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A therapeutic human IgG4 monoclonal antibody that depletes target cells in humans

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SUMMARY

It is traditionally held that human IgG4 MoAbs should not deplete target cells *in vivo*, as this isotype is inactive in a number of *in vitro* assays that measure effector function. We have previously challenged this dogma, and the current study was designed to investigate the *in vivo* biological effects in humans of a MoAb of human IgG4 isotype. Nine patients with refractory rheumatoid arthritis (RA) fulfilling ARA criteria, and one with ankylosing spondylitis (AS) received a human IgG4 Campath-1 MoAb (with specificity against the pan-lymphocyte antigen CD52) as part of a two-stage therapeutic protocol. In stage 1, patients received a single dose of this MoAb. Stage 2, starting 48 h later, comprised a 5-day course of a human IgG1 Campath-1 MoAb with identical V-region (CAMPATH-1H), as previously used in the management of RA patients. The intervening 48 h provided a window of opportunity to monitor the biological effects of the IgG4 MoAb for comparison with the IgG1. The two MoAbs were also compared for *in vitro* biological activity. IgG4 depleted peripheral blood lymphocytes (PBL), albeit less efficiently than IgG1. It produced a first-dose reaction of similar intensity, although associated circulating tumour necrosis factor-alpha (TNF-α) levels were lower. TNF-α release from whole blood *in vitro* was also greater with the IgG1 MoAb. The study design did not permit conclusions to be drawn regarding therapeutic efficacy of the IgG4 MoAb. In summary, a human IgG4 Campath-1 MoAb depletes target cells *in vivo*. Importantly, this study demonstrates for the first time in humans that *in vitro* assays may not predict the *in vivo* effector function of therapeutic MoAbs.

Keywords monoclonal antibody Fc receptors cytokine Campath rheumatoid arthritis

INTRODUCTION

An increasing range and number of MoAbs are being used therapeutically in rheumatic diseases. Clinical efficacy has been generally unimpressive, however, and we have previously argued that human treatment regimes should be based on those effective in animal models of autoimmunity [1,2]. Ignoring the rules derived from such models could result in a premature curtailment of MoAb immunotherapy studies.

It is now fashionable to produce 'designer' MoAbs using recombinant DNA technology, suited to particular therapeutic situations. For example, a MoAb that efficiently kills target cells is ideal for cancer therapy, whereas a 'blocking' MoAb may be more suitable for immunotherapy of autoimmune disease. There is also a potential misconception in the creation of designer MoAbs, however: that their biological activity *in vivo* (responsible for beneficial and adverse effects) is routinely predictable from *in vitro* assays. For example, the human IgG1 isotype efficiently utilizes complement- and antibody-dependent cell-mediated cytotoxicity (ADCC) to kill target cells *in vitro*, whereas IgG4 is inactive [3,4] and the same hierarchy of killing is assumed to hold *in vivo*. The validity of such assumptions has been questioned, however, and recent studies have highlighted ambiguities. For example, there was no clear relationship between *in vivo* depletion and *in vitro* effector function when rat anti-mouse CD4 MoAbs were administered to mice [5]. Polymorphisms within the human population add a further complication. Thus, Greenwood *et al*. demonstrated an unexpected polymorphism in ADCC with human IgG4 [6]. Using mononuclear cells from normal subjects they found that, whereas in some individuals IgG4 killed poorly, as expected, in others it was as active as the potent IgG1. Interestingly, human IgG4 was also as potent as IgG1 at killing target cells in an *in vivo* mouse model of immunotherapy [7]. As human IgG4 T cell MoAbs are now being produced as 'blocking' MoAbs for therapeutic use [8], we decided to investigate their *in vivo* biological activity.

CD52, the Campath-1 antigen, is a small GPI-anchored glycoprotein which is expressed at high density on virtually all human

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lymphocytes and monocytes [9]. Perhaps due to its proximity to the cell membrane, it provides one of the best targets known for MoAb-mediated lysis by humoral or cellular mechanisms [3,10]. This characteristic has been exploited *in vivo* in the therapy of subsets of patients with lymphoma [11] and autoimmunity [12–14] using CAMPATH-1H, a CD52 MoAb of human IgG1 isotype. Although a potentially useful MoAb, the degree and duration of lymphopenia produced by CAMPATH-1H was unexpected [15,16], and almost certainly unnecessary for therapy of autoimmune disease, where non-depleting MoAbs are at least as efficacious [17]. A further unwanted effect was the 'first-dose' reaction comprising fever, chills, nausea and headache. Such reactions are a consequence of cytokine release [18,19], but their relationship to lymphocyte depletion is uncertain.

If human IgG4 were non-lytic *in vivo*, an IgG4 Campath-1 MoAb could provide lymphocyte blockade without lymphopenia. Furthermore, the limited allotypy of human IgG4 should reduce its immunogenicity in comparison with other human isotypes [20]. We designed a study to measure the biological effects of an IgG4 Campath-1 MoAb *in vivo*, investigating lymphocyte depletion, cytokine release, and first dose reaction with subsequent intrapatient comparison with CAMPATH-1H. *In vitro* assays were performed using patients' mononuclear cells for comparison with *in vivo* results.

PATIENTS AND METHODS

Monoclonal antibodies

CAMPATH-1H (IgG1-C1H) was produced in Chinese hamster ovary cells [21] grown in a hollow-fibre continuous culture system (Acusyst-junior; Endotronics Inc, Minneapolis, MN) and was purified on protein A followed by ion-exchange chromatography on S-Sepharose. Monomeric MoAb was subsequently purified on a gel filtration column (Superdex 200) before formulation in PBS. After sterility and endotoxin checks it was stored at -70°C prior to administration. The IgG4 version (IgG4-C1H) was produced in Y0 myeloma cells [22] in an identical culture system. Due to a lower yield this preparation was not subject to gel filtration. The two MoAbs have an identical variable (antigen-binding) region (V-region).

Patients

Ten patients were chosen for study. Nine had rheumatoid arthritis (RA) fulfilling the American Rheumatism Association criteria [23] and the tenth had ankylosing spondylitis (AS) with severe peripheral joint involvement. Entry criteria for the study were the presence of active and refractory disease as defined previously [12]. Disease-modifying anti-rheumatic drugs (DMARDs) were stopped at least 4 weeks before day 0 of the study, but patients were permitted to continue non-steroidal anti-inflammatory drugs and an existing dose of prednisolone (up to 10 mg daily). Approval of the local ethical committee and informed consent of the patients were obtained before treatment.

Treatment protocol (Fig. 1)

Patients were admitted to hospital for MoAb therapy and received both IgG4 and IgG1 Campath-1 MoAbs in a two-phase protocol. Each dose of MoAb was diluted in 500 ml normal saline and administered by i.v. infusion over 4 h. Phase 1 comprised the administration of 12 mg IgG4-C1H on day 0. Phase 2 started 48 h later, and comprised 12 mg IgG1-C1H on day 2 and then 40 mg daily through days 3–6. (One patient had a 96-h break between phases 1 and 2 to avoid the first administration of IgG1-C1H on a week-end, and this patient erroneously received a larger first dose of IgG1-C1H (40 mg).) All patients therefore received 5 days therapy with IgG1-C1H as previously shown to provide benefit in uncontrolled studies in RA [1,12].

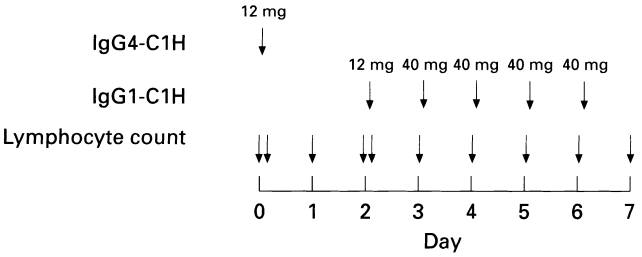

Fig. 1. MoAb administration protocol. Patients were administered 12 mg IgG4-C1H on day 0, following baseline bloods. Peripheral blood lymphocyte counts were monitored during the ensuing 48 h, and 12 mg IgG1-C1H were given on day 2; 40 mg IgG1-C1H were given on the subsequent 4 days.

Monitoring adverse effects

Vital signs were recorded every 15 min during infusions. Adverse reactions were fully documented with regard to timing and symptomatology. First-dose 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 and 38·5°C, and/or rigor; $+++ =$ temperature rise to >38·5°C and/or hypotension and/or chest tightness. Hypotension was classified as a fall in systolic BP of greater than 30 mmHg to a systolic BP of 90 mmHg or below on two successive readings. Blood was drawn during any adverse reactions for subsequent cytokine measurements.

Lymphocyte counts

Lymphocyte counts were measured daily before each infusion, and post-infusion on days 0 and 2 (Fig. 1). Subsets were determined when total counts were sufficient by single-colour immunofluorescence for CD4, CD8, CD16 (natural killer (NK) cells) and CD19 (B cells).

In vitro *whole blood assay*

This assay measured tumour necrosis factor-alpha (TNF-α) release from patients' blood during incubation with therapeutic MoAb *in vitro*. Heparinized or defibrinated blood (0·5 ml) was incubated with each MoAb for 4 h at 37°C. Cells and plasma were then separated by centrifugation and TNF-α concentration determined in the plasma fraction as described below.

Cytokine measurements

Serum samples from blood taken during 'first-dose' reactions, and plasma samples from *in vitro* whole blood assays, were tested for TNF-α using a sandwich ELISA (R + D Systems, Abingdon, UK; sensitivity 4·4 pg/ml). Serum samples were also analysed for interferon-gamma (IFN-γ) using a sandwich ELISA (R + D Systems; sensitivity 3 pg/ml).

Antiglobulin response

Serum samples (taken on day 21 and then monthly for 6 months) were tested for antiglobulin reactivity in a double-capture ELISA [24]. This assay used IgG1-C1H as capture and detection MoAb.

Consequently, an anti-idiotype response and an anti-IgG1 anti-isotype response could be differentiated by selective absorption. An anti-IgG4 anti-isotype response would not have been detected, but the study was designed to minimize the likelihood of such a response (see Discussion). Furthermore, significant anti-isotype responses have not been reported in recipients of humanized MoAbs [1,12,13].

Antibody levels

Blood was drawn immediately upon completion of IgG4-C1H infusion, and on days 1 and 2, for determination of serum MoAb concentration by immunofluorescence. Human peripheral blood lymphocytes (PBL; 5×10^5) from a normal donor (suspended in wash buffer (PBS containing 0·2% bovine serum albumin and 0·01% sodium azide)) were incubated for 1 h on ice with an equal volume of patient serum (heat-inactivated at 56°C for 30 min). After extensive washing, bound MoAb was sought with FITC-conjugated monoclonal mouse (anti-human IgG4) (Sigma, Poole, UK; no. F9890), diluted 1:100 in wash buffer containing 10% heat-inactivated normal rabbit serum. Cells were fixed, and relative fluorescence intensity measured using a FACScan (Becton Dickinson, Mountain View, CA). A standard curve obtained using known concentrations of IgG4-C1H (diluted in heat-inactivated normal human serum) enabled determination of absolute serum concentrations. The assay sensitivity was 80 ng/ml of IgG4-C1H.

ADCC

Prior to therapy, fresh peripheral blood mononuclear cells (PBMC) were isolated from each patient by density gradient centrifugation. They were assessed for their ability to mediate ADCC using the two forms of therapeutic MoAb, as previously described [6]. This assay measures killing mediated via FcγRIII [25].

Proliferation assays

Prior to therapy, PBMC were also assayed for proliferation in the presence of murine IgG1 and IgG2a anti-human CD3 MoAbs. PBMC were resuspended in Dulbecco's modified Eagles' medium (DMEM) containing 10% (v/v) heat-inactivated human AB serum. Cells (10^4–10^5) were added to round-bottomed microtitre plates containing titrations of the above CD3 MoAbs, or aglycosyl human IgG1 anti-CD3. Proliferation was measured after 72 h at 37°C by ^{3}H-thymidine incorporation. This assay correlates with a known polymorphism in human FcγRII. Approximately 70% of a normal population proliferate to both murine MoAbs, the remainder only with mouse IgG2a [26]. The aglycosyl human IgG1 is non-mitogenic [27].

RESULTS

Patients

Nine patients had RA, and one AS. Median age was 53·5 years, disease duration 11 years, and DMARDs used, four. Eight of the nine RA sufferers were seropositive for rheumatoid factor and four were taking prednisolone (3–10 mg/day). In addition to RA, patient 8 had insulin-dependent diabetes mellitus and patient 3 inflammatory bowel disease (IBD).

Lymphocytes (Fig. 2a)

PBL fell from a mean value of $1{\cdot}95 \times 10^9/l$ pre-treatment to $0{\cdot}27 \times 10^9/l$ immediately post-treatment with IgG4-C1H (86% depletion). There was an increase to a mean of $0{\cdot}52 \times 10^9/l$ at 24 h (73% depletion) and $0{\cdot}67 \times 10^9/l$ at 48 h (66% depletion). At that time seven patients had PBL between 0·31 and $0{\cdot}63 \times 10^9/l$, whilst three had counts remaining above $1 \times 10^9/l$. Percentage depletion at 48 h varied between 17% and 84% for individual patients (Table 1). Three patients (3, 6 and 10) were taking 5 mg or more of prednisolone during the study, and depletion at 48 h was >80% in these patients. No other clinical features appeared to relate to the degree of depletion with IgG4-C1H. After 12 mg IgG1-C1H, PBL fell to a mean value of $0{\cdot}11 \times 10^9/l$ (83% depletion from pre-IgG1-C1H value). Following 5 days of this MoAb, counts remained low for several months, consistent with previous reports (data not shown).

Subsets

Figure 2b shows mean lymphocyte subset values of all patients before treatment with IgG4-C1H and of three patients 48 h after therapy. (Total PBL were too low for accurate subset analysis in six patients at the latter timepoint and there was minimal depletion in a further patient in association with a low circulating MoAb level (*vide infra*).) In the patients analysed, $CD4^+$ cells were depleted disproportionately, forming 55% of PBL before treatment and 25% after. In contrast, the $CD8^+$ subset comprised 22% before and 39% after treatment.

First dose reaction

Reactions on initial dosing were similar in type and severity with both MoAbs and usually comprised chills and fever (Table 2). Two patients (7 and 10) developed significant hypotension with IgG4-C1H compared with one with IgG1-C1H (patient 8, who received a higher initial dose of MoAb). Two patients experienced no reaction with IgG4-C1H, whereas all patients reacted to IgG1-C1H. In only one patient was the reaction to IgG4-C1H more severe than that to IgG1-C1H.

Cytokine release

TNF-α release into the circulation was higher following IgG1-C1H, although the range of values was broad. Table 3a shows median values at the start of first-dose reactions and at the end of

Table 1. Antibody-dependent cell-mediated cytotoxicity (ADCC) and proliferation assays. ADCC results illustrate percent killing at an E:T ratio of 25:1 (0 = no killing). Proliferation is expressed relative to a negative control*

	ADCC		Proliferation		
Patient	IgG4	IgG1	mIgG1	mIgG2a	Percent depletion (IgG4)
1	24	19	+	+	47
2	0	0	−	+	47
3	9	15	+	+	84
4	0	0	N/A	N/A	47
5	0	0	+	+	53
6	0	0	+	+	83
7	16	15	+	+	70
8	10	19	+	+	17
9	0	0	+	+	71
10	20	38	+	+	82

* Median (range) of absolute ct/min with each MoAb: mIgG1 (excluding patient 2): 22 020 (3155–112 631); mIgG2a: 20 249 (4393–90 452); aglycosyl hIgG1 (negative control): 269 (107–1428).

N/A, Not available. Percentage depletion with IgG4 refers to depletion at 48 h.

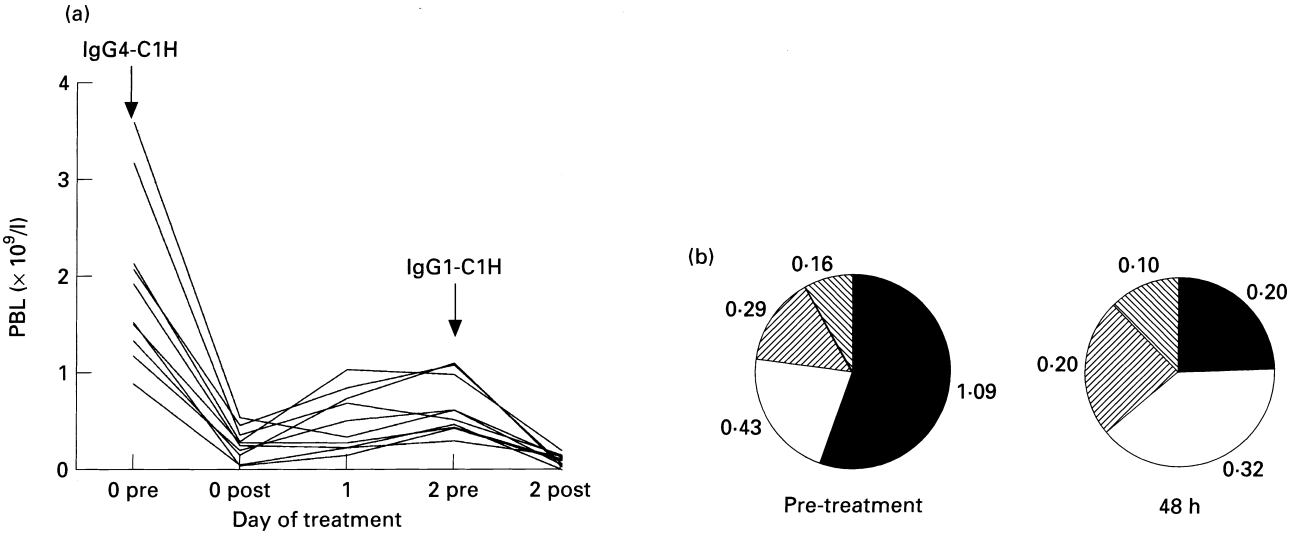

Fig. 2. (a) Peripheral blood lymphocyte (PBL) counts during the first 2 days of the therapeutic protocol. Blood was analysed before and after administration of both MoAbs. Each line represents an individual patient. (Note that the abscissa is not to scale). (b) Pie charts illustrating PBL subsets before and 48 h after treatment with IgG4-C1H. Only three patients were suitable for post-treatment analysis (see text). Number represent the mean count for each subset. Staining was for CD4, CD8, CD19 (B cells) and CD16 (natural killer (NK) cells). ■, CD4; □, CD8; ▨, B cells; ▧, NK cells.

MoAb infusions. Although IgG1-C1H was associated with higher circulating TNF-α levels at the end of infusions, this was not so at the start of first-dose symptoms, although data were only available for four IgG1-C1H patients at the earlier time-point. Very high levels of TNF-α (>1000 pg/ml) were measured in patient 8 following an initial dose of 40 mg IgG1-C1H. This patient had a more marked reaction to IgG1-C1H, in keeping with observations that the first-dose reaction to this MoAb was dose-related [1]. IgG1-C1H data for this patient are excluded from Table 3a. TNF-α release from whole blood *in vitro* was approximately 10 times greater with IgG1-C1H (Table 3b). There was no discernible relationship between *in vitro* and *in vivo* TNF-α release for individual patients and, apart from patient 8, there was no relationship between TNF-α release *in vivo* and the magnitude of the first-dose reaction to either MoAb.

Post-infusion serum samples from nine patients were also analysed for levels of IFN-γ. Low levels (<10 pg/ml) were detected in four patients after IgG1-C1H (including patient 8), but none was detected after IgG4-C1H (data not shown).

Table 2. First-dose reactions to IgG4–C1H and IgG1-C1H

Patient	IgG4	IgG1
1	+++	+
2	0	++
3	+++	+++
4	+++	+++
5	++	++
6	+	++
7	+++	+++
8*	++	+++
9	0	+
10	+++	+++

*This patient received a first dose of 40 mg IgG1-C1H with a 96-h break between phases 1 and 2 (see text).

+, Temp $\leqslant$ 37·5°C; ++, temp 37·6–38·5°C; +++, temp >38·5°C or hypotension (fall in systolic BP >30 mmHg to systolic BP $\leqslant$ 90 mmHg) or chest tightness.

Chill without fever = +; rigor without fever = ++.

The mean time to onset of symptoms was 3·3 ± 1 h for IgG4-C1H and 1·9 ± 1 h for IgG1-C1H.

ADCC and proliferation assays

Table 1 illustrates the results of ADCC and proliferation assays. Significant ADCC was observed in five patients. Of these, three showed higher levels of killing with IgG1, with equivalence in the other two (patients 1 and 7, equivalence defined as values within 30% of each other). At an effector:target ratio of 25:1, killing with IgG4-C1H ranged from 9% to 24%, but neither the absolute value nor potency relative to IgG1-C1H predicted *in vivo* depletion (Table 1). Neither rheumatoid factor status nor concurrent treatment with prednisolone appeared to influence these results. All but one of the patients tested proliferated to both mouse IgG1 and mouse IgG2a anti-CD3 MoAbs. There was no relationship between these data and depletion with IgG4-C1H *in vivo*.

Antiglobulin responses

A weak anti-idiotype response was detected in one patient, 7 weeks after treatment, equivalent to approximately 100 ng/ml of the monoclonal anti-CD52 anti-idiotype YID 13.9 [24].

Serum IgG4-C1H levels

Serum MoAb levels immediately post-IgG4-C1H infusion ranged between 0·09 and 0·91 μg/ml. There was a trend for lymphocyte depletion to vary with peak MoAb levels, but this did not reach statistical significance ($r = 0{\cdot}565$, $P = 0{\cdot}089$, Fig. 3).

Clinical data

The clinical efficacy of the therapeutic regime was similar to that reported previously in uncontrolled studies of CAMPATH-1H

Table 3. Tumour necrosis factor-alpha (TNF-α) release *in vivo* (a) and *in vitro* (b)

a. *In vivo**

IgG4		IgG1	
Start of symptoms	End of infusion	Start of symptoms	End of infusion
35 (19–296) $n = 8$	49 (16–272) $n = 9$	246·5 (153–1264) $n = 4$	126·5 (62–297) $n = 8$

*$P = 0{\cdot}05$ for difference between circulating TNF-α levels at the end of infusions. No significant difference between circulating TNF-α levels at start of symptoms.

P values calculated by log-rank method.

b. *In vitro*†

IgG4		IgG1	
2·5 μg/ml	10·0 μg/ml	2·5 μg/ml	10·0 μg/ml
8 (0–51) $n = 9$	22 (2–162) $n = 9$	95 (4–335) $n = 9$	266 (8–1007) $n = 9$

†$P \leqslant 0{\cdot}004$ for difference in TNF-α release by 2·5 μg of MoAb; $P \leqslant 0{\cdot}02$ for difference in TNF-α release by 10 μg of MoAb.

P values calculated by log-rank method.

TNF-α was measured in blood at the start of 'first-dose' reactions, and at the end of the first infusion of each MoAb. TNF-α was also measured in whole blood *in vitro*, following incubation with each MoAb. Data show median values (range) in pg/ml, and *n* represents number of samples analysed. Patient 8 was excluded from the IgG1-C1H analysis in (a); this patient received a 40 mg first dose of IgG1-C1H.

[1,12] and will not be discussed further (data not shown). Furthermore, the study design did not allow an assessment of therapeutic potential for IgG4-C1H if used alone.

Adverse reactions were noted in six patients. Four suffered minor infections (Herpes labialis ($n = 3$), urinary tract infection (UTI) ($n = 2$), and oropharyngeal candidiasis ($n = 1$)) during the first 8 weeks post-treatment. Patient 5 developed dyspnoea and deteriorating lung function around day 100. Chest x-ray remained normal and investigations did not reveal an infectious cause, although urinary direct early antigen fluorescence (DEAFF) test was positive for cytomegalovirus (CMV). Symptoms settled spontaneously without specific therapy, and bronchoscopy was not performed. Patient 3 was well until day 55, when there was a flare of IBD and a rapid deterioration of renal function. No cause was found for the renal failure, which recovered following a period of haemodialysis, broad-spectrum antibiotics and treatment for IBD.

DISCUSSION

This study was designed to investigate the *in vivo* biological activity of a therapeutic MoAb of IgG4 isotype, specifically its ability to deplete PBL and to provoke a cytokine-release reaction. Our protocol provided a 48-h window for monitoring these effects. An extended time period between administration of the two MoAbs would have provided additional data, but an isolated infusion of IgG4-C1H may have encouraged the development of an anti-globulin response which could have reduced the subsequent efficacy of IgG1-C1H [13]. An inter-patient comparison of the two MoAbs may also have provided additional information, but it was felt necessary that all patients received 5 days therapy with IgG1-C1H, since the latter MoAb had provided benefit in uncontrolled studies in RA, whereas IgG4-C1H was previously untested *in vivo*. Within this framework, our results recommend caution in the design of MoAbs for therapy, demonstrating that *in vitro* data do not automatically predict *in vivo* activity.

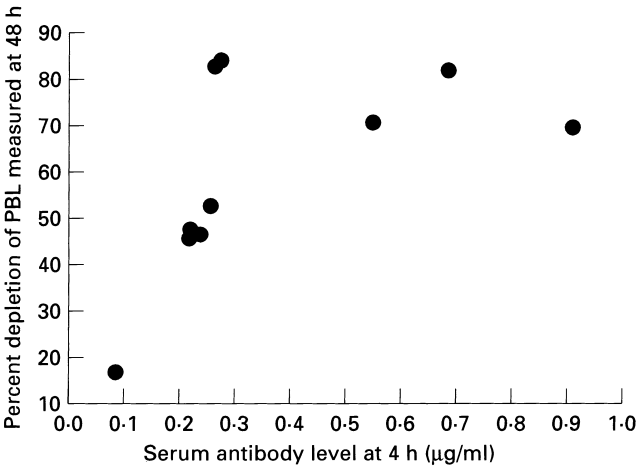

Fig. 3. Relationship between peak serum IgG4-C1H levels, and depletion of circulating lymphocytes measured at 48 h. Each point represents an individual patient. $r = 0{\cdot}565$, $P = 0{\cdot}089$.

Although IgG4-C1H demonstrated limited effector function *in vitro* [4,10], the existence of a polymorphism for ADCC with this MoAb [6], together with the ability of an IgG4 CD8 MoAb to deplete murine target cells *in vivo* [7], led us to examine the biological properties of IgG4-C1H in human subjects. In fact, a peripheral blood lymphopenia was present immediately following infusion of IgG4-C1H, with a mean 86% depletion (Fig. 1). Subsequently there was some recovery of PBL, with 73% depletion at 24 h and 66% at 48 h, presumably reflecting the return of 'sequestered' cells to the circulation. The study design did not permit monitoring of PBL beyond 48 h to look for further change, but there was no significant difference between counts at 24 and 48 h, suggesting stabilization (log rank test). Furthermore, more consistent depletion may have been seen with higher MoAb doses, in view of a possible relationship between peak serum MoAb concentration and lymphopenia (Fig. 3). (IgG1-C1H was not administered until day 5 in patient 8, but minimal depletion occurred in this patient, PBL being close to baseline at 48 h.) In previous studies patients with lymphoid malignancies received Campath-1 MoAbs differing only in isotype to those used here [11]. Non-depleting rat IgM and IgG2a versions provoked an immediate fall in PBL, but a rebound to pretreatment values was documented within hours of the injection. Depleting rat IgG2b gave slower repletion, similar to that seen with IgG4-C1H in the current study. Elsewhere, patients have received single doses of IgG1-C1H [16,28] and, regardless of route of administration,

10 mg or more produced at least 90% depletion of PBL with slow recovery, median PBL exceeding 500 cells/mm^3 after a minimum of 2 months.

Thus, IgG4-C1H has depleting powers intermediate between potent IgG1-C1H and non-depleting IgM and IgG2a Campath-1 MoAbs, although its mechanism of action remains uncertain. IgG4-C1H does not bind complement C1q, nor activate C3 or C4 [4]. Whilst not excluding alternative pathway activation, this makes complement-mediated clearance an unlikely mode of action. Furthermore, the IgM Campath-1 MoAb consumed complement without depleting, whereas the IgG2b version depleted but did not reduce total haemolytic complement levels [11]. Neither *in vitro* proliferation (FcγRII) nor ADCC (FcγRIII) predicted *in vivo* outcome (Table 1), but as peak MoAb levels varied almost 10-fold, with a possible link to depletion (Fig. 3), a role for these receptors cannot be excluded definitively. Furthermore, only five patients displayed significant ADCC, and poor ADCC has previously been noted in RA patients. This has been attributed in part to rheumatoid factor-containing immune complexes binding and modulating FcγRIII [29], but the rheumatoid factor-negative RA sufferer in our study also did not support ADCC. Of Fcγ receptors, human IgG4 binds most strongly to FcγRI [30], but whilst it seems most likely that depletion occurred via this interaction, non-conventional effector mechanisms should be considered as well. For example, glucocorticoids, activating stimuli and a variety of MoAbs induce apoptosis of mature lymphocytes [31], and in the current study, patients who were receiving more than 5 mg prednisolone daily had all cleared more than 80% of PBL within 48 h of IgG4-C1H administration. Furthermore, a high proportion of CD4$^+$ lymphocytes are activated in RA, and MoAb-induced apoptosis could also explain the disproportionate depletion of CD4$^+$ PBL (Fig. 2a).

The 'first-dose reaction' to IgG4-C1H was similar in severity to that seen following IgG1-C1H 48 h later (Table 2). These reactions are not directly comparable, however, as 'tachyphylaxis' usually develops after initial dosing with Campath-1 MoAbs and minimal reactions occur on subsequent days [1,12]. In fact, the data support IgG4-C1H being less toxic than IgG1-C1H: equal toxicity should have resulted in a lesser reaction to IgG1-C1H. Furthermore, two patients did not react to IgG4-C1H, whereas all patients receiving more than 1 mg IgG1-C1H via the i.v. route developed a reaction [1,16].

TNF-α was measured in blood taken during first-dose reactions and at the end of infusions (Table 3). Levels measured at the end of infusions were significantly higher for IgG1-C1H. Although the mean 'peak' TNF-α level was seven times higher with IgG1-C1H, this did not reach statistical significance because the range of values was broad and data were only available for four IgG1-C1H patients. In patients with multiple sclerosis receiving 12 or 20 mg IgG1-C1H for the first time (without a prior dose of IgG4-C1H), circulating TNF-α levels ranged from approximately 200 pg to $>$1 ng/ml at the time of first-dose symptoms, similar to the range of values measured in our patients [19]. In the current study, mean time to onset of symptoms was 3·3 h with IgG4-C1H but only 1·9 h with IgG1-C1H and, as cytokines have a short serum half-life, a time course may have provided a better comparison between the isotypes. Patient 8 received a higher first dose of IgG1-C1H, suffered a more severe reaction and had higher circulating TNF-α levels, but with this exception there was no correlation between cytokine levels and reaction severity to either MoAb for individual patients (data not shown; patient 8 was excluded from the IgG1-C1H analysis in Table 3a). More TNF-α was released from the whole blood incubated *in vitro* with IgG1-C1H than with IgG4-C1H (Table 3).

Thus, IgG4-C1H depletes PBL with an associated first-dose reaction and release of TNF-α into the circulation. *In vitro* it also causes release of TNF-α from whole blood, but in all respects it is less potent than IgG1-C1H. Cytokine release and first-dose symptoms are dependent upon cross-linking of lymphocytes and monocytes by Fc receptors [27], and the differences between IgG4- and IgG1-C1H may therefore reflect their relative affinities for Fc receptors, particularly FcγRI [30]. Our observations challenge the traditional view of IgG4 as an inactive isotype, although target antigen also has a profound effect on effector function [32–34] and Campath-1 provides an excellent target for MoAb-mediated lysis [9]. IgG4 MoAbs of other specificities may not deplete as effectively, but, if a pure blocking effect is required *in vivo*, an aglycosyl MoAb variant [7,27], or a mutant engineered to prevent Fc receptor binding [35], may be better choices.

The incidence of adverse reactions in this study was similar to previous reports, and a weak anti-idiotype response was detected in just one patient. A significant drawback of IgG1-C1H therapy for autoimmunity is the long duration of lymphopenia following treatment [15,16]. Whilst the full significance of this is uncertain, there is an infection risk [1,16,28] which detracts from the otherwise impressive clinical results achieved in a variety of settings [12–14]. Animal studies suggest that a combination of lymphocyte depletion followed by blockade may be required to control a large autoreactive lymphocyte pool [17]. Furthermore, B cells, T cells and monocytes are all involved in the immunopathogenesis of RA, and all express CD52. In this context it would be interesting to explore further the lymphocytotoxic properties of IgG4-C1H. A MoAb which provided dose-dependent lymphocyte depletion could prove ideal for the immunotherapy of RA, by facilitating subsequent blockade with non-depleting MoAbs of appropriate specificity.

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