The Effect of Intravenous Administration of a Chimeric Anti-IgE Antibody on Serum IgE Levels in Atopic Subjects: Efficacy, Safety, and Pharmacokinetics

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Abstract

CGP 51901 is a non-anaphylactogenic mouse/human chimeric anti–human IgE antibody that binds to free IgE and surface IgE of IgE-expressing B cells but not to IgE bound to high affinity IgE receptors (FcεR1) on mast cells and basophils or low affinity IgE receptors (FcεR2) on other cells. A phase 1 double-blind, placebo-controlled, single dose study with doses of 3, 10, 30, and 100 mg of CGP 51901 was conducted in 33 pollen-sensitive subjects who had raised levels of serum IgE and received either intravenous CGP 51901 or placebo. The administration of CGP 51901 was well tolerated and resulted in a decrease of serum free IgE levels in a dose-dependent manner, with suppression after 100 mg of CGP 51901 reaching > 96%. Time of recovery to 50% of baseline IgE correlated with the dose of administered antibody and ranged from a mean of 1.3 d for the 3 mg to 39 d for the 100 mg dose. Total IgE, comprised of free and complexed IgE, increased as stored and newly synthesized IgE bound to CGP 51901. Complexed IgE was eliminated at a rate comparable with the terminal half-life of free CGP 51901 (11–13 d at all doses). Only one subject showed a weak antibody response against CGP 51901. We conclude that the use of anti–human IgE antibody is safe and effective in reducing serum IgE levels in atopic individuals and provides a potential therapeutic approach to the treatment of atopic diseases. (*J. Clin. Invest.* 1997. 99:879–887.) Key words: IgE • atopic hypersensitivity • rhinitis • monoclonal antibodies • chimeric proteins

Introduction

Atopic diseases are a major cause of morbidity and mortality. The prevalence of both asthma and allergic rhinitis are rising and at present are estimated to be 10–20% (1). In the United States asthma alone has been estimated to affect between 9 and 12 million people, lead to 1.81 million emergency room visits per year, and cause an annual loss of nearly 3 million working days (2).

Epidemiological studies have suggested a link between circulating IgE and atopic disease. With regard to asthma, levels of circulating IgE have been shown to correlate with symptom severity (3), bronchial hyperresponsiveness (4, 5) and risk of emergency room admissions (6). In seasonal rhinitis levels of circulating IgE have been shown to increase in parallel with symptoms during the pollen season (7, 8).

IgE binds to the α chain of the high affinity receptor (FcεR1)[1] on mast cells and basophils and to the low affinity receptor (FcεR2) on monocytes/macrophages, lymphocytes, epithelial cells, and dendritic cells. Allergen cross-linking of IgE bound to mast cells results in activation of these cells and release of a range of preformed and newly generated vasoactive and bronchoconstrictive substances leading to the immediate hypersensitivity type responses such as the early phase of airway obstruction that follows experimental allergen challenge. The identification of the cytokines IL-4, IL-5, IL-6, and TNFα within the mast cell (9) and demonstration of their release after IgE cross-linking (10–12) also suggests an important role for IgE in the late phase of airway obstruction and the associated increase in bronchial hyperresponsiveness.

Recent work has highlighted the potential importance of IgE with regard to T cell responsiveness. Antigen–IgE complexes bound to FcεR2, and possibly the FcεR1 receptors recently identified on antigen presenting cells (13, 14), have been shown to amplify the T cell response to allergen by facilitating antigen presentation to antigen-specific T cells (15), and, consistent with this, a recent study has shown that treatment of mice with a monoclonal anti–mouse IgE antibody inhibits the T cell production of IL-4 and the airway eosinophilia resulting from challenge with house dust mite antigen (16). This response was not mast cell-dependent since mast cell-deficient mice develop a normal eosinophilic response to antigen provocation (17) but rather, it appeared to be mediated through the low-affinity IgE receptor. It would appear that IgE, acting directly through FcεR2, plays a central role in the induction of a Th2-type response and forms part of a positive feedback loop leading to further increases in IgE and causing airway eosinophilia. Therapy effectively targeted against IgE, that interferes with its binding to both high and low-affinity receptors, should inhibit this amplification as well as reducing the early and late phase airway responses through inhibition of mast cell degranulation. Such a therapy would therefore be specifically directed against a central component of the inflammatory response in contrast to currently available treatments that are not specific, are often limited by side effects and have failed to

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1. *Abbreviations used in this paper:* FcεR1, high affinity IgE receptor; FcεR2, low affinity IgE receptor; HRP, horseradish peroxidase; PBSTT, PBS plus 0.5 ml/liter Tween 20 and 0.1 g/liter Thimerosal.

stem the increase in morbidity and mortality associated with atopic diseases.

CGP 51901 is a mouse/human chimeric anti-IgE antibody produced by recombinant DNA methodology. It consists of the heavy and light chain variable regions of a parent murine antibody and the heavy and light chain constant regions of the human κ and γ1 antibody isotypes (18). It binds to the low- and high-affinity receptor-binding portions of human IgE located in the Cϵ3 domain. It therefore binds to circulating IgE and IgE expressed on B cells since the IgE molecule is anchored within the B cell membrane with the Cϵ3 domain exposed. CGP 51901 does not bind to IgE bound to mast cells and basophils or cells bearing the FcϵR2 receptor since the epitope on IgE against which it is directed is attached to and thus hidden within the receptor. Consequently it does not trigger anaphylaxis nor cause lysis of cells bearing IgE receptors. Chimerization of the antibody results in a reduction of its potential immunogenicity in man and an increase in its expected half-life (19) since 90% of the antibody response is directed against the constant region (20). The utilization of the γ1 constant region optimizes its potential for complement and cell-mediated lysis of IgE-expressing B cells (21).

After extensive in vitro and animal experiments we have hypothesized that administration of CGP 51901 to humans would be safe and effective in reducing levels of circulating IgE. We have therefore performed a phase 1 study in which we have administered CGP 51901 intravenously in increasing doses to atopic, non-asthmatic individuals sensitive to grass pollen. Volunteers were monitored for effects on serum levels of IgE as well as levels of CGP 51901, development of anti-CGP 51901 antibodies and other clinical and laboratory markers of safety. As a secondary outcome variable, effects on skin prick tests to pollen allergen were also measured.

Methods

This was a double-blind, placebo-controlled randomized, single dose, sequential group study of ascending doses of 3, 10, 30, and 100 mg of CGP 51901. The study was approved by the Southampton University and Hospital Joint Ethics Committee and was conducted in accordance with the Declaration of Helsinki. All volunteers gave their written informed consent.

33 male volunteers aged 18 to 60 years participated in the study. All had positive skin prick tests to mixed grass pollens, a positive radio allergen absorbance test (RAST) to the same grass pollen mix, and total serum IgE levels between 100 IU/ml and 850 IU/ml at the time of screening. None had a history of asthma or non-seasonal rhinitis, parasitic infections, previous anaphylactic reactions or allergy to mice. None were taking phenothiazines, oral corticosteroids or tricyclic anti-depressants and none suffered from any other concomitant disease. As a further safety measure an intradermal skin test to CGP 51901 was performed on each volunteer and any with a positive reaction to CGP 51901 (a wheal 3 mm greater than the negative, saline control) was excluded from the study. The protocol did not include skin testing with CGP 51901 after the infusion since the repeated intradermal administration of a protein is known to be highly effective in stimulating an immune response.

Skin prick testing. Skin prick testing was performed using a combination of 5 grass pollens (Ky Blue, Meadow Fescue, Orchard, Per Rye, Redtop, Sweet Vermal, Timothy) (Dome/Hollister Stier, Washington) using the puncture technique (22). Tests were considered positive if they caused a wheal with a diameter 3 mm greater than that of the negative, saline control. For follow up the area of the skin test wheals were measured by planimetry.

Screening visit. After taking a full medical history and performing a physical examination an electrocardiograph was performed and 35 ml of blood drawn for differential cell count, liver function tests, urea and electrolytes, and levels of total serum IgE, complement C4 and circulating globulins. An abnormal result (exceeding 2 standard deviations) for any of these variables (except IgE) led to exclusion from the study.

Study design. Volunteers were dosed in groups of nine, within each group six receiving intravenous CGP 51901 in 40 ml of phosphate buffered saline (PBS) with 0.02% polysorbate 80 and three receiving placebo consisting of 40 ml of PBS with 0.02% polysorbate 80 only. Initially 0.1 mg of CGP 51901 or an equivalent volume of placebo was infused via a syringe pump (IVAC P2000, IVAC Ltd., Basingstoke, UK) over 5 min. Volunteers were then monitored for signs of hemodynamic compromise over 30 min and, if stable, the remainder of the intravenous dose was administered over a further 30 min. Blood was drawn for measurements of IgE and CGP 51901 levels as well as immune and biochemical parameters after 30 min, 1, 2, 4, 6, 8, and 24 h and then at 5, 8, 11, 15, 22, 29, 43, 57 d and 3 mo after dosing. Volunteers were then seen at monthly intervals to monitor recovery of IgE levels. Skin prick tests to grass pollen were performed at all visits from day 5 onwards.

Measurement of CGP 51901. Serum levels of CGP 51901 were measured by an enzyme linked immunosorbent assay (ELISA) based on a monoclonal anti-idiotypic antibody, (CA69-76-5) to CGP 51901 which was conjugated to horseradish peroxidase (HRP) and acted both as a capturing and signalling antibody. Briefly, Immulon 2 Microtiter plates were coated overnight at room temperature with 100 μl/well of an anti-idiotypic antibody-coating solution with an antibody concentration of 0.5 μg/ml in CMF-PBS-pHix (calcium and magnesium free phosphate buffered saline containing 80 μl/liter pHix buffer preservative obtained from Socochim SA, Lausanne, Switzerland). After removal of the coating solution, unoccupied binding sites were blocked by incubation at 22°C with 200 μl/well of Blotto (PBS plus 0.5 ml/liter Tween 20 and 0.1 g/liter Thimerosal containing 5% non-fat dry milk powder for 2 h (23, 24). The plates were then washed four times with 200 μl/well of PBS plus 0.5 ml/liter Tween 20 (Fluka Chemicals AG, CH-9470 Buchs, Switzerland) and 0.1 g/liter Thimerosal (PBSTT) (Sigma Chemical Co., St. Louis, MO). To prepare the standards, CGP 51901 was diluted in Blotto at concentrations ranging from 2 ng/ml to 50 ng/ml. 10 μl of either standard, serum sample or control were then added in triplicate to the appropriate wells already containing 40 μl of Blotto. After 3 h at room temperature the solution was decanted, the plates washed again and equilibrated at 22°C for 1 h in the dark with 50 μl of the HRP conjugate solution diluted to 0.25 μg/ml in Blotto. After decanting and washing, the wells were incubated with 100 μl of freshly prepared tetramethylbenzidine (0.1 ml of a 1% tetramethylbenzidine (E Merck, Zurich, Switzerland) in dimethylformamide, 0.2 ml 1 M sodium acetate and 12 μl 3% H_2O_2 added to 90.7 ml deionized water) for 30 min at 22°C in the dark. The reaction was stopped by adding 50 μl/well of 1 M H_2SO_4. Absorbance was measured at 405 nm in a microplate reader (Molecular Devices, Type V_{max}, Paul Bucher, Analytil und Biotecnologie, Basel, Switzerland) and the data processed with RIASmart™ software package (Canberra Packard Int SA, Zurich, Switzerland) using a smooth line curve-fitting program. The working range of the assay was between 2 and 50 ng/ml.

Measurement of serum total IgE. The assay for total IgE was a chemiluminescence sandwich immunoassay based on a mouse anti-human IgE mAb covalently coupled to paramagnetic particles (Ciba Corning Diagnostics, East Walpole, MA), on magnetic separation of bound from unbound reagents and on a revealing anti-IgE mAb labelled with an acridinium ester (Ciba-Geigy Ltd, Basel, Switzerland). This assay measures both uncomplexed (free) IgE and IgE complexed with CGP 51901. CGP 51901 binding to IgE does not interfere with the binding of either the capturing or the revealing antibody of the assay. Since the assay was designed to measure both free and CGP 51901-complexed IgE, all samples were preincubated with a

large molar excess of CGP 51901 before the assay in order to establish qualitative IgE complexing and therefore reduce heterogeneity of the reactant. For the assay, 50 μl of serum were mixed with 50 μl of CGP 51901 in phosphate buffered saline containing 0.5 g/liter Tween 20 (Fluka Chemie AG, Switzerland), 10 g/liter bovine serum albumin (Fluka Chemie AG, Switzerland) and 0.2 ml/liter of 10% NaN_3 (E Merck, Zurich, Switzerland) resulting in a final concentration of 40 μg/ml of CGP 51901. After preincubation for 1 h at 37°C, 25 μl of this solution was mixed with 500 μl of solid phase suspension and the mixture incubated for 2 h at room temperature. The unbound material was removed and the solid phase washed once with 1 ml of deionized water before equilibration with the Lite Reagent prepared from a purified anti–human IgE mAb–AB 669-6-7 (Asthma and Allergy Research, Ciba-Geigy Ltd.) coupled to an acridinium ester (Ciba Corning Diagnostics). After 3 h incubation at room temperature, excess solution was removed and the bound material washed twice with 750 μl of millipore water and placed in a luminometer (Magic Lite Analyzer II, Ciba Corning Diagnostics). The standard curve, based on a built-in master curve, was adjusted to the condition of the assay by means of a two-point calibration system. The working range of the assay is between 2 and 460 IU/ml.

Measurement of free IgE in serum. Free IgE was measured using an ELISA technique with CGP 51901 as the capture antibody. This avoids detection of IgE that is already complexed with CGP 51901 and thus measures both baseline IgE levels before infusion of CGP 51901 and remaining free (uncomplexed) IgE after infusion. A biotinylated monoclonal anti-IgE which was specific for an epitope of the IgE molecule different from that of CGP 51901 was used as the revealing antibody. To prepare IgE standards, a serum of known IgE concentration was diluted with PBS/BSA/TW (Phosphate buffered saline containing 1% bovine serum albumin (Fluka Chemie AG, Switzerland), 0.5 ml/liter Tween 20 and 0.2 ml/liter of a 10% NaN_3 solution) to concentrations between 1 and 144 IU/ml. M-129 B Dynatech 96-well ELISA-plates were coated overnight at room temperature with 50 μl/well of CGP 51901 coating solution (1 μg/ml in coating buffer). The coating solution was removed and unoccupied sites blocked by incubating overnight at 4°C with 200 μl/well of assay buffer. After washing three times with 200 μl/well of PBSTT, standard point solutions, controls (50 μl) and test sera (5 μl in 45 μl of buffer) were added to the appropriate wells in triplicate and incubated for 1 h at room temperature. The plates were washed again and, after adding 50 μl/well of a 1:5000 dilution of the biotinylated antibody, incubated again for 1 h at room temperature. After washing they were incubated for a further 1 h at room temperature with 50 μl/well of avidin alkaline phosphatase (Calbiochem, 0.5 mg/ml, diluted 1:2000 in substrate buffer consisting of 97 ml/liter diethanolamine (E Merck, Darmstadt, Germany). 0.2 ml/liter of 10% NaN_3 (E Merck, Zurich, Switzerland) and 0.1 g $MgCl2.6H_2O$ (E Merck) dissolved in 900 ml deionized water with the pH adjusted to 9.8 with 10 M HCl). The washing was repeated and followed by addition of 150 μl/well of p-nitrophenyl phosphate (Sigma Chemical Co.) in substrate buffer. The mixture was incubated at room temperature for 20 min, the reaction stopped by adding 50 μl/well of 1N NaOH, and the optical density was read at 405 nm using a Dynatech MR 7000 ELISA reader (Microtec Produkte AG, Embrash-Embraport, Switzerland). The working range of the assay was between 9 and 500 IU/ml.

Analysis of serum IgE by HPLC. Serum samples were analyzed on a Beckman System Gold HPLC (Beckman, Nyon, Switzerland) using a Sorbax Bio-Series GF-450 molecular sieve column (Rockland Technologies, Newport, DE) equilibrated and developed in 0.2 M sodium phosphate buffer, pH 7.2, at a flow rate of 0.5 ml/min. 50 μl of serum were injected per column run. Fractions of 200 μl were collected and analyzed for IgE by the chemiluminescence assay for total IgE.

Measurement of serum anti-CGP 51901. The antibody response to CGP 51901 was assayed using a double-antibody capture ELISA based on CGP 51901 as the capture antibody and the revealing antibody. Endogenous IgE was blocked by pre-incubating samples with an excess of a mouse anti-IgE monoclonal antibody which binds to the same epitope as CGP 51901 and has a higher affinity for IgE. Standards were prepared using dilutions of Ab 69-76-5 in Blotto (0–60 ng/ml). Wells of Immulon 2 Microtest plates were coated overnight at room temperature with 100 μl/well of CGP 51901 in CMF-PBS-pHix (0.5 μg/ml). The coating-solution was removed and unoccupied binding sites were blocked by overnight incubation at 4°C with 200 μl/well of Blotto. The plate was then washed four times with 200 μl/well PBSTT. To neutralize endogenous IgE 50 μl of test serum was preincubated for 1 h at room temperature with 50 μl of Blotto containing mAb 669-1251 (Ciba-Geigy, Ltd.) (30 μl/well) and 20 μl of this solution was added to each well and incubated with 30 μl/well of Blotto for 2 h at room temperature. The plate was then washed again and 100 μl/well of HRP-CGP 51901 (0.3 μg/ml in Blotto) were added and incubated for 1 h at room temperature. After washing, 100 μl/well of peroxidase substrate (TMB) solution were added and incubated for 30 min at room temperature. The reaction was stopped by adding 50 μl/well of 1 N H_2SO_4 and the plates read at 450 nm using a Dynatech MR7000 ELISA reader. The concentrations of anti-CGP 51901 were expressed in ng equivalents/ml using the anti-idiotypic mAb against anti-CGP 51901 (Ab 69-76-5) as the reference. The lower limit of detection was 5 ng equivalents/ml of anti-idiotypic mAb.

All the assays were validated using normal sera to which known concentrations of IgE, CGP 51901 and IgE-CGP 51901 complexes had been added. Within the working range of the curve, intra and interassay coefficients of variation were $< 10\%$.

Basophil histamine release. To test the effect of administration of CGP 51901 on the release of mediators of the immediate phase reaction, 40 ml of blood was taken for assay of basophil histamine release immediately before administration of the infusion and at 6 h and 8 d after the infusion. The leukocytes were then separated into layers by density centrifugation (25). Briefly, the 40 ml of whole blood was diluted 1:1.5 with 0.9% saline and a cushion of 1.080 g/cm^3 Percoll (Pharmacia, Sollentuna, Sweden) was carefully underlaid followed by centrifugation for 13 min at 175 *g*. The upper layer was harvested and centrifuged at 100 *g* for 8 min. The cells were then washed twice in PAG (1,4–25 mM piperazinediethanesulphonic acid buffer, 110 mM NaCl and 5 mM KCl at pH 7.3 containing 0.003% wt/vol human serum albumin and 0.1% wt/vol D-glucose) and resuspended in PAGCM (PAG supplemented with 1 mM $MgCl_2$ and 1 mM $CaCl_2$). For histamine release assay cells were incubated at 37°C in the presence of buffer alone, 25% trichloroacetic acid, anti-IgE (Serotec, Oxford, UK) (0.01–1 μg/ml), formyl-methionyl-leucyl-alanine (Sigma Ltd., Poole, UK) (1 μM) or calcium ionophore A23187 (Sigma Ltd.) (1 μg/ml). Mediator release was allowed to proceed for 45 min and the cell solutions were then centrifuged at 150 *g* for 3 min. The supernatants were harvested. 250 μl of 25% trichloroacetic acid was added to each sample in order to precipitate protein and the supernatants were frozen. Histamine content of the samples was later determined by the automated fluorometric analysis method of Siraganian (26).

To determine the total histamine content within a sample, 0.5 ml of whole blood was lysed with 0.5 ml of 25% trichloroacetic acid and the solution was then centrifuged at 150 *g* for 3 min. The supernatants were harvested and analyzed for histamine content as above.

Pharmacokinetic modeling. Measurements of serum CGP 51901 were fitted to a two compartment open model by means of PC-SAS version 6.11 The relationship between the half-life of the first phase of elimination of CGP 51901 and baseline free IgE was calculated using the Analytical Tools Regression option of Excel version 4.0.

Results

All the volunteers completed all visits up to 3 mo after dosing.

Baseline characteristics. There was no significant difference between the volunteers receiving placebo and those receiving CGP 51901 in terms of age or baseline serum IgE levels (Mann-Whitney U test) (Table I).

Table I. Age of Volunteers and Baseline IgE for Placebo and Each Dosing Group

Group	Mean age (range)	Baseline IgE IU/ml (range)
Placebo	22.2	295.1
	(18–30)	(170–674)
3 mg	34.5	290
	(18–48)	(80*–446)
10 mg	31.5	359
	(20–49)	(99*–978*)
30 mg	19.8	239
	(18–22)	(131–521)
100 mg	34.2	395
	(19–52)	(168–1079*)

*IgE value was between 100–850 IU/ml at screening.

Intradermal skin testing to CGP 51901. There were no positive intradermal skin test reactions to CGP 51901.

Clinical effects. Throughout and after the infusion all volunteers remained hemodynamically stable with no signs or symptoms of anaphylaxis, serum sickness or allergic reactions. One patient receiving 100 mg of CGP 51901 developed a mild increase in complement C4 levels to 0.6 g/liter (upper limit of normal = 0.35 g/liter) at 6 and 8 h which returned to normal by 24 h.

Free IgE. In volunteers receiving placebo levels of free IgE were monitored for up to 375 d. Based on the mixed effect modelling of log (IgE), IgE levels in the volunteers receiving placebo fluctuated with a within subject variation of 28% and a between subject variation of 32%.

After infusion of CGP 51901 there was an immediate fall in serum levels of free IgE. Fig. 1, *a* and *b* show the time-course of free IgE and total IgE levels in one volunteer receiving 3 mg of CGP 51901 and one receiving 100 mg of CGP 51901, respectively. The initial reduction in the level of free IgE in the volunteer receiving 3 mg of CGP 51901 (Fig. 1 *a*) was from 335 IU/ml (baseline) to 41 IU/ml, and the reduction in the volunteer receiving 100 mg of CGP 51901 (Fig. 1 *b*) was from 226 IU/ml (baseline) to non-detectable levels within 0.5 h after infusion. These results are representative of all volunteers receiving these doses. Minimum concentrations (6.1–31.3% of baseline values) of free IgE were observed between 0.5 and 23 h in those receiving 3 mg and 10 mg of CGP 51901 respectively. In those volunteers receiving 30 mg of CGP 51901 the range of minimum free IgE was from 8% to 16.8% of baseline in the first 24 h and in the 100 mg group minimum free IgE was < 1% to 6.7% of baseline. Table II shows the extent of free IgE reduction and the time points at which minimum free IgE levels were reached for each dosing group. The mean times required for restoring levels of free IgE to within 50% of baseline were 1.3, 5.3, 15.6, and 39 d for those receiving 3, 10, 30, and 100 mg doses, respectively. Analysis of covariance (ANCOVA) demonstrated significant differences between minimum free IgE concentrations for all CGP 51901-treated volunteers compared with those receiving placebo ($P < 0.0001$ for all doses).

Table II. Mean Values of Maximum Total IgE (Mean C tot_{max}) and Minimum Free IgE (Mean C $free_{min}$) for Each Dosing Group and the Mean Time at Which Maximum Total (Mean T tot_{max}) and Minimum Free (Mean T $free_{min}$) Were Reached for Each Group (only Including Actively Treated Volunteers)

	Total IgE		Free IgE	
CGP dose	Mean T tot_{max} (range) h	Mean C tot_{max} (range) IU/ml	Mean T $free_{min}$ (range) h	Mean C $free_{min}$ (range) IU/ml
3 mg	53.9	495.8	5.7	53.8
	(6–96.17)	(289–671)	(0.5–23.67)	(25–70)
10 mg	136.3	700.8	3.17	41.7
	(20.8–334.6)	(332–1743)	(0.5–8)	(27–60)
30 mg	540.3	1147.3	0.5	27
	(101.3–648.8)	(443–2033)	(0.5–0.52)	(13–43)
100 mg	585.1	2317.0	2.3	7.3
	(502.6–719.3)	(1310–5497)	(0.48–7.98)	(0–10)

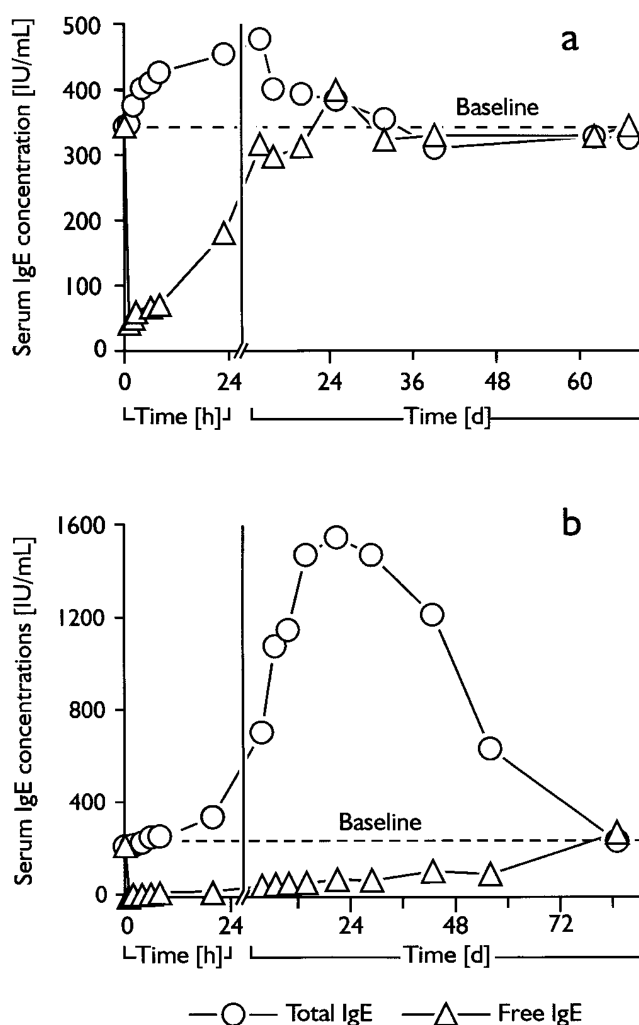

Figure 1. Serum concentrations of total (○) and free (△) IgE measured in a volunteer before (time 0) and after receiving an infusion of (*a*) 3 mg CGP 51901 and (*b*) 100 mg CGP 51901. Baseline (----) represents concentration of serum IgE before the commencement of the infusion.

Serum total IgE. Fig. 1, *a* and *b,* shows the time-course of levels of total IgE (circulating free + circulating IgE complexed to CGP 51901) in two representative volunteers receiving 3 and 100 mg of CGP 51901, respectively. After infusion of

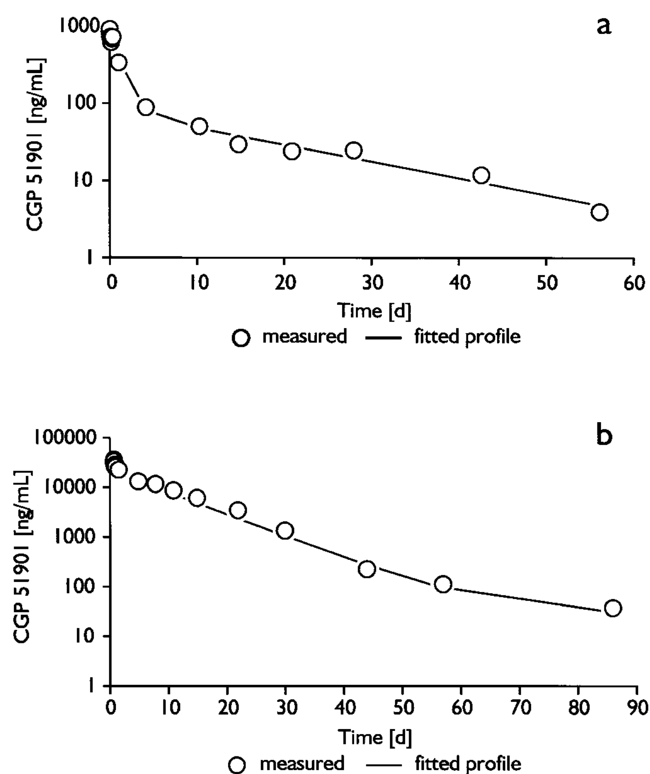

Figure 2. Serum concentrations of CGP 51901 in a volunteer after infusion of (*a*) 3 mg and (*b*) 100 mg of CGP 51901. (○) measured values; (—) fitted profile.

CGP 51901 the concentrations of total IgE increased in all treated volunteers. Maximum levels were up to 3.8-fold of baseline IgE in those receiving 3 mg of CGP 51901, 4-fold in those receiving 10 mg, 6.1-fold in those receiving 30 mg and 11.4-fold in the those receiving 100 mg of CGP 51901. Levels reached the maximum between 6 h and 4 d for those receiving 3 mg of CGP 51901, between 1 and 14 d for those receiving 10 mg, between 4 and 21 d for those receiving 30 mg and between 21 and 30 d for those receiving 100 mg. Maximum total IgE levels and the times at which these levels were observed are shown in Table II.

Serum CGP 51901. Fig. 2, *a* and *b,* illustrates the time-course of CGP 51901 levels in one volunteer receiving 3 mg of CGP 51901 and one receiving 100 mg, respectively. They are representative of the CGP 51901 levels in all volunteers receiving these doses of CGP 51901. Elimination of CGP 51901 closely fitted the computer-generated curve for a 2-compartment open model (27). The initial phase of elimination (α half-life) was negatively correlated with baseline IgE levels at all doses (Fig. 3) and positively correlated with the dose of CGP 51901. The second or terminal phase of elimination (β half-life) was similar for all doses ranging from 11–12 d, and was independent of both the baseline IgE and dose of CGP 51901. This phase was observed in all volunteers when CGP 51901 had reached a concentration approximately equivalent to 1 K_d (~ 100 ng/ml).

HPLC: analysis of serum IgE. The pharmacodynamic effects of CGP 51901 on serum IgE were further explored by HPLC analysis of serum samples at selected time-points. Fig. 4 shows the results from a volunteer treated with 30 mg of CGP 51901. This shows that the increase in total IgE between 1 and 10 d after infusion of CGP 51901 is associated with accumulation of complexes of IgE-CGP 51901. Between 1 and 4 d these are of heterogeneous sizes, presumably corresponding to bimolecular to tetramolecular complexes, whereas after 4 d tetramolecular complexes dominate. The serum half-life of IgE-CGP 51901 complexes has been estimated to be between 10 and 14 d based on numerous HPLC analyses and on the kinetics of serum total IgE decline after elimination of free CGP 51901 to or below its K_d concentration. Some complex dissociation occurs during dilution and separation during chromatography and this will cause trailing of originally complexed IgE into the peak area for free IgE thus leading to increased apparent levels of free IgE. Therefore, free IgE up to 10 d after treatment is less than is apparent in Fig. 4.

Anti-CGP. Only one volunteer, who received 10 mg of CGP 51901, demonstrated measurable levels of anti-CGP 51901 antibodies of 143, 130 and 121 ng equivalents/ml on days 13, 28, and 56, respectively. The elimination pharmacokinetics of CGP 51901 in this volunteer appeared similar to those of other individuals who received the 10 mg dose.

Basophil histamine releasibility. Fig. 5 *a* shows the effect of infusion of CGP 51901 on basophil histamine release in response to ex vivo challenge with anti-IgE. Two volunteers (one receiving placebo and one receiving 10 mg of CGP 51901) have been excluded from this analysis since their basophils released negligible (< 5%) histamine after challenge by all stimuli and so any change fell within the variability of background histamine release. There was no significant change in histamine release after treatment with either CGP 51901 or placebo in comparison with pretreatment levels and this was true for all doses of CGP 51901 administered. There were also no changes in spontaneous release of histamine from basophils (data not shown). In addition there were no changes observed in basophil histamine release in response to calcium ionophore A23187 or to the bacterial peptide fMLP at any of the time points for any of the doses tested (data not shown). Basal and anti-IgE induced histamine release was higher in the group receiving 100 mg of CGP 51901. This was due to one individual who had exceptionally high levels of basal histamine release (> 80%). If this individual is removed form the analysis there is still no significant effect of administration of CGP 51901 on basophil histamine release.

Total histamine content within each blood sample was also calculated. Fig. 5 *b* illustrates the histamine concentration, which represents both stored and secreted histamine expressed per ml of blood. There were no changes observed in total histamine levels for placebo and 10 and 30 mg of CGP 51901. Histamine levels were increased after administration of 100 mg of CGP 51901 but this did not reach statistical significance and was due to increases in 2 out of the 5 volunteers receiving this dose. Follow-up studies on these volunteers indicated that histamine levels remained high at 57 d in one individual but returned to baseline in the other.

Skin prick tests. There was no significant difference in skin test reactivity to mixed grass pollen between those volunteers receiving CGP 51901 and those receiving placebo.

Discussion

In this first study of its kind we have shown that intravenous administration of an anti–human IgE antibody is safe and effective in reducing free IgE levels in atopic human volunteers.

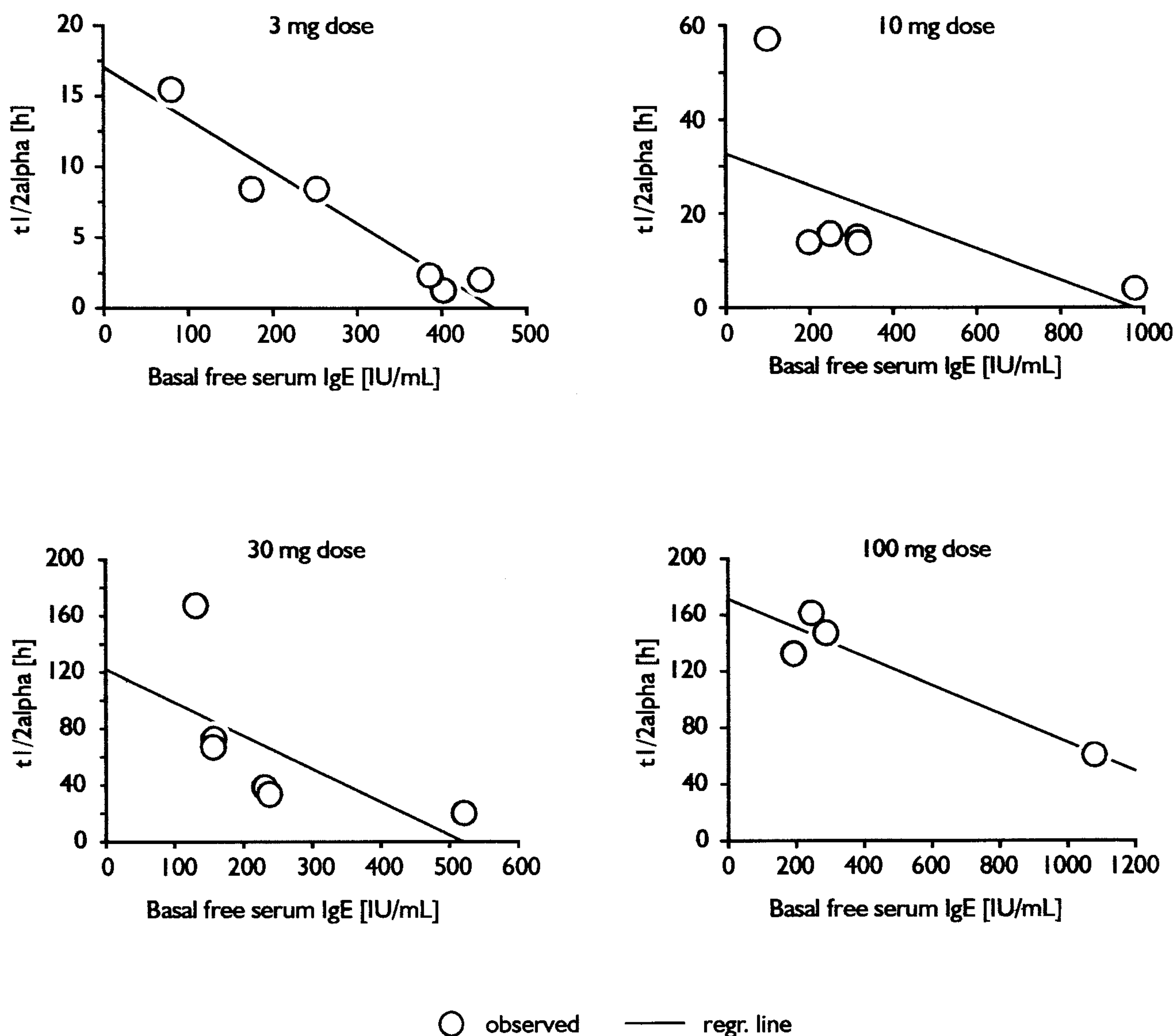

Figure 3. Relationship between the half-life of first phase elimination ($t_{1/2}$) of CGP 51901 and baseline free IgE for all doses of CGP 51901.

CGP 51901 was developed from an antibody selected from a panel of murine antibodies generated against human IgE (18). The parent antibody (TES-C21) was shown to bind with high affinity to free IgE and IgE expressed on the surface of B cells but not to induce histamine release from IgE-bearing basophils (18). Preincubation of IgE with the parent antibody prevented its binding to both high and low affinity IgE receptors and TES-C21 has been shown not to bind to cells bearing IgE already bound to high or low affinity receptors. This suggests that TES-C21 binds at or near the Fc receptor binding site of the IgE molecule located in its Cε3 domain (28), to an epitope that is concealed when IgE binds to FcεR1 and FcεR2 but is accessible on membrane anchored IgE of IgE expressing B cells. CGP 51901 is composed of the variable heavy (H) and light (L) chain regions of TES-C21 linked to the human γ1 and κ constant regions. It has been shown to have the same binding properties as its parent murine antibody (18) and in vitro studies have demonstrated that it does not bind to other immunoglobulin isotypes nor to other circulating cells (18).

We have demonstrated that CGP 51901 is highly effective in reducing the levels of circulating free IgE by trapping IgE in the form of stable IgE-CGP 51901 complexes. The rapid reduction of free IgE after infusion of CGP 51901 is compatible with the fast association rate constants that are characteristic for high affinity antibodies. The time for free IgE to return to baseline was dependent on the dose of CGP 51901 and was negatively correlated with baseline IgE levels. At the highest dose (100 mg) the amount of CGP51901 infused was between 15 to 100 times in excess of IgE in the total plasma volume at the time of infusion. At this dose total IgE levels (free and complexed) increased up to 11-fold of baseline levels within 21 to 30 d. A pre-existing reservoir of IgE, including FcεR-bound IgE could contribute to some of the early rise in total IgE levels. However the prolonged and substantial rise of total IgE, in the form of IgE-CGP 51901 complexes, appears to be mainly the result of continuous de novo synthesis of IgE at a rate compatible with the known kinetics of IgE turnover at which up to 80% of circulating IgE can be synthesized per day. While the short catabolic half-life of free IgE ($t_{1/2}$ 1–2 d) (29) in equilibrium with de novo synthesis is responsible for maintaining IgE

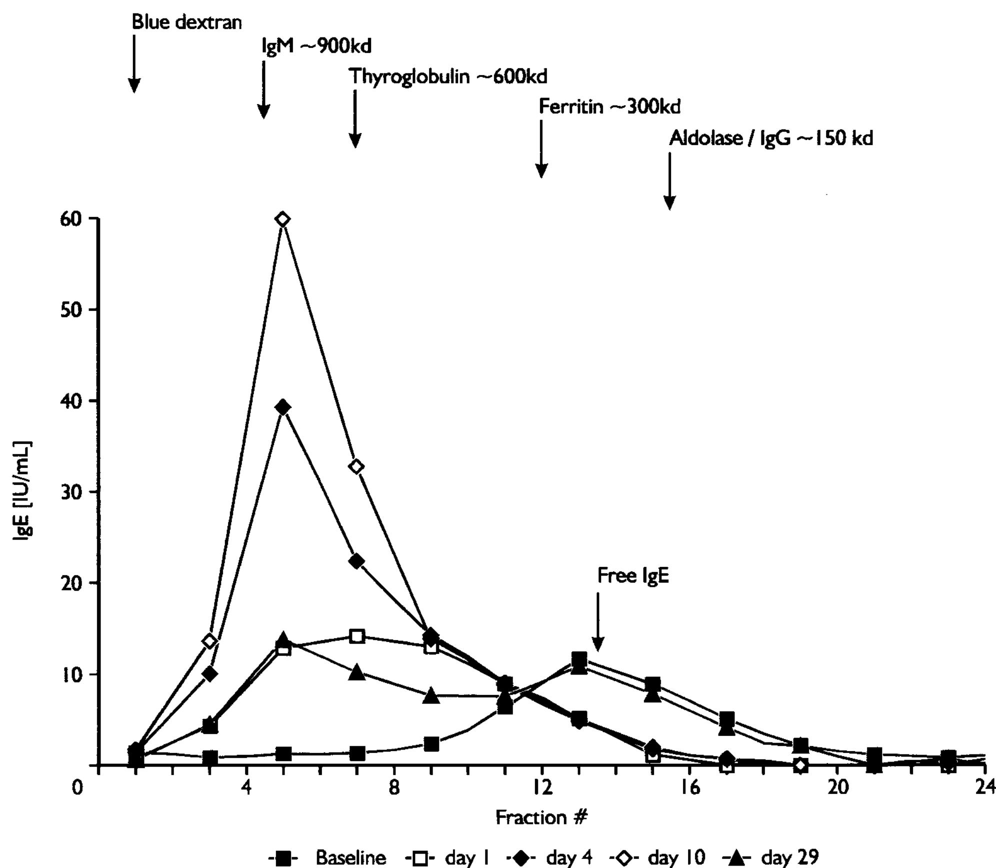

Figure 4. HPLC analyses of serum samples from a volunteer treated with 30 mg of CGP 51901. Baseline (◆) is serum obtained immediately before infusion of CGP 51901. The elution positions of reference proteins (with mol wt in kD) and blue dextran (exclusion volume) used to calibrate the column are indicated by arrows. All column fractions were analysed for IgE concentration using the assay for total IgE. For clarity only the measurements of the uneven fractions were plotted.

levels relatively constant in the physiological state we conclude that the much slower elimination rate of IgE-CGP 51901 complexes ($t_{1/2}$ 10–14 d) causes their accumulation in the circulation resulting in the observed rise of total IgE levels after CGP 51901 administration.

There was no clinical evidence that administration of CGP 51901 or the subsequent formation of CGP 51901-IgE complexes had an effect on mast cell or basophil histamine release which is in keeping with in vitro observations that CGP 51901 and IgE-CGP 51901 complexes do not bind to IgE on the surface of basophils and do not increase basophil histamine releasability (18). This was confirmed by our ex vivo studies of histamine release from basophils isolated before and after infusion of CGP 51901. There was no increase in either spontaneous basophil histamine releasability or that induced by anti-IgE or fMLP. An increase in total blood histamine levels was seen only after infusion of 100 mg of CGP 51901 but did not reach statistical significance and was seen in two volunteers only. The basophils of these volunteers showed no increase in histamine releasability in vitro and there was no detectable increase in absolute basophil numbers. Thus CGP 51901 does not bind to IgE on basophils to cause histamine release nor does it "prime" the cells for subsequent activation. It should be noted however that isolated basophils showed no decrease in histamine releasability after administration of CGP 51901 suggesting that a single dose of up to 100 mg had not depleted basophils of IgE within 8 h of the infusion.

Previously described chimeric antibodies have kinetics of elimination that fit a two-compartment model in agreement with the known kinetics of human IgG (30, 31). The α phase elimination is due to antibody equilibration with the extravascular compartment and has a half-life of between 12 and 24 h and the β phase is due to catabolism and has a half-life of about 10–14 d for chimeric IgG1. Although we have initially used two compartment kinetics to describe elimination of CGP 51901 it is clear from Fig. 2 that the division into α and β half-life is not entirely applicable to CGP 51901 since an intermediate phase seems to exist between the initial and terminal phases of elimination. We propose that CGP 51901 is eliminated from the intravascular compartment by equilibration with the extravascular compartment (distribution), catabolism by the reticuloendothelial system, and also by complexing with newly formed IgE. The first phase of elimination is predominantly due to distribution, the intermediate phase predominantly due to complexing with IgE and the terminal phase, which begins when the concentration of CGP 51901 reaches that of its K_d, due to catabolism. Thus the distribution phase occurs within the first 1–2 d whereas the half-life of the complexing phase is dependent on both the dose of CGP 51901 administered and levels of baseline IgE. The half-life of the catabolism phase appears to be independent of either levels of baseline free IgE or dose of CGP 51901 and was 11.8 d, which is shorter than the reported half-life of IgG1 (32) but is consistent with previous observations with chimeric antibodies (31). This pattern of elimination should apply in general to monoclonal antibodies directed against continuously synthesized molecules such as IgE.

Despite the large fall in free IgE we have not demonstrated any change in skin prick test reactions to grass pollen although variability of the skin tests reduced the power of the study to detect any changes. The half-life of IgE on mast cells has been estimated at 13 d (33) and so it is probable that, unless CGP

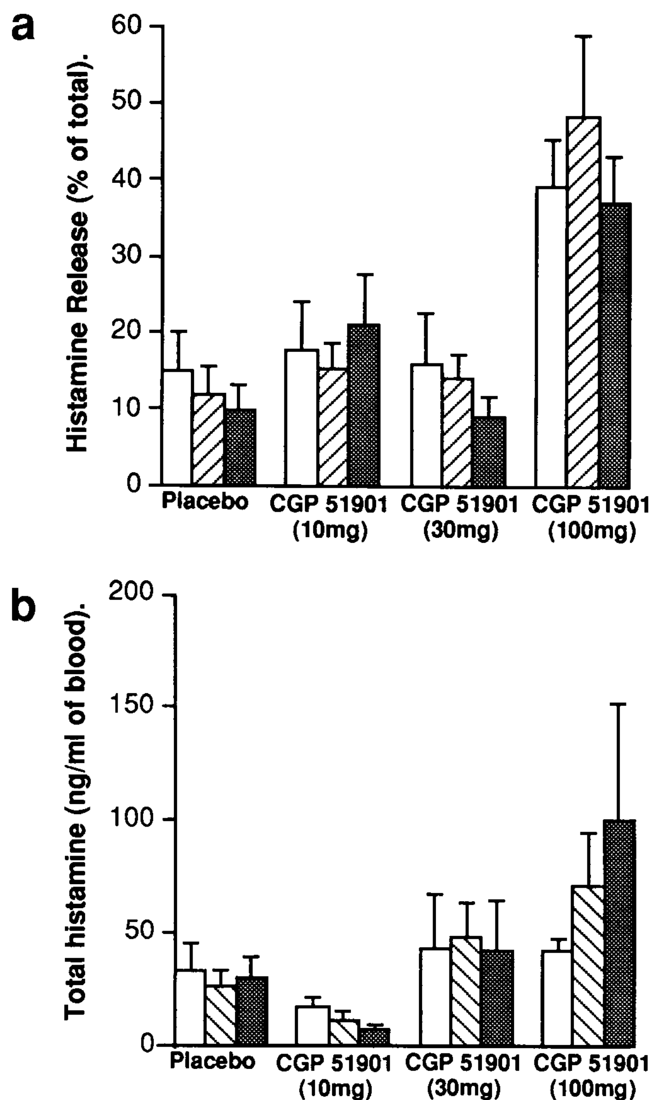

Figure 5. (*a*) The effect of administration of placebo and CGP 51901 on anti-IgE induced histamine release from circulating basophils. Blood samples were obtained before administration of CGP 51901 or placebo (*open bars*), and 6 h (*hatched bars*), and 8 h (*shaded bars*) after treatment. Basophils were challenged with anti-IgE (1 μg/ml) and incubated at 37°C for 45 min and histamine release was then determined. Results are expressed as mean±standard error of the mean. (*b*) The effect of placebo and CGP 51901 on total blood histamine content. Blood samples were obtained before infusion of CGP 51901 or placebo (*open bars*), and 6 h (*hatched bars*) and 8 h (*shaded bars*) after treatment. Aliquots of whole blood were lysed with 25% trichloroacetic acid and histamine content with the supernatants determined. Results are expressed as mean±standard error of the mean.

51901 is able to strip IgE off FcϵR1 receptors, which our data on basophil histamine releasibility suggests is unlikely, multiple dosing, resulting in depletion of free IgE for more than 2 weeks, will be required to produce an effect on IgE-mediated mast cell function in the skin or elsewhere.

Infusion of CGP 51901 was well tolerated by all subjects. Only one volunteer developed significant although low levels of anti-CGP 51901 antibodies and there were no clinically significant consequences of antibody development. None of the volunteers experienced a cutaneous reaction to intradermal injection of CGP 51901 nor symptoms or signs of anaphylaxis after its administration. These findings support the view obtained from previous in vitro studies that CGP 51901 does not cross-link receptor bound IgE. The low immunogenicity of this chimeric antibody is in keeping with the fusion of a mouse variable region to a human constant region since 90% of the antibody response after repeated administration of a xenogeneic monoclonal antibody is directed against the constant region (20). However, some antibody response is to be expected in view of previous reports of immune responses to mouse/human chimeric antibodies which may adversely effect the pharmacokinetics of repeat infusions (34). In this single dose study the level of anti-CGP antibody detected, in one volunteer receiving 10 mg of CGP 51901, would not have been enough to adversely effect the pharmacokinetics of even a 3 mg dose of CGP 51901. Nevertheless, further studies will be needed to determine whether repeated administration of CGP 51901 might induce higher immune responses that would significantly reduce the efficacy of long-term treatment.

By its nature CGP 51901 forms complexes with IgE and therefore offers a potential for the development of circulating immune complexes with possible adverse effects. This, however, is unlikely due to the small size of the tetrameric CGP 51901-IgE complexes formed and the relatively low concentration of complexes accumulating even in individuals with very high IgE levels. The greatest increase in total serum IgE level was from 1000 IU/ml to 5500 IU/ml and was observed in a volunteer who was treated with 100 mg CGP 91501. This corresponds to about 24 μg/ml of IgE/CGP 51901 complexes, which falls within the lower range of natural immune complexes in healthy individuals (35). Because the amount of total serum IgE and IgE-CGP 51901 complexes that accumulate are dependent on de novo synthesis of IgE the maximum accumulation of IgE-CGP 51901 immune complexes is not expected to increase beyond that observed for the 100 mg single dose of CGP 51901. It should also be noted that only one subject in this study developed any changes in complement levels which was a small rise that resolved by 24 h.

Finally, the long-term safety implications of administration of CGP 51901 are important if this form of treatment is to enter clinical practice. One putative role of IgE is protection against parasitic disease (36). However, protection against parasites is not solely an IgE-dependent response but also involves cellular and other humoral components (37). IgE-deficient mice infected with helminths show no difference in worm burden after primary infection or resistance to secondary infection when compared to IgE-producing control mice (38). Other studies have shown that when infected with *Leishmania* high IgE responding BALB/c mice have a higher mortality than low IgE responding BALB/c mice. Furthermore, administration of an anti-IL4 antibody to the high-IgE responders, which knocks out their IgE response, reduces their mortality to that of the low responding group (39). Similarly another study demonstrated that anti-IgE treatment was beneficial in *Schistosoma* infected mice (40). Some authors have suggested that atopy may protect against the development of neoplasia (41, 42) but others have failed to corroborate these findings (43, 44) and they relate the diminished risk of cancer to atopy and not specifically to IgE levels. It must also be remembered that CGP 51901 reduces circulating levels of IgE in atopic individuals to low levels commonly seen in non-atopic individuals.

In conclusion the chimeric antibody, CGP 51901, was effec-

tive in reducing levels of circulating free IgE for a prolonged period after a single intravenous administration. It was well tolerated and did not cause serum sickness or immune complex disease. Therefore CGP 51901 warrants further evaluation in phase II studies for asthma and allergic rhinitis where IgE is considered to play a key triggering role.

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