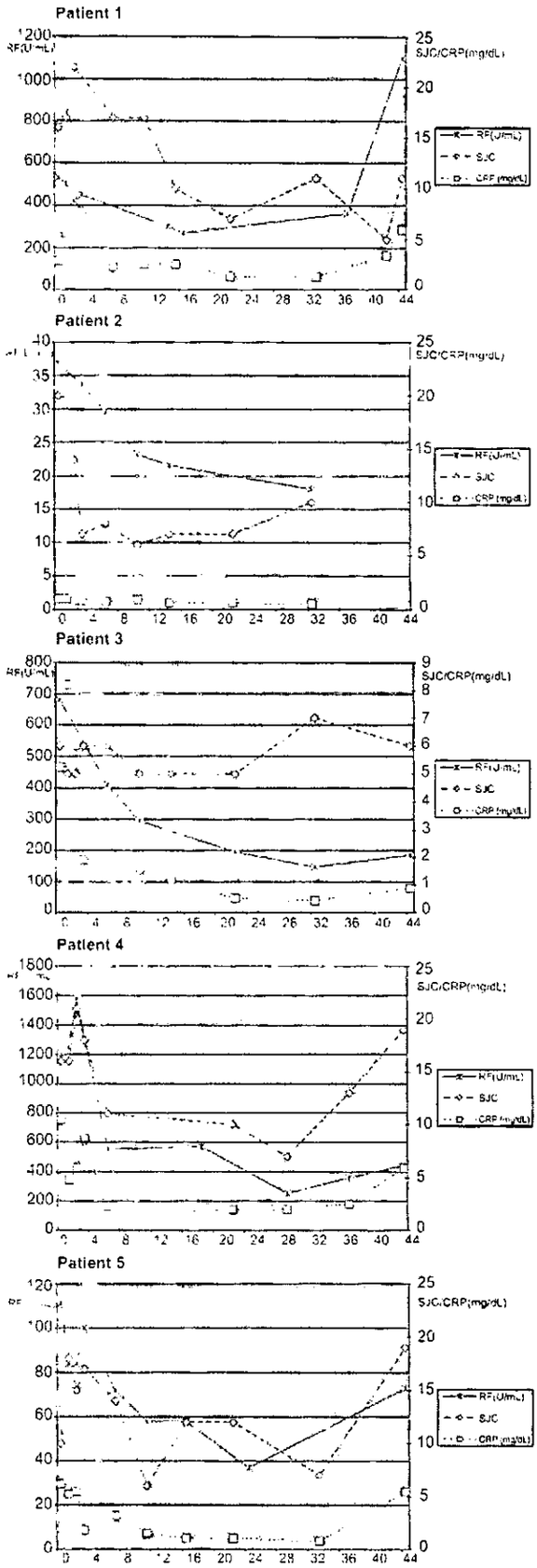

Figure 1. Time-course of SJC, CRP (mg/dL) and RF (U/mL) for each patient (Patients 1–5) during the study.

Patient 1. A decline of DAS28 ≥ 1 was observed in all patients, in 4/5 patients the DAS28 declined ≥ 1.7 (Table 1). The group of patients started with a mean DAS28 score of 6.2, which declined by Week 22 to 4.1. Improvement in different clinical parameters could be detected as early as 4 weeks after beginning the treatment. The maximal response was usually reached at 12 weeks, and persisted for up to 32 weeks. Figure 1 shows the time-course for each patient of swollen joint count (SJC), rheumatoid factor (RF) and CRP. Patients 1, 3, 4, and 5 started with elevated CRP levels, which significantly decreased after 4 weeks in the study. There was a parallel fall in RF levels. The decline in swollen joint counts (SJC) was variable. Only two patients (Patients 1 and 2) had a 50% reduction in SJC. Patient 3 showed no change in number of swollen joints, but showed a good reduction in serological parameters and pain assessment. Patient 2 left the study in Week 32, requesting additional therapy despite a measurable decline in the DAS28 (Table 1).

Patients 1, 4 and 5 after Week 32 experienced a gradual rise in disease activity (mean DAS28 of the remaining four patients: Week 0: 6.0; Week 22: 3.85; Week 44: 5.6). Patient 3 for more than 44 weeks showed a stable good response, receiving only 5 mg prednisolone/day. Fifty weeks after treatment he relapsed, with active disease at Week 74.

Immunobiological parameters

Serum immunoglobulin and autoantibodies. IgG and IgA levels remained stable (data not shown), with a falling trend of serum IgM (Figure 2A). However, RF activity declined significantly and there was a parallel increase in disease activity at Week 32 (Figure 2A). Surprisingly, Patients 3 and 4, who were not treated with MTX, showed a more pronounced and lasting decrease of RF levels (Figure 1). We observed no significant changes in specific antibodies directed against measles, mumps, and rubella virus, and diphtheria or tetanus toxoid.

B lymphocytes. Peripheral blood B-cell counts declined rapidly during treatment (Figure 2B). B-cell recovery started between 16 and 22 weeks after the end of anti-CD20 therapy. In Patients 3 and 4, who were not treated with MTX during the study period, a more rapid reconstitution of peripheral B cells was observed. In 3/4 patients observed for at least 44 weeks, the increase of peripheral B cells coincided with the clinical relapse.

Discussion

Enhanced B-cell function is increasingly appreciated as an important pathogenic event in such autoimmune diseases as RA or SLE (16). This open study adds more evidence for B-cell depletion as a treatment

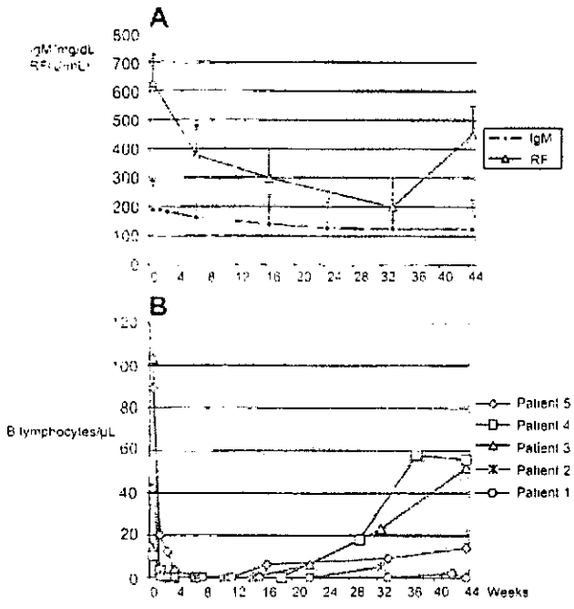

Figure 2. (A) Rf (U/mL) and IgM (mg/dL) levels of all patients finishing the study (Patients 1, and 3–5) indicated as mean values. (B) B-cell counts (cells/µL) for Patients 1–5 following B-cell depletion.

option for RA. Rituximab was administered as 375 mg/m^2 monoclonal antibody infused once a week for four consecutive weeks. The treatment was well tolerated. Over the study period of 44 weeks we did not observe any protracted toxicity attributable to B-cell depletion. In addition, we did not see a significant fall in polyclonal immunoglobulin levels (Figure 2A). These findings are in line with the presence of long-lived CD20-negative plasma cells, which are not targeted by anti-CD20 treatment. In addition, our previous experience with a rituximab-treated SLE patient, who was boosted with tetanus toxoid 2 months after the end of rituximab therapy, indicates that a secondary antibody response can still be elicited, despite the depletion of peripheral B cells (17).

After therapy, a fast decrease of peripheral B-cell numbers was observed (Figure 2B). We have previously shown, in single patients using the same protocol, that B-cell depletion extends to the spleen and bone marrow (17). B cells repopulated the peripheral blood between 4 and 5 months after treatment. The substantial individual time difference observed could be related to ongoing MTX treatment, since these patients had a prolonged B-cell regeneration phase (Figure 2B, Patients 1, 2, and 5).

Evaluation of our open study shows a remarkable reduction in disease activity (DAS28), in single patients as well as in the total patient group. In particular, Patients 3 and 4, who had longstanding active disease refractory to a variety of regimens — including TNF-α blockade — experienced >6 months of very low disease activity after anti-CD20 treatment, which required only additional low-dose corticosteroid therapy (Table 1). Nevertheless, not all efficacy variables improved to the same extent. Serological parameters, like CRP, RF and ESR, decreased quite regularly, whereas clinical parameters, like SJC, showed a reduction of >50% in only two patients. These limited observations may relate to the pathological mechanisms targeted by rituximab treatment.

B-cell targeted therapy regimens were first introduced in 2001, when Edwards published the first results of combining treatment with cyclophosphamide and rituximab (13). Very recently, they extended this approach and reported on 22 single cases using different dosing protocols (14). In our study, we used rituximab as a single agent in a dose at least twice as high, either in addition to ongoing MTX or with no DMARD at all. Application of very high amounts of glucocorticoids for the first 3 weeks might explain the somewhat faster onset of response in the previous studies (13, 14). Our data are very much in line with the recent study by de Vita, who used equivalent rituximab amounts and reported comparable results in five patients (15). In the absence, so far, of multicentre studies, the similar results from these three independent studies favour the significance of each of these limited reports.

Interestingly, we observed a quite rapid fall in RF, followed by a more delayed clinical benefit, reaching a maximum at about 3 months (Figure 1). In addition, RF levels increased again in parallel with disease activity after 6–9 month. Also, the repopulation of B cells seems to be connected to relapse of disease, even though peripheral blood may not ideally reflect B cell repopulation in central immunologic organs or synovial tissue.

In summary, our results provide additional evidence for the concept of targeting B cells as an option for RA treatment. B-cell depletion by rituximab is safe, and results in a significant response in a group of patients who have a very unfavourable prognosis because of their resistance to a variety of treatment regimens, including TNF-α blockade.

Acknowledgements

We acknowledge Hoffmann La-Roche for supplying rituximab. This work was supported by the IZKF of the University of Würzburg (BMBF 01KS 9603).

References

1. Weyand CM. New insights in the pathogenesis of rheumatoid arthritis. Rheumatology 2000;39:3–8.
2. Hovdenes J. B-cell growth-promoting activity in supernatants from CD4$^+$ cells from synovial fluid and peripheral blood of patients with rheumatoid arthritis and other inflammatory arthritides. Scand J Rheumatol 1989;18:385–92.

3. Mellbye OJ, Vartdal F, Pahle J, Mollnes TE. IgG and IgA subclass distribution of total immunoglobulin and rheumatoid factors in rheumatoid tissue plasma cells. Scand J Rheumatol 1990;19:333–40.
4. Shiokawa S, Matsumato N, Nishimura J. Clonal analysis of B cells in the synovial membrane of patients with rheumatoid arthritis. Scand J Rheumatol 2003;32:12–8.
5. Takemura S, Klimiuk PA, Braun A, Goronzy JJ, Weyand CM. T cell activation in rheumatoid synovium is B cell dependent. J Immunol 2001;167:4710–18.
6. Reff ME, Carner K, Chambers KS, Chinn PC, Leonard JE, Raab R, et al. Depletion of B cells in vivo by a chimeric mouse human monoclonal antibody to CD20. Blood 1994;83:435–45.
7. Sacchi S, Federico GM, Dastoli C, Fiorani G, Vinci V, Casolari B. Treatment of B-cell non-Hodgkin's lymphoma with anti CD20 monoclonal antibody Rituximab. Crit Rev Oncol Hematol 2001;37:13–25.
8. Stasi R, Pagano A, Stipa E, Amadori S. Rituximab chimeric anti-CD20 monoclonal antibody treatment for adults with chronic idiopathic thrombocytopenic purpura. Blood 2001;98:952–7.
9. Specks U, Fervenza CF, McDonald TJ, Hogan MCE. Response of Wegener's granulomatosis to anti-CD20 chimeric monoclonal antibody therapy. Arthritis Rheum 2001;44: 2836–40.
10. Arzoo K, Sadeghi S, Liebman HA. Treatment of refractory antibody mediated autoimmune disorders with an anti-CD20 monoclonal anbibody (rituximab). Ann Rheum Dis 2002: 62:922–4.
11. Petschner F, Walker UA, Schmitt-Graff A, Uhl M, Peter HH. Catastrophic systemic lupus erythematosus with Rosai–Dorfman sinus histiocytosis. Successful treatment with anti-CD20/rituximab. Dtsch Med Wochenschr 2001;126: 998–1001.
12. Perrotta S, Locatelli F, La Manna A, Cennamo L, De Stefano P, Nobili B. Anti-CD20 monoclonal antibody (Rituximab) for life-threatening autoimmune haemolytic anemia in a patient with systemic lupus erythematosus. Br J Haematol 2002;116: 465–7.
13. Edwards JCW, Cambridge G. Sustained improvement in rheumatoid arthritis following a protocol designed to deplete B lymphocytes. Rheumatology 2001;40:205–11.
14. Leandro MJ, Edwards JCW, Cambridge G. Clinical outcome in 22 patients with rheumatoid arthritis treated with B lymphocyte depletion. Ann Rheum Dis 2002;61:883–8.
15. De Vita S, Zaja F, Sacco S, De Candia A, Fanin R, Ferraccioli G. Efficacy of selective B cell blockade in the treatment of rheumatoid arthritis. Arthritis Rheum 2002: 46:2029–33.
16. Lipsky PE. Systemic lupus erythematosus: an autoimmune disease of B cell hyperactivity. Nat Immunol 2001;9: 764–6.
17. Kneitz C, Wilhelm M, Tony HP. Effective B-cell depletion with rituximab in the treatment of autoimmune diseases. Immunobiology 2002;206:519–27.

Genentech

martes, abr 5, 2005

Phase III Study Shows Rituxan Significantly Improves Symptoms in Patients with Rheumatoid Arthritis Who Inadequately Responded to Anti-TNF Therapies

- Third Randomized Trial to Evaluate Efficacy and Safety of Rituxan in RA --

South San Francisco, Calif., Cambridge, Mass. and Basel, Switzerland -- 5 de abril de 2005 - - Genentech, Inc. (NYSE: DNA), Biogen Idec (Nasdaq: BIIB) and Roche (SWX Zurich) announced today that a Phase III clinical study of Rituxan® (Rituximab) met its primary endpoint of a greater proportion of Rituxan-treated patients achieving an American College of Rheumatology (ACR) 20 response at week 24, compared to placebo. The study included patients with active rheumatoid arthritis (RA) who have had an inadequate response or were intolerant to prior treatment with one or more anti-TNF therapies.

In this study, known as REFLEX (Randomized Evaluation of Long-term Efficacy of Rituximab in RA), patients who received a single treatment course of two infusions of Rituxan with a stable dose of methotrexate (MTX) displayed a statistically significant improvement in symptoms compared to patients who received placebo and MTX. Further analyses of the data are ongoing and will be submitted for presentation at an upcoming medical meeting.

A preliminary analysis of the data did not reveal any unexpected safety signals. The most common side effects in the Rituxan arm included headache, upper respiratory tract infection and nasopharyngitis. The reported rate of serious adverse events was comparable across the two treatment arms.

"These results continue to support the potential of Rituxan as a new therapeutic option for RA," said Burt Adelman, M.D., executive vice president, development, Biogen Idec. "We look forward to sharing the REFLEX data in our discussions with the FDA."

"These are the first Phase III Rituxan data to demonstrate clinical improvement in this difficult-to-treat RA patient population. The findings add to the growing body of evidence that selectively targeting B cells may provide an important new treatment approach for this debilitating disease," said Hal Barron, M.D., Genentech's senior vice president, development and chief medical officer. "While we are encouraged that the preliminary safety results are similar to previous studies, we recognize the importance of monitoring long-term safety in RA patients treated with Rituxan."

These new Phase III data follow recent positive preliminary findings from a Phase IIb study that evaluated the efficacy and safety of Rituxan in moderate-to-severe RA patients who failed prior treatment with at least one disease-modifying anti-rheumatic drug (DMARD).

About the Study
A total of 520 patients from the United States, Canada and Europe were randomized to receive either Rituxan (1000 mg i.v. on days one and 15) or placebo in this multi-center, double-blind, placebo-controlled study. All patients received a stable dose of MTX and a two-week course of corticosteroids.

ACR 20 indicates a 20 percent improvement in the number of swollen and tender joints, as well as a 20 percent improvement compared with baseline in three of five disease-activity measures: patient assessment, physician assessment, pain scale, Health Assessment Questionnaire and the value for one acute phase reactant (erythrocyte sedimentation rate or C-reactive protein).

About RA
RA is a debilitating autoimmune disease that affects more than two million Americans1 and hinders the daily activities of sufferers. RA occurs when the immune system inappropriately attacks joint tissue, causing chronic inflammation and irreversible destruction of cartilage, tendons and bones, often resulting in disability. While RA has traditionally been considered a T-cell mediated disease, emerging research suggests that other immune cells called B cells may play multiple roles in the pathophysiology of RA including autoantibody production, T-cell activation and cytokine production. Common RA symptoms include inflammation of the joints, swelling, fatigue, stiffness and pain. Additionally, since RA is a systemic disease, it can have effects in other tissues such as the lungs, eyes and bone marrow.

About Rituxan
Rituxan is a therapeutic antibody that targets and selectively depletes peripheral CD20 positive B cells without targeting stem cells or existing plasma cells. B cells may play multiple roles in the pathophysiology of RA. Rituxan is also being investigated in other autoimmune diseases including

lupus, multiple sclerosis and ANCA associated vasculitis.

Rituxan received initial FDA approval in November 1997 for the treatment of relapsed or refractory low-grade or follicular, CD20 positive, B cell non-Hodgkin's lymphoma (NHL). It also was approved in the European Union (EU) under the trade name MabThera® in June 1998. Genentech and Biogen Idec co-market Rituxan in the United States and Roche markets MabThera in the rest of the world, except Japan, where Rituxan is co-marketed with Zenyaku Kogyo Co. Ltd. Rituxan has been used to treat more than 380,000 patients worldwide. For a copy of the Rituxan full prescribing information, including Boxed Warning, please call 1-800-821-8590 or visit http://www.gene.com.

Rituxan Safety Profile in NHL
In NHL patients, the majority of patients experience infusion-related symptoms with their first Rituxan infusion. These symptoms include but are not limited to: flu-like fever, chills/rigors, nausea, urticaria, headache, bronchospasm, angioedema and hypotension. These symptoms vary in severity and generally are reversible with medical intervention. In rare instances, severe and fatal infusion-related reactions have occurred, nearly all of which have been associated with the first Rituxan infusion. These events appear as manifestations of an infusion-related complex and include hypoxia, pulmonary infiltrates, acute respiratory distress syndrome, myocardial infarction, ventricular fibrillation, cardiogenic shock and tumor lysis syndrome. Patients who develop clinically significant infusion-related cardiopulmonary events should have their Rituxan infusion discontinued and receive medical treatment.

In rare instances, severe mucocutaneous skin reactions have occurred that may be associated with Rituxan therapy. Many of these reactions have been described as paraneoplastic pemphigus and are known to be associated with various B cell lymphomas, particularly NHL and chronic lymphocytic leukemia. Patients who develop a severe mucocutaneous skin reaction should have Rituxan discontinued and receive appropriate medical treatment, including a skin biopsy to guide therapy.

About Genentech
Genentech is a leading biotechnology company that discovers, develops, manufactures and commercializes biotherapeutics for significant unmet medical needs. A considerable number of the currently approved biotechnology products originated from or are based on Genentech science. Genentech manufactures and commercializes multiple biotechnology products directly in the United States and licenses several additional products to other companies. The company has headquarters in South San Francisco, California and is traded on the New York Stock Exchange under the symbol DNA. For additional information about the company, please visit http://www.gene.com.

About Biogen Idec
Biogen Idec creates new standards of care in oncology and immunology. As a global leader in the development, manufacturing and commercialization of novel therapies, Biogen Idec transforms scientific discoveries into advances in human healthcare. For product labeling, press releases and additional information about the company, please visit www.biogenidec.com.

About Roche
Headquartered in Basel, Switzerland, Roche is one of the world's leading research-focused healthcare groups in the fields of pharmaceuticals and diagnostics. As a supplier of innovative products and services for the early detection, prevention, diagnosis and treatment of disease, the Group contributes on a broad range of fronts to improving people's health and quality of life. Roche is a world leader in diagnostics, the leading supplier of medicines for cancer and transplantation and a market leader in virology. In 2004 sales by the Pharmaceuticals Division totaled 21.7 billion Swiss francs, while the Diagnostics Division posted sales of 7.8 billion Swiss francs. Roche employs roughly 65,000 people in 150 countries and has R&D agreements and strategic alliances with numerous partners, including majority ownership interests in Genentech and Chugai.

(1) American College of Rheumatology, 2005

ARTHRITIS & RHEUMATISM
Vol. 46, No. 8, August 2002, pp 2029–2033
DOI 10.1002/art.10467

Efficacy of Selective B Cell Blockade in the Treatment of Rheumatoid Arthritis

Evidence for a Pathogenetic Role of B Cells

Salvatore De Vita, Francesco Zaja, Stefania Sacco, Alessandro De Candia, Renato Fanin, and Gianfranco Ferraccioli

Objective. **The pathogenetic role of B cells in rheumatoid arthritis (RA) is under debate, but it is currently believed to be marginal. The availability of selective anti–B cell treatment provides a unique opportunity to clarify this issue. This study was undertaken to investigate the effects of B cell blockade in the treatment of refractory RA, and to evaluate the implications with regard to the role of B cells in the disease.**

Methods. **Five female patients with active, evolving erosive RA were treated with rituximab, an anti-CD20 chimeric monoclonal antibody. All 5 patients had been nonresponders to combination therapy with methotrexate plus cyclosporin A. Two of the 5 had also failed to respond to anti–tumor necrosis factor α therapy. All of these treatments were discontinued 1 month before institution of anti-CD20 therapy.**

Results. **Marked clinical improvement was observed in 2 patients (American College of Rheumatology 70% response [ACR70] and ACR50, respectively), starting at the end of the second month after institution of anti-CD20 therapy (month 2) and lasting until month 10 in 1 patient (articular relapse) and month 12 in the other (last followup). ACR20 response was observed in 2 additional patients, lasting until month 5 and month 7, respectively (articular relapse in both). Decrease or normalization of serum C-reactive protein and rheumatoid factor levels were observed in these patients. In contrast, patient 3 had no response to the treatment. RA synovitis and evolving erosive damage were decreased in patients exhibiting a major response, as demonstrated by imaging studies.**

Conclusion. **Our finding of the clinical efficacy of selective B cell blockade indicates that B cells play a critical role in rheumatoid synovitis, at least in a subset of patients. Qualitative or quantitative differences in B cell commitment in RA pathobiology might have a function in the different responses observed.**

The key biologic targets of the currently recommended treatments for rheumatoid arthritis (RA), including aggressive combination therapy and tumor necrosis factor α (TNFα) blockade, are represented by T cells, monocytes, macrophages, and synovial fibroblasts (1). A fraction of RA patients remain nonresponders to such treatments, however, due to still-undefined mechanisms of resistance. Because of the high prevalence of RA, the clinical impact of this subgroup is important.

B cells have been shown to participate in chronic rheumatoid synovitis. They undergo antigen-dependent clonal expansion, affinity maturation, and differentiation into plasma cells, and produce rheumatoid factor (RF), a well-recognized prognostic factor for aggressive RA (2,3). Although B cells are believed to have a pathogenetic role in rheumatoid synovitis, including immune complex–mediated inflammation and antigen presentation, this is currently believed to be marginal with respect to T cell and synoviocyte dysregulation. This has led to the belief that treatments directed at B cells alone, if they exist, are unlikely to be effective as monotherapy in RA (2). However, results of very recent studies with human RA synovium–SCID mouse chime-

Salvatore De Vita, MD, Francesco Zaja, MD, Stefania Sacco, MD, Alessandro De Candia, MD, Renato Fanin, MD, Gianfranco Ferraccioli, MD: University of Udine, Udine, Italy.

Address correspondence and reprint requests to Gianfranco Ferraccioli, MD, Department of Rheumatology, DPMSC, University of Udine, P.zza S. Maria della Misericordia 1, 33100 Udine, Italy. E-mail: gf.ferraccioli@med.unud.it.

Submitted for publication August 13, 2001; accepted in revised form May 3, 2002.

Table 1. Baseline clinical features of the patients studied*

Patient	Age, sex	Years of RA, functional, anatomic class	Serum RF, IU/ml	Extraarticular manifestations	Drugs previously ineffective	Drugs administered with anti-CD20†
1	51 F	3, II, II	183	Sjögren's syndrome, parotid myoepithelial sialadenitis (B cell monoclonal), osteoporosis	Gold, HCQ, MTX + HCQ, MTX + CSA + HCQ	HCQ, pred. (5 mg/day)
2	67 F	40, III, IV	1,620	Peripheral neuropathy, mixed cryoglobulinemia, rheumatoid nodules, osteoporosis	MTX, MTX + CSA	Pred. 7.5 mg/day, NSAIDs
3	52 F	12, III, IV	–	Anemia, osteoporosis	HCQ, SSZ, MTX + CSA, CSA + SSZ, MTX + IFLX	Pred. 5 mg/day, NSAIDs
4	65 F	7, III, III	127	Rheumatoid nodules, osteoporosis	HCQ, MTX, MTX + CSA, MTX + SSZ, MTX + IFLX	Pred. 5 mg/day, NSAIDs
5	73 F	40, IV, IV	48	Anemia, osteoporosis, rheumatoid nodules, lymphadenopathy	HCQ, MTX, MTX + CSA	Pred. 5 mg/day, NSAIDs

* RA = rheumatoid arthritis; RF = rheumatoid factor (evaluated by nephelometry; considered positive if >40 IU/ml according to reference laboratory values); HCQ = hydroxychloroquine (6 mg/kg/day); MTX = methotrexate (15–20 mg/week); CSA = cyclosporin A (3–5 mg/kg/day); SSZ = sulfasalazine (4–6 gm/day); IFLX = infliximab (0.3 mg/kg at weeks 0, 2, 6, and every 8 weeks).
† Lack of prednisone (pred.) efficacy in higher dosages (up to 25 mg/day) was also noted in all patients, when given in conjunction with drugs that were previously ineffective. After anti-CD20 therapy, prednisone was discontinued in patients 2 and 4 and reduced to 2.5 mg/day in patient 1, nonsteroidal antiinflammatory drugs (NSAIDs) were discontinued in patients 1, 2, 4, and 5, and no additional treatments were introduced (see text).

ras are consistent with the notion of a central role of B cells in RA synovitis (4).

In this study, 5 patients with RA that had not been responsive to therapies targeted to the T cell/macrophage cell-mediated immune response were treated with anti-CD20 monoclonal antibody to achieve selective blockade of B cells. This proved clinically effective in 4 of the 5 patients. This finding is consistent with published data from the murine model (4) and supports the notion that B cells play a critical pathogenetic role in rheumatoid synovitis, at least in a subset of patients.

PATIENTS AND METHODS

Five female patients with RA fulfilling the criteria of the American College of Rheumatology (ACR; formerly, the American Rheumatism Association) (5) gave informed consent to treatment with rituximab (Mabthera; Roche, Milan, Italy), an anti-CD20 chimeric monoclonal antibody containing human IgG1 and kappa constant regions with murine variable regions (6). Clinical characteristics of the patients are shown in Table 1. All of the patients had very active, evolving erosive articular disease, which had not responded to combination immunosuppressive therapy. Patient 1 also had a monoclonal non-neoplastic B cell lymphoproliferative disorder of the right parotid gland, and this supported the initiation of anti-CD20 treatment (7,8). Patients 3 and 4 had also been treated unsuccessfully (nonresponse) with anti-TNFα (Table 1).

All of the above-mentioned treatments were discontinued 1 month before initiation of anti-CD20 therapy, which consisted of 4 weekly intravenous infusions of 375 mg/m^2, as in treatment of B cell lymphoma (7). Only low-dose steroids, nonsteroidal antiinflammatory drugs (NSAIDs), or antimalarial drugs could be continued during the trial. Patients underwent close clinical and laboratory followup during and after anti-CD20 treatment, including monthly measurement/evaluation of serum C-reactive protein (CRP), RF, immunoglobulins, and peripheral blood B cells, as well as assessment of joint involvement by means of conventional radiography, magnetic resonance imaging (MRI), and ultrasonography.

In 3 patients (patients 1, 3, and 4), as well as in 7 controls with B cell lymphoma, serum concentrations of rituximab were determined at weekly intervals, from baseline to the end of the fifth week after the last infusion of the drug. A previously validated enzyme-linked immunosorbent assay (9) was used for these determinations.

RESULTS

A marked and sustained clinical improvement was observed in patients 1 and 2, starting at the end of the second month after the initiation of anti-CD20 therapy (month 2), with an ACR 70% response (ACR70) and ACR50, respectively. This lasted until month 10 in patient 1 (at which time there was an articular relapse) and until month 12 in patient 2 (the time of the last followup). ACR 20% response was

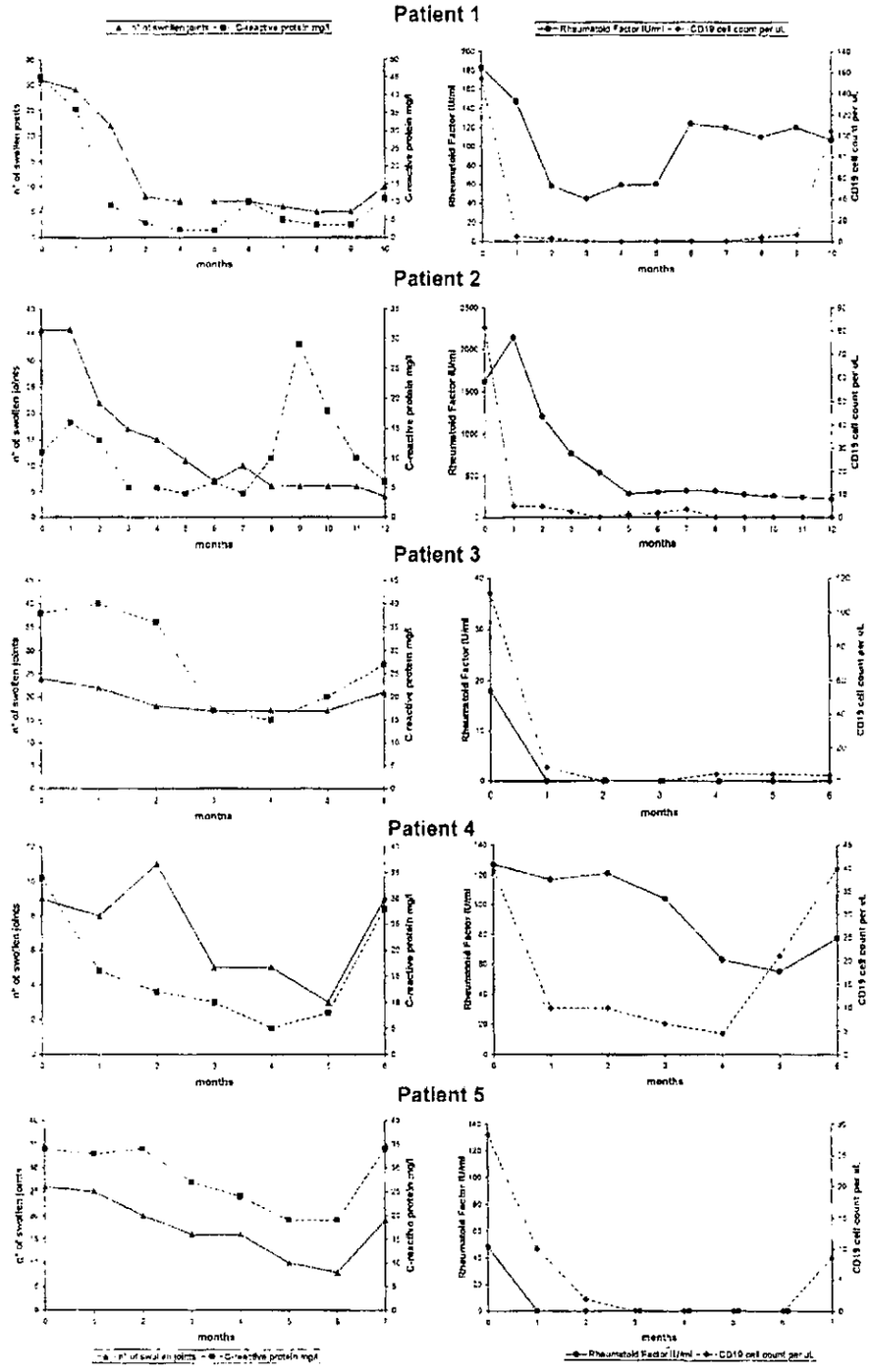

Figure 1. Clinical and biologic effects of anti-CD20 therapy in the rheumatoid arthritis patients studied. C-reactive protein levels were considered abnormal if >5 mg/liter. Rheumatoid factor (evaluated by nephelometry) was considered positive if >20 IU/ml (according to reference laboratory values).

observed from month 3 to month 5 in patient 4 and from month 2 to month 7 in patient 5, with subsequent articular relapse in both. Maximum improvement in disease activity score (DAS) (10) from baseline was observed at month 8 in patient 1 (from 7.1 to 3.4), at month 6 in patient 2 (from 7.7 to 3.9), at month 4 in patient 4 (from 5.2 to 4.2), and at month 6 in patient 5 (from 9.1 to 6.1) (11).

Concomitant with the clinical response in these patients, NSAID treatment was withdrawn in all 4, and daily prednisone treatment was withdrawn in 2, reduced to 2.5 mg/day in 1 (patient 1), and unchanged at 5 mg/day in 1 (patient 5). Normalization or reduction of CRP levels, as well as a >50% decrease in serum RF levels or seroconversion to RF negativity, were also observed in all 4 patients (Figure 1).

In contrast to the other patients, patient 3 exhibited no improvement with the anti-CD20 treatment. Her DAS increased from 6.5 to 7.3, and she did not have a response according to the ACR 20% improvement criteria.

B cell depletion in the peripheral blood was noted in all of the patients studied (Figure 1). No relevant changes were observed in any patient in white blood cell, red blood cell, or platelet counts, hemoglobin levels, or the findings of other routine laboratory tests. A 30–60% decrease in serum IgM levels (but remaining within the normal range) was observed in patients 1, 2, 3, and 5 within month 6, in conjunction with a slight decrease (<30%) in serum IgG and IgA levels; no such decrease was noted in patient 4.

No minor or major adverse events were observed during treatment or followup, with the exception of mild fever and nausea during the first drug infusion in patients 1 and 3, respectively. Episodes of *Escherichia coli* cystitis occurred in patient 2 (at month 9) and in patient 5 (during the entire study period), but had been recorded also in the year preceding anti-CD20 treatment.

The target inflamed joint (wrist in patients 1 and 2; knee in patients 3, 4, and 5) was assessed by MRI and color Doppler ultrasonography at baseline and at month 6, and showed a marked decrease in RA synovitis and synovial vascularization in patients 1 and 2. Only synovial vascularization was decreased in patient 4, and no decrease was observed in patients 3 and 5 (synovitis and vascularization were also mild at baseline in patient 5). Lack of progression of erosive damage in the wrist was also noted by MRI in patients 1 and 2. The number of eroded joints as seen on hand and foot radiographs increased from 24 (baseline) to 25 (month 9) in patient 1, from 14 (baseline) to 15 (month 6) in patient 4, and from 20 (baseline) to 23 (month 6) in patient 3. Due to the severe hand and foot involvement in patients 2 and 5, who had longstanding RA, an accurate comparison of the number of eroded joints could not be performed.

No relationship between higher serum concentrations of rituximab and response of RA to therapy (12) was observed (data not shown).

DISCUSSION

In this study, selective B cell blockade was observed to be clinically efficacious in the treatment of RA, consistent with the notion that B cells have a crucial biologic role in some rheumatoid synovitis. Anti-CD20 therapy (a safe therapeutic option in patients with B cell lymphoma) (7,8) proved clinically beneficial in 4 of 5 patients with aggressive, refractory RA, indicating that B cells were critical in sustaining chronic inflammation and disease activity in such patients. In fact, the CD20 antigen is absent in other cells implicated in chronic RA synovitis, and additional pathogenetic mechanisms (B cell independent) were insufficient (in responders) to maintain the level of articular disease activity. Furthermore, previous treatments targeted to T cell/synoviocyte cell-mediated immune response had proved ineffective in the patients studied herein. Biologic evidence of anti–B cell activity was observed, i.e., B cell depletion in the peripheral blood and decrease in serum RF titer. This was accompanied by clinical and laboratory improvement in the absence of concomitant treatments that might substantially impair B cell number and function (Figure 1). Such a link between decreased RF levels and clinical response is well recognized with other drugs that are effective in RA, and may reflect the biologic relevance of B cell blockade also in the course of other treatments that do not directly target the B cells (1,2).

Decreases in RA synovitis and in evolving erosive damage were noted in patients who had a major response to anti-CD20. Definite conclusions could not be drawn from previous preliminary findings with regard to anti-CD20 therapy in RA, mainly because such therapy was used in conjunction with cyclophosphamide and intermittent high-dose steroids (13).

The mechanisms by which B cell blockade may prove clinically effective in RA remain ill-defined at present. A role of B cells in immune complex–mediated synovitis and in antigen presentation has been proposed (2,3). In human synovium–SCID mouse chimeras, T cell activation was shown to be B cell dependent, and antigen-presenting cells other than B cells could not

substitute in maintaining T cell activation (4). Thus, B cells may be crucial for stimulation of proinflammatory T cells in the RA synovial microenvironment.

A particular sensitivity to anti-CD20 therapy among selected RA patients may be hypothesized. Pathology studies have documented differential patterns of B cell activation in different subsets of RA synovitis (2–4). Our responder patients were all RF positive, while the nonresponder patient was RF negative. In addition, patients 1 and 2 had extraarticular features usually associated with RF positivity (2,8). Whether quantitative or qualitative differences in B cell commitment are involved in RA pathobiology needs to be assessed in future research. Other issues include the link with TNFα-mediated inflammation and optimal drug administration (7,12,14).

While our demonstration of the efficacy of selective B cell blockade was useful to highlight the biologic role of B cells in rheumatoid synovitis, the clinical impact of anti-CD20 therapy, alone or in combination, in RA clearly requires additional investigation. The response demonstrated in the majority of our patients might be consequent to the selection of patients who had been nonresponders to standard treatments, particularly of those with a more relevant B cell commitment. Additional biologic research on rheumatoid B cells and clinical trials, including tissue biopsy studies, will be crucial to clarify these issues.

ACKNOWLEDGMENTS

We wish to acknowledge the fundamental role of Dr. I. Iacona and Prof. M. Regazzi (Department of Pharmacology, IRCCS, S. Matteo Hospital, Pavia, Italy) in generating the pharmacokinetic data on rituximab.

REFERENCES

1. Choy EH, Panayi GS. Cytokine pathways and joint inflammation in rheumatoid arthritis. N Engl J Med 2001;344:907–16.
2. Zhang Z, Bridges SL Jr. Pathogenesis of rheumatoid arthritis: role of B lymphocytes. Rheum Dis Clin North Am 2001;27:335–53.
3. Kim HJ, Berek C. B cells in rheumatoid arthritis. Arthritis Res 2000;2:126–31.
4. Takemura S, Klimiuk PA, Braun A, Goronzy JJ, Weyand CM. T cell activation in rheumatoid synovium is B cell dependent. J Immunol 2001;167:4710–8.
5. 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.
6. Reff ME, Carner K, Chambers KS, Chinn PC, Leonard JE, Raab R, et al. Depletion of B cells in vivo by chimeric mouse human monoclonal antibody to CD20. Blood 1994;83:435–45.
7. Maloney DG, Grillo-López AJ, White CA, Liles TM, Roystan I, Varus C, et al. IDEC-C2B8 (rituximab) anti-CD20 monoclonal antibody therapy in patients with relapsed low-grade non-Hodgkin's lymphoma. Blood 1997;90:2188–95.
8. De Vita S, Boiocchi M, Sorrentino D, Carbone A, Avellini C, Dolcetti R, et al. Characterization of prelymphomatous stages of lymphoproliferation in Sjögren's syndrome. Arthritis Rheum 1997; 40:318–31.
9. Iacona I, Lazzarino M, Avanzini MA, Rupolo M, Arcaini L, Astori C, et al. Rituximab (IDEC-C2B8): validation of a sensitive enzyme-linked immunoassay applied to a clinical pharmacokinetic study. Ther Drug Monit 2000;22:295–301.
10. Van der Heijde DMFM, van 't Hof MA, van Riel PLCM, Theunisse LM, Lubberts EW, van Leeuwen MA, et al. Judging disease activity in clinical practice in rheumatoid arthritis: first step in the development of a disease activity score. Ann Rheum Dis 1990;49:916–20.
11. Van Gestel AM, Prevoo MLL, van 't Hof MA, van Rijswijk MH, van de Putte LBA, van Riel PLCM. Development and validation of the European League Against Rheumatism response criteria for rheumatoid arthritis: comparison with the preliminary American College of Rheumatology and the World Health Organization/International League Against Rheumatism criteria. Arthritis Rheum 1996;39:34–40.
12. Berinstein NL, Grillo-Lopez AJ, White CA, Bence Bruckler I, Maloney D, Czuczman M, et al. Association of serum rituximab (IDEC-C2B8) concentration and anti-tumor response in the treatment of recurrent low-grade or follicular non-Hodgkin's lymphoma. Ann Oncol 1998;9:995–1001.
13. Edwards JCW, Cambridge G. Sustained improvement in rheumatoid arthritis following a protocol designed to deplete B lymphocytes. Rheumatology (Oxford) 2001;40:205–11.
14. Zaja F, Iacona I, Masolini P, Russo D, Sperotto A, Prosdocimo S, et al. B-cell depletion with rituximab as treatment for immune hemolytic anemia and chronic thrombocytopenia. Haematologica 2002;87:189–95.