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Humanized anti-CD4 monoclonal antibody therapy of autoimmune and inflammatory disease

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SUMMARY

We have investigated the biological and therapeutic properties of a humanized anti-CD4 MoAb, hIgG1-CD4, in patients with refractory psoriasis and rheumatoid arthritis (RA). hIgG1-CD4 is a modulating, non-depleting MoAb, which induced a first-dose reaction in most patients treated. It provided brief symptomatic relief in both conditions, and psoriasis appeared easier to control with conventional agents after MoAb therapy. At the doses used, hIgG1-CD4 did not synergize therapeutically with the pan-lymphocyte MoAb CAMPATH-1H (C1H) in patients with RA treated sequentially with both agents. There were no serious adverse effects definitely attributable to therapy. Our results are compared with those of other CD4 MoAb studies, and factors influencing the outcome of therapy are discussed.

Keywords anti-CD4 immunotherapy CAMPATH psoriasis rheumatoid arthritis

INTRODUCTION

Helper (usually $CD4^+$) T cells co-ordinate immune responses, both physiological and pathological. Consequently, manipulation of these cells with MoAbs can modulate ongoing immune reactions. In animals, CD4 MoAbs are immunosuppressive or tolerogenic, depending upon the circumstances surrounding their administration, and rules for effective tolerance induction have been established in a number of models of human autoimmune disease [1–3]. Thus, it is now possible to use CD4 MoAbs to tolerize activated T cells, and furthermore, skin graft tolerance has been achieved across complete MHC barriers. In these experimental models, the intensity of anti-CD4 therapy required (dose, duration) is related to the degree of antigen mismatch or degree of priming present in the system, often requiring the addition of CD8 MoAbs to fully control rejection [4]. Whereas in early experiments depleting MoAbs were used for tolerance induction, it is now recognized that non-depleting MoAbs may be more effective agents [4]. It is uncertain how MoAbs induce tolerance, but once tolerance is established, regulatory mechanisms then maintain the tolerant state. It has been emphasized that, in the transplant situation, such mechanisms of 'infectious tolerance' are essential if tolerance is to be maintained life-long [5].

Thus far it has not been possible to translate these impressive animal data to the clinic. In man, a variety of depleting and non-depleting CD4 MoAbs have been used to treat rheumatoid arthritis (RA) [6–11], psoriasis [12], systemic lupus erythematosus (SLE) [13], and multiple sclerosis [14,15], but only temporary symptomatic relief has been achieved in open studies. Furthermore, two placebo-controlled studies of a depleting mouse–human chimeric CD4 MoAb in RA failed to show any therapeutic benefit [16,17], although a third study employing a non-depleting macaque–human chimeric MoAb did report significant benefit over placebo at the end of a 4-week treatment course [18]. In contrast to these results, a rat anti-human CD4 MoAb synergized with CAMPATH-1H (C1H), a humanized CD52 MoAb, and resulted in long-term remissions in some patients with previously refractory and life-threatening systemic vasculitis who had gained only short-term benefit from C1H alone [19,20]. It is unclear whether the dramatic responses reported were attributable to particular qualities of the CD4 MoAb, to vasculitis being a more sensitive therapeutic target, or to prior treatment with the lymphocytotoxic MoAb C1H easing the task of the CD4 MoAb by 'debulking' the autoreactive lymphocyte load.

A humanized version of the CD4 MoAb successfully administered to vasculitis patients is now available [21]. Whilst retaining antigen specificity, humanization reduces immunogenicity and may alter effector function [22], two important characteristics of a therapeutic MoAb. We have administered this humanized CD4 MoAb to patients with refractory psoriasis and RA, two diseases in which $CD4^+$ lymphocytes are felt to play an important pathogenic role [23,24]. Some of the RA patients received combination

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therapy with C1H, the regime used in systemic vasculitis. We documented both the biological and clinical activity of the CD4 MoAb.

PATIENTS AND METHODS

Monoclonal antibodies

The humanization procedures were described previously [21,22]. The humanized CD4 MoAb was derived from the rat MoAb YNB 46.1.8 [25] and was of human IgG1 isotype. It is referred to hereafter as hIgG1-CD4. Therapeutic-grade MoAb was produced in Chinese hamster ovary cells grown in a hollow-fibre continuous culture system (Acusyst-Junior, Endotronics Inc, Minneapolis, MN) and was purified by affinity chromatography on protein A-Sepharose fast-flow, followed by ion exchange on S-Sepharose and gel filtration using Sephadex 200. It was formulated in PBS and after sterility and endotoxin checks was stored at $-70°C$ before administration. C1H was described previously [26].

Patients

To be eligible for this study, patients had either psoriasis or RA. Psoriatic patients had clinically active disease, with Psoriasis Area Severity Index (PASI [27]) $\geqslant 9$. Either they were intolerant of, or their disease was refractory to, a number of standard treatments. RA patients had disease which fulfilled the American Rheumatism Association criteria [28], apart from one patient who had polyarticular juvenile chronic arthritis (JCA). Entry criteria for RA patients were the presence of active disease as defined by three of the following four criteria: Ritchie articular index [29] >10 (maximum score 78), early morning stiffness >45 min, erythrocyte sedimentation rate (ESR) or C-reactive protein (CRP) >30, 26-joint swollen joint score >10. For inclusion, their disease had been refractory to at least two disease-modifying anti-rheumatic drugs (DMARDs). Psoriasis patients were permitted to continue topical treatments during the study, but any systemic treatment was stopped at least 4 weeks before MoAb administration. RA patients were permitted to continue non-steroidal anti-inflammatory drugs (NSAIDs) and prednisolone (up to 10 mg daily), provided the dosage was constant for the 4 weeks before dosing day 1. Ethical approval was obtained for the studies, and informed consent from each patient.

Treatment protocols

All patients were admitted to hospital for treatment. A number of protocols were used and MoAb doses were based on those previously administered to patients with RA or vasculitis. Psoriasis patients received either 20 mg of MoAb per day for 10 days (200 mg in total) or 100 mg per day for 5 days (500 mg in total). Three RA patients received 40 mg per day for 5 days. Five RA patients and one with JCA received combination therapy comprising C1H 40 mg per day for 5 days and then hIgG1-CD4 40 mg per day for the subsequent 5 days. These patients had previously received a course of C1H alone (40 mg per day for 5 days) or a combination of C1H and an IgG4 chimeric version of that MoAb [30].

hIgG1-CD4 was diluted in normal saline or 5% dextrose and administered by i.v. infusion. Initial doses were diluted in 500 ml and administered over 4 h, but if there were no adverse reactions, subsequent doses were diluted in a smaller volume (100–250 ml) and infused over a shorter time period (1–2 h). C1H was diluted in 500 ml normal saline and administered over 4 h.

Patients left hospital 24 h after the last MoAb infusion. They were assessed 2 weeks later, and then at 2–4 week intervals. At each visit they underwent a disease activity assessment as at study entry, and blood was drawn for analysis (see below).

Adverse reactions

Temperature, blood pressure and pulse were monitored at 15-min intervals during and after infusions, until stable. Adverse reactions to MoAb infusions were graded as previously described [30] (Table 2). Potential delayed adverse reactions (e.g. infective episodes) were recorded at each clinic attendance following therapy. Blood was drawn for full blood count (FBC), renal and hepatic function before and after therapy, and at each follow-up visit.

Lymphocytes

Lymphocyte counts were measured daily during therapy, and at each follow-up visit. In addition to total counts, T cell subsets ($CD3^+$, $CD4^+$, $CD8^+$, $CD16^+$ (natural killer (NK) cells)) were measured using dual-colour immunofluorescence and a lysed whole blood technique. Antibody pairs used were CD3/DR, CD4/CD8 and CD3/$CD16^+$ CD56, and all reagents were from the Becton Dickinson (Mountain View, CA) Simultest range. B cells were measured using a FITC-conjugated CD19 MoAb (Leu-12; Becton Dickinson). Aliquots of whole blood were incubated with the appropriate MoAbs at room temperature for 15 min, following which 2 ml of FACSlyse were added to each tube. After a further 10-min incubation, tubes were centrifuged and cells fixed with 1% formaldehyde. Cells were analysed using a Becton Dickinson FACScan and Simultest software. Preliminary experiments established that the epitope bound by the CD4 MoAb used for subset analysis (Leu-3a) did not overlap with that bound by hIgG1-CD4.

Modulation of lymphocyte surface CD4 was determined using Leu-3a by relating mean fluorescence intensity (MFI) with this MoAb to MFI using a CD3 MoAb (CD3 MFI did not modulate with treatment). Percentage modulation was then expressed as:

$$\frac{\text{Post-treatment CD4 MFI/CD3 MFI}}{\text{Pre-treatment CD4 MFI/CD3 MFI}} \times 100$$

$CD4^+$ lymphocyte coating was also assessed by dual-colour immunofluorescence. Peripheral blood mononuclear cells (PBMC) were prepared by dextran sedimentation and washed twice with PBS/1% (w/v) albumin/0·01% (w/v) sodium azide (wash buffer). PBMC were resuspended at 5×10^6 cells/ml and stained with FITC-conjugated Leu-3a and PE-conjugated anti-human IgG. Cells were incubated for 30 min on ice, washed twice with wash buffer and residual erythrocytes lysed with FACSlyse. Cells were analysed using LYSIS-II software, and the percentage of $CD4^+$ lymphocytes dual-stained with anti-human IgG recorded.

Antiglobulin responses

Post-treatment serum samples were assayed for antiglobulin reactivity against hIgG1-CD4 using a variant of a previously described double-capture ELISA [31]. In the current assay, hIgG1-CD4 was used as capture and detection MoAb. Goat anti-human IgG MoAb (Sigma I-2316; Poole, UK) was used as a positive control, and the sensitivity of the assay was approximately 500 ng/ml.

Table 1. Characteristics of patients treated

Patient	Age (years)	Sex	Disease duration (years)	Previous therapy*	Seropositive (RA only)
Psoriasis					
1	36	M	9	Pu	N/A
2	57	F	24	Az, CsA, E, H, M, Pu	N/A
3	45	F	38	E, M, Pu	N/A
4	78	F	8	CsA, Ct, D, E, G, M, Pu	N/A
5‡	79	F	40	Az, G, M, Pu	N/A
6‡	76	F	60	CsA, E, M	N/A
7‡	64	M	40	CsA, E, M, Pu, R	N/A
8	39	F	17	Ct, E, M, Pu	N/A
9‡	60	M	10	CsA, E, M, Pu	N/A
Rheumatoid arthritis					
10	64	M	14	Au, P, S	+
11	71	M	4	Au, Az, M, S	+
12	65	F	13	Au, M, P, S	+
13§	53	F	9	Au, Az, Cy, CsA, M, P, Q, S	+
14§	14†	F	6	Au, M, P, S	-
15§	47	M	8	Au, Az, M	-
16§	35	M	6	Au, M, OG, P, S	-
17§	54	F	36	Au, M, P, Q	+
18§	43	F	11	Au, S	+

* Au, i.m. gold; Az, azathioprine; CsA, cyclosporin A; Ct, calcipitriol; Cy, cyclophosphamide; D, dapsone; E, etretinate; G, systemic glucocorticoids; H, hydroxyurea; M, methotrexate; OG, oral gold; Pe, penicillamine; Pu, PUVA; Q, hydroxochloroquine; R, Razoxane; S, sulphasalazine.

† Juvenile chronic arthritis.

‡ Psoriatic arthritis.

§ Patients 13–18 received combination therapy with C1H and hIgG1-CD4.

N/A, Not applicable.

Serum antibody concentration

Blood was drawn from patients before and after treatment on each day of therapy. Serum hIgG1-CD4 concentration was measured by immunofluorescence as previously described [30] using baby hamster kidney cells stably transfected with the human CD4 antigen for staining (kindly provided by Dr M. Tone, University of Oxford, UK), and FITC-conjugated monoclonal anti-human IgG1 immunglobulin (Sigma F0767) as detection reagent. In approximately 20% of patients, a high level of background staining precluded an accurate assessment of serum MoAb levels. In the remaining patients, assay sensitivity was approximately 500 pg/ml.

Measurement of tumour necrosis factor-alpha

Blood was drawn from patients at the end of infusions and serum was assayed for tumour necrosis factor-alpha (TNF-α) using a sandwich ELISA (R&D Systems, Abingdon, UK). This assay was sensitive to 4·4 pg/ml of TNF-α.

RESULTS

Eighteen patients were treated in the current series (Table 1). Twelve received hIgG1-CD4 alone and six in combination with C1H. Nine patients had psoriasis, eight RA and one JCA. Four of the psoriasis patients had an arthropathy. Concurrent medical conditions included diabetes mellitus, asthma, migraine, angina, diverticulitis and multiple myeloma. All patients conformed to entry criteria apart from one patient with psoriasis who continued treatment with oral triamcinolone, which had been prescribed for 5 years.

Clinical

First-dose reactions. Patients receiving sequential therapy with C1H and hIgG1-CD4 experienced a first-dose reaction with C1H [26], but did not react to the first dose of hIgG1-CD4. Patients receiving hIgG1-CD4 alone, however, experienced first-dose reactions with a trend towards a dose–response relationship (Table 2). The reactions were qualitatively similar to those reported with other MoAbs [30]. Patient 8, however, experienced a very early reaction, which occurred within 15 min of the infusion starting, and comprised a sensation of throat fullness, shortness of breath and chills but no fever or wheeze. Also, patient 2 experienced recurrent hypotensive episodes during infusions 2, 4, 8 and 10, which occurred at different times and recovered with slowing of infusion rate. Patient 6, with a history of migraine headaches, suffered a severe, refractory episode of migraine on dosing day 1 and refused further MoAb therapy. This was the only patient not to complete treatment. One patient (10) was seated rather than supine during initial MoAb infusion and suffered a vaso-vagal reaction.

Clinical response A. Psoriasis, Table 3. One psoriasis patient improved dramatically following treatment, with PASI falling from 18·3 pre-treatment to 4·7 at 2 weeks. Four additional patients (5, 7, 8 and 9) showed improvements in PASI of 18–42%, lasting for up to 1 month. Two of these and one additional patient

Table 2. Adverse reactions during the first dose of hIgG1-CD4, and post-therapy circulating tumour necrosis factor-alpha (TNF-α) levels

Patient	Dose (mg)	First-dose reaction	TNF-α level (pg/ml)
1	20	++	0
2	20	++	0
3	20	++	6
4	20	+	6
5	100	+++	156·3
6	100	– (migraine)	NA
7	100	++	0
8	100	+++	7
9	100	+++	122
10	40	(+++)*	0
11	40	++	73
12	40	++	28

*Patient developed hypotension as a consequence of a vaso-vagal reaction.

Reactions were graded according to the following scale: 0, no reaction; +, temperature rise up to 37·5°C, and/or chills; ++, temperature rise to between 37·6°C and 38·5°C, and/or rigor; +++, temperature rise to greater than 38·5°C and/or hypotension and/or chest tightness.

Hypotension was defined as a fall in systolic blood pressure of >30 mmHg to a value of 90 mmHg or below on two successive readings 15 min apart.

TNF-α was measured in serum samples taken at the end of infusions.

NA, Not available.

subsequently benefited from previously ineffective treatments: patient 5 to topical steroids and coal tar cream, patient 3 to etretinate, and patient 7 to topical steroids plus PUVA. Patient 2 did not improve with MoAb treatment, and in two others it was not possible to assess the impact of MoAb therapy due to confounding factors. One of these patients (6) received a single dose of hIgG1-CD4 and then improved following the initiation of calcipotriol treatment which had not been used previously. The other had myeloma and required variable steroid dosage to control symptoms attributable to that disease. Thus at the doses of hIgG1-CD4 administered, psoriasis either improved or became easier to control in six of seven patients in whom therapy could be assessed. Of five patients demonstrating improvement with MoAb alone, four received the higher dose schedule.

B. RA, Fig. 1. The clinical effects of hIgG1-CD4 were dramatic but transient in patients with RA at the dose administered. Patients receiving hIgG1-CD4 alone showed significant reductions in arthritis activity during treatment, as exemplified by Ritchie and joint scores, but improvement was of brief duration with return towards baseline by 3 weeks. There were similar improvements in morning stiffness and visual analogue score for pain, but no consistent changes in ESR or CRP (data not shown). hIgG1-CD4 did not synergize with C1H in patients receiving combination therapy with the two MoAbs (data not shown). In four of six patients improvements in disease activity were of similar duration whether or not they received hIgG1-CD4 with C1H. The response to combination treatment was less sustained than the response to C1H alone in a fifth patient, and a further patient developed polymyalgia rheumatica (PMR) after combination therapy and received prednisolone before a comparison could be made. Thus, hIgG1-CD4 appeared to provide symptomatic relief in patients with RA, but this was of brief duration and, in contrast to patients with systemic vasculitis, there was no synergy with C1H at the doses used. Of three patients with psoriatic arthropathy that received 500 mg of hIgG1-CD4, two experienced a subjective improvement in their joints lasting for 1 month and 3 months.

Adverse reactions. Apart from first-dose reactions, hIgG1-CD4 was well tolerated. Two RA patients developed mild purpuric rashes during therapy (with normal platelet counts), lasting 7 days and 1 day, respectively. Two patients developed proximal muscle aches and pains 3 weeks after therapy. In one, PMR was diagnosed at week 10 and in the other non-specific RA changes were seen on muscle biopsy at week 3. Patient 9

Table 3. Clinical course of psoriasis patients

Patient	Dose (mg)	Pre-treatment	End of treatment	2 weeks	1 month	2 months	3 months	Comments
1	200	18·3	7·4	4·7	6·4	3·0	4·2	Improved with treatment.
2	200	10·2	NR	9	NR	NR	NR	No improvement.
3	200	10·4	14·3	15·4	NR	0	NR	Psoriasis worsened during treatment. Etretinate restarted and psoriasis subsequently cleared.
4	200	Erythrodermic	NR	NR	NR	NR	NR	Not assessable: variable glucocorticoid dose for multiple myeloma.
5	500	19·2	11·4	(14)	(16)	6·1	NR	Partial response. Topical steroids and coal tar restarted at 1 month. Arthritis improved to day 37.
6	100	13·5	8·7	4·6	NR	7·1	NR	Only one dose of MoAb (migraine). Topical steroids and calcipotriol commenced day 2.
7	500	16·7	16·5	13·7	16·8	NR	5·8	Mild, transient improvement. Marked improvement with topical steroids + PUVA from 1 month. Joints improved to day 90.
8	500	9·0	7·1	NR	6·6	10·4	NR	Mild, transient improvement.
9	500	19·2	NR	NR	14	NR	NR	Mild improvement in skin. No improvement in joints.

Values represent Psoriasis Area Severity Index (PASI). NR, Not recorded. Values in parentheses = observation by different observer to baseline.

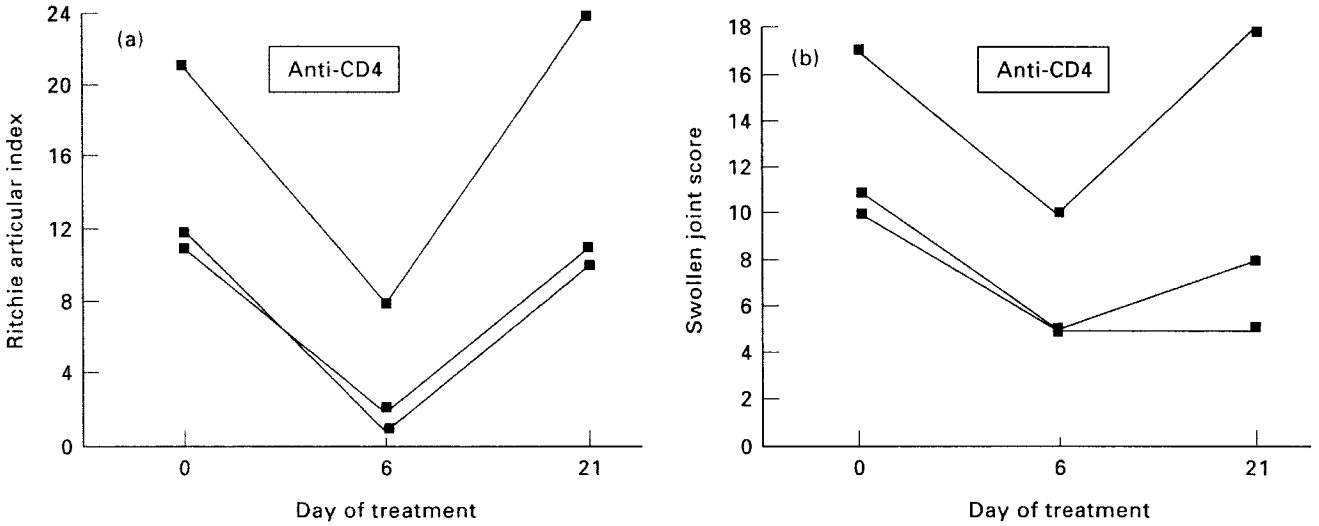

Fig. 1. Ritchie articular index (a) and swollen joint score (b) in rheumatoid arthritis (RA) patients receiving hIgG1-CD4 MoAb therapy. Each line represents an individual patient.

developed bacterial cholangitis during treatment, but liver function tests suggested that this was developing before therapy and may even have accounted for fever and rigors on the first dosing day. Subsequent investigation suggested that a gallstone had passed spontaneously and the illness responded appropriately to antibiotic treatment. There were no other perturbations of renal or hepatic function in any patient.

The only definite opportunistic infection was a case of mucosal candidiasis following combination therapy in a patient who was also diabetic, and this responded to topical anti-fungal therapy. A further combination therapy patient developed a self-limiting illness 3 weeks post-treatment, comprising malaize and morbilliform rash lasting for 4 days. Serology was consistent with a recent measles infection despite a history of measles during childhood. One patient who received hIgG1-CD4 alone developed a febrile illness with diarrhoea and arthralgias on day 20 which lasted for 10 days. Hospitalization was not necessary and family members suffered similar symptoms.

There was one serious adverse event which could not definitely be attributed to MoAb therapy. A patient with an 8-year history of RA, previously treated with gold, methotrexate and azathioprine, received two courses of MoAb therapy. C1H alone (40 mg per day for 5 days) provided symptomatic relief lasting for 3 months. The patient then received combination therapy with C1H and hIgG1-CD4 with a further 3 months of benefit. Upon relapse, azathioprine was recommenced and slowly increased to a dose of 3 mg/kg per day. Approximately 7 months into azathioprine treatment the patient became unwell with general malaize, nausea, weight loss and jaundice. Investigations revealed the presence of widespread non-Hodgkin's lymphoma, and the patient died before therapy could be instigated.

Laboratory

Peripheral blood lymphocyte counts (Fig. 2). Administration of hIgG1-CD4 resulted in a transient fall in $CD4^+$ peripheral blood lymphocyte count (PBLC). Twenty-four hours after the last dose of MoAb, mean $CD4^+$ PBLC was 75% of baseline in psoriasis patients receiving 200 mg of MoAb and 31% of baseline in the 500 mg cohort. There was then a recovery towards baseline, although two 500 mg patients continued to show PBLC marginally below the normal range at 70 and 80 days, respectively. This was also true of one patient in the 200 mg cohort who started with a subnormal PBLC. Similar effects were observed in RA patients receiving CD4 MoAb alone (24 h post-treatment values were 59% of baseline and at 1 month mean PBLC had returned to normal). Apart from a transient fall in NK cell counts, other lymphocyte subsets were unaffected by therapy. Due to the lymphocytotoxic properties of Campath-1 MoAbs and consequent post-treatment lymphopenia, it was not possible to monitor lymphocyte subsets in patients receiving combination therapy.

CD4 modulation. There was dramatic and temporary modulation of lymphocyte surface CD4 antigen during treatment with hIgG1-CD4 (Fig. 3). Modulation was more marked when 500 mg of MoAb were administered compared with 200 mg, presumably reflecting a higher degree of cross-linking at the cell surface. In patients receiving 500 mg hIgG1-CD4 over 5 days, modulation persisted for more than 10 days after the end of treatment, but was back to baseline at 1 month (Fig. 3).

Thus, hIgG1-CD4 resulted in transient disappearance of $CD4^+$ lymphocytes from peripheral blood, but levels of expression of the CD4 molecule were potently modulated during treatment.

Coating of peripheral blood $CD4^+$ lymphocytes. Between 0% and 80% of $CD4^+$ lymphocytes demonstrated surface IgG in blood taken before dosing on day 2 from patients receiving 20 mg of MoAb daily (data not shown). This broad range may have reflected differences in circulating MoAb concentration between patients (although simultaneous serum concentrations were below the assay's sensitivity) or differences in sequestration of MoAb-coated cells. Greater than 90% of $CD4^+$ PBL were coated with hIgG1-CD4 in equivalent samples from two high-dose (100 mg per day) patients.

Antiglobulin response. Blood was taken from patients at each follow-up clinic visit and no antiglobulins to hIgG1-CD4 were detected in any patient. A weak positive response was detected at baseline in some seropositive RA patients, but this background

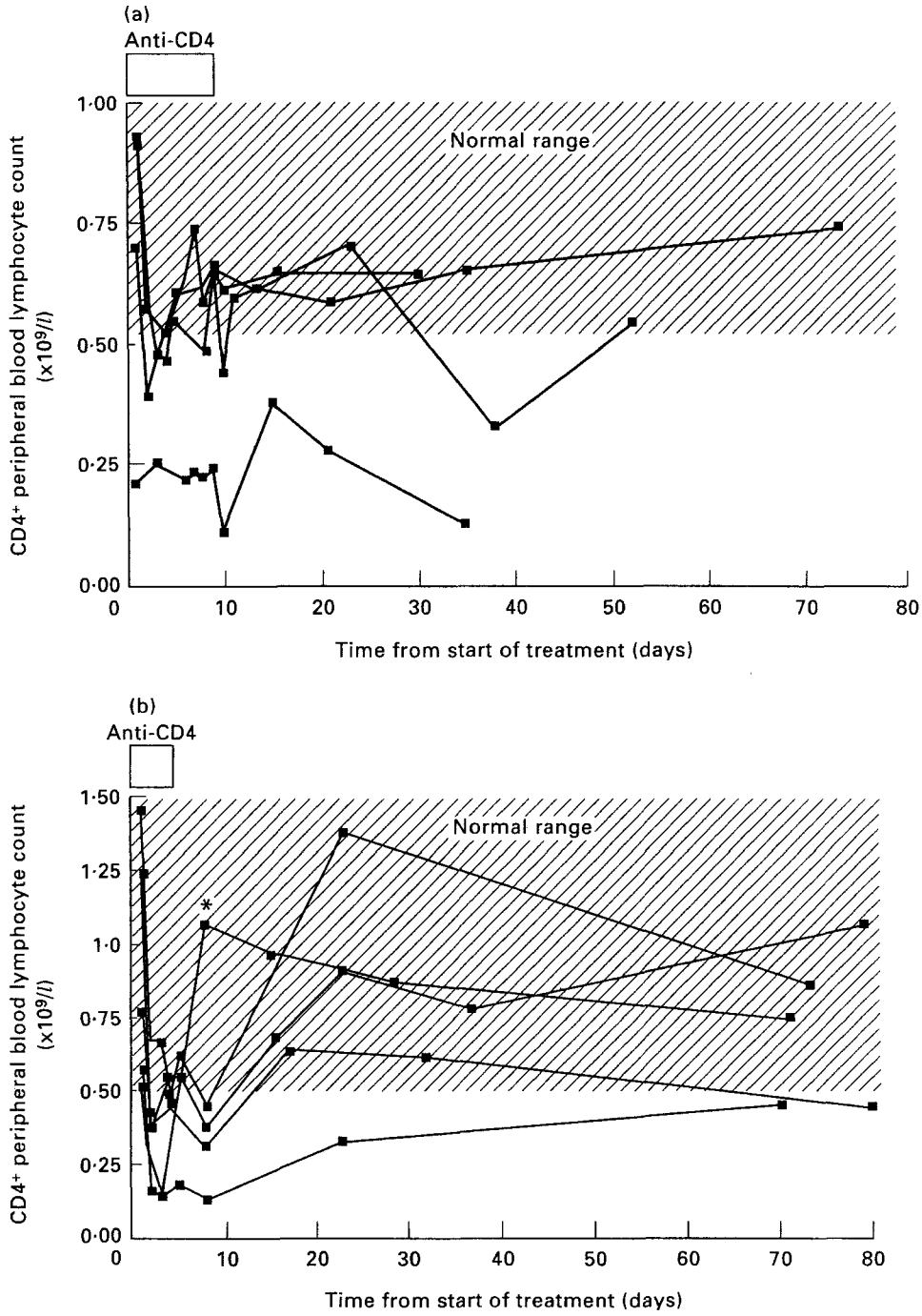

Fig. 2. Peripheral blood $CD4^+$ lymphocyte counts in psoriasis patients receiving (a) 200 mg and (b) 500 mg hIgG1-CD4 MoAb. Each line corresponds to an individual patient. *Received only 1 dose of treatment.

reading was non-specifically absorbable using a number of unrelated rodent MoAbs.

Serum hIgG1-CD4 concentrations. Peak serum MoAb concentrations ranged from 2 μg/ml to >100 μg/ml (data not shown). MoAb did not accumulate in psoriasis patients receiving 20 mg per day, in whom trough levels were undetectable and post-infusion levels varied from 2 to 4 μg/ml. In contrast, concentrations up to 150 μg/ml were recorded at the end of the treatment course in patients receiving 100 mg per day. Data were available for five RA patients, in whom there was some accumulation during treatment at 40 mg per day to peak levels of 10–50 μg/ml. A prior course of C1H did not obviously affect serum levels. Of note, a patient who previously received an IgG4 version of C1H with minimal biological effect and a low serum level [30] also achieved very low levels of hIgG1-CD4 (<2 μg/ml after 40 mg per day for 5 days). Our data did not permit half life calculations.

TNF-α was measured in blood taken at the end of first infusions of hIgG1-CD4. Four samples demonstrated TNF-α

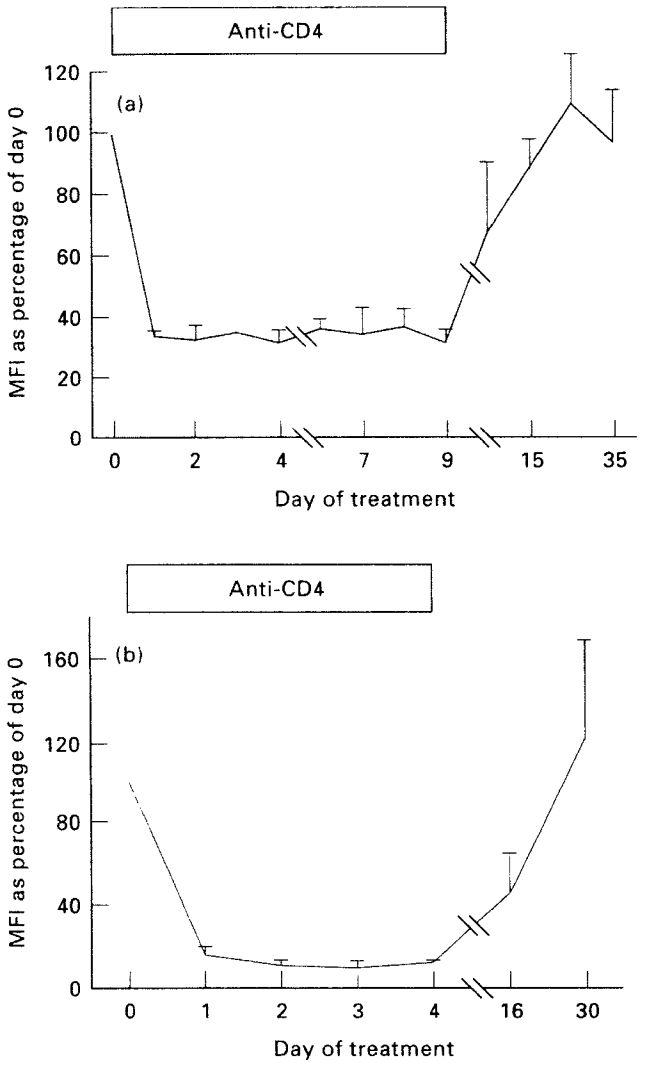

Fig. 3. Modulation of CD4 antigen on $CD4^+$ peripheral blood lymphocytes of psoriasis patients receiving (a) 200 mg and (b) 500 mg hIgG1-CD4 MoAb (mean + s.d. of mean fluorescence intensity (MFI) as percentage of pretreatment value, see Patients and Methods).

levels > 10 pg/ml (Table 2), and each donor experienced a moderate or severe first-dose reaction. TNF-α was not detected in serum taken from patient 2 during a hypotensive episode.

DISCUSSION

hIgG1-CD4 is the first fully humanized rat CD4 MoAb to be used therapeutically. It is a non-depleting, modulating MoAb of low immunogenicity which provokes a typical first-dose reaction in most recipients. $CD4^+$ PBL fell during treatment, with subsequent recovery to counts close to baseline and usually within the normal range (Fig. 2). $CD4^+$ PBL counts remained mildly depressed in a few patients, particularly those receiving a higher dose of MoAb, but these findings should be interpreted within the considerable day-to-day variation in lymphocyte counts. Furthermore, reduced $CD4^+$ PBL counts have previously been reported after treatment with placebo in a blinded study of CD4 MoAb therapy [16].

The lytic capacity of a MoAb resides in a number of factors, including MoAb isotype, and the antigen and epitope bound [32–36]. In contrast to hIgG1-CD4, a chimeric CD4 MoAb of human IgG1 isotype (cM-T412) caused prolonged depletion of circulating $CD4^+$ lymphocytes lasting beyond 30 months [37]. The marked difference in lymphocytotoxicity between these MoAbs must reside either in antigenic factors such as epitope specificity or affinity, or in undefined intrinsic features of the MoAb itself. Unfortunately it is not currently possible to use *in vitro* tests to reliably predict *in vivo* cytotoxicity either in animals or in man [38]. For example, a human IgG4 MoAb had significant depleting activity *in vivo* which was not predicted by standard *in vitro* testing [30].

All rodent CD4 MoAbs were immunogenic in man, and in one case IgE antiglobulins resulted in anaphylaxis [7]. The immunogenicity of the chimeric human–mouse MoAb cM-T412 was lower, although 75% of patients developed antiglobulins in one study [39]. Humanization further reduces immunogenicity, probably by removing T cell epitopes from within the MoAb framework [40]. Furthermore, CD4 MoAbs are not immunogenic in rodents provided a sufficient dose is administered, which contrasts with the strong immunogenicity of MoAbs to other lymphocyte surface antigens [41,42]. CD4 is also present on monocytes in man, however, which might improve the presentation of CD4 MoAbs to the immune system and so enhance their immunogenicity [40]. Despite this possibility, no antiglobulins were elicited by hIgG1-CD4 in the current study.

First-dose reactions reflect the systemic release of cytokines provoked by MoAb infusion [43,44]. Complement activation may contribute, but the association of first-dose symptoms with a MoAb of human IgG4 isotype excludes complement involvement in that circumstance [30]. Furthermore, animal models suggest cross-linking of target and effector cell via MoAb–Fc receptor interactions to be a more likely mechanism [45], and it follows that, like target cell depletion, the magnitude of the first-dose reaction will be influenced by both MoAb and antigenic factors. MoAb (isotype) dependency is exemplified by the relatively minor reaction to an IgG4 version of C1H (in comparison with C1H of IgG1 isotype) in our previous study [30]. In contrast, antigenic factors probably underlie the different reactions provoked by two IgG1 MoAbs, hIgG1-CD4 (nil to severe in the current study) and C1H (always severe [26]), although differences between study populations should also be considered.

In a previous paper we showed a strong association between circulating TNF-α levels and first-dose reactions in patients with multiple sclerosis receiving C1H [43]. The inconsistent relationship following hIgG1-CD4 infusion in the current study may simply reflect the timing of blood sampling which was not specifically tailored to first-dose reactions. Elevated levels of TNF-α were detected in four post-treatment blood samples, however, taken from patients receiving 40 or 100 mg of hIgG1-CD4 (Table 2), and these levels were similar to those measured at that time point in RA patients following 12 mg of IgG4 or IgG1 versions of C1H [30]. We previously reported a good correlation between *in vitro* and *in vivo* TNF-α release by MoAbs, but hIgG1-CD4 (at 2·5 and 10 μg/ml) did not release TNF-α *in vitro* [46]. Since TNF-α was only detected following higher doses of hIgG1-CD4 *in vivo*, however, it is possible that higher MoAb concentrations may also have caused TNF-α release *in vitro*.

Circulating levels of hIgG1-CD4 rose with increasing MoAb dose. There was no accumulation with 20 mg daily, but higher doses resulted in steadily rising trough levels, especially apparent at 100 mg per day. Prior administration of C1H, which caused peripheral blood lymphopenia, did not seem to influence serum

hIgG1-CD4 levels. By removing a 'sink' of CD4 antigen, lymphopenia might have resulted in higher serum levels. Circulating lymphocytes account for a small fraction of the total, however, and we have previously argued that C1H may not deplete lymphoid tissue [47]. Very low serum levels of hIgG1-CD4 were found in one recipient who had previously achieved low levels with an IgG4 version of C1H [30]. This patient had RA with a high level of IgM rheumatoid factor (>400 U/ml on several occasions) which could theoretically have promoted clearance of therapeutic MoAb.

Our studies with hIgG1-CD4 were unblinded and open-label but, notwithstanding this limitation, therapy was associated with transient relief of both skin and joint symptoms. Symptomatic relief of joint pain was longer lasting in psoriatic arthritis, although these patients received a higher dose of MoAb. Also, joint symptoms improved for longer than skin lesions in patients with psoriasis. In RA, there was no apparent synergy with C1H. In a previous report, three patients with severe psoriasis received a murine IgG1 CD4 MoAb for 10 days in doses varying from 0·2 to 0·8 mg/kg per day, a higher dose than our patients received [12]. That MoAb also induced modulation but not depletion of $CD4^+$ PBL, and each patient experienced a mild first-dose reaction comprising minor chills and fever. Clinical improvement peaked between days 20 and 30, consistent with our experience with hIgG1-CD4. There have been numerous open studies of CD4 MoAb therapy in RA. As with our study, patients had refractory disease and treatment was with brief courses of murine or chimeric MoAb [6–11]. Murine MoAbs were either non- or mildly depleting, usually with modulation of surface CD4, and all were generally well tolerated. First-dose reactions were more frequent with the chimeric MoAb, which was also significantly lymphocytotoxic. It is not possible to compare directly the various studies for efficacy due to variation in outcome parameters and lack of placebo controls, but most reported symptomatic relief and, in some, a dose–response effect was apparent. These effects lasted from a few weeks to several months, with rare patients achieving disease remission, although two of three controlled trials of CD4 therapy in RA have failed to show a beneficial effect of treatment [15–17].

These clinical results are far removed from the impressive tolerogenic effects of a wide range of CD4 MoAbs applied to animal models of human autoimmunity. In a recently published theory based on the results of extensive animal data, it is argued that CD4 MoAbs' principal tolerogenic action is to simply coat $CD4^+$ lymphocytes and thereby block ongoing auto- or alloimmune responses [48]. This seems to allow the targeted immune system to default back to its natural tolerant state with the emergence of regulatory $CD4^+$ cells to ensure tolerance is robust and lasting. From a clinical perspective the critical features of the model are dose and duration of therapy, and it follows that MoAb therapy may be required for several weeks in patients with autoimmune diseases, at doses sufficient to target all autoreactive lymphocytes, including those sequestered in target tissues. A further ramification of the theory is that tolerance may be difficult to achieve in an inflammatory setting because inflammation up-regulates immune responses [49]. These factors should be taken into consideration in the design of future CD4 MoAb studies.

Theoretically, non-depleting MoAb therapy should not predispose to a long-term risk of opportunistic infection or tumour [48]. In our hands there were a few minor infections occurring soon after hIgG1-CD4 therapy alone, and this mirrors the experience with other CD4 MoAbs. One of our patients developed a lymphoma after receiving C1H (on two occasions) and hIgG1-CD4, but there is an increased incidence of immune neoplasms in RA patients, particularly in those with refractory disease and in association with azathioprine therapy [50,51]. Thus MoAb therapy cannot be definitely implicated in our case but future studies should include case controls (matched for age, disease duration and previous therapy) who continue conventional therapies as comparators for infection and tumour risk.

Used appropriately, hIgG1-CD4 may be an ideal MoAb for human therapy. It is non-immunogenic, at least after one course, and non-depleting at the doses used in this study. We would advocate further investigation of hIgG1-CD4 in autoimmunity, in higher dosage and longer courses, within the context of a controlled therapeutic trial. Such studies are already underway and dose-ranging data have recently been reported in abstract form [52].

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