Clin Exp Immunol 2002; **130**:93–100

Preclinical efficacy and safety of pascolizumab (SB 240683): a humanized anti-interleukin-4 antibody with therapeutic potential in asthma

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(Accepted for publication 25 July 2002)

SUMMARY

The type 2 helper T cell (T_H2) cytokine interleukin (IL)-4 is thought to play a central role in the early stages of asthma. In an effort to develop an antibody treatment for asthma that neutralizes the effects of IL-4, a murine monoclonal antibody, 3B9, was generated with specificity for human IL-4. *In vitro* studies demonstrated that 3B9 inhibited IL-4-dependent events including IL-5 synthesis, T_H2 cell activation and up-regulation of immunoglobulin E expression. 3B9 was then humanized (pascolizumab, SB 240683) to reduce immunogenicity in humans. SB 240683 demonstrated species specificity for both monkey and human IL-4 with no reactivity to mouse, rat, cow, goat or horse IL-4. Pascolizumab inhibited the response of human and monkey T cells to monkey IL-4 and effectively neutralized IL-4 bioactivity when tested against several IL-4-responsive human cell lines. Affinity studies demonstrated rapid IL-4 binding by pascolizumab with a slow dissociation rate. *In vivo* pharmacokinetic and chronic safety testing in cynomolgus monkeys demonstrated that pascolizumab was well tolerated, and no adverse clinical responses occurred after up to 9 months of treatment. Three monkeys developed an anti-idiotypic response that resulted in rapid pascolizumab clearance. However, in the chronic dosing study the antibody response was transient and not associated with clinical events. In conclusion, pascolizumab is a humanized anti-IL-4 monoclonal antibody that can inhibit upstream and downstream events associated with asthma, including T_H2 cell activation and immunoglobulin E production. Clinical trials are under way to test the clinical efficacy of pascolizumab for asthma.

Keywords asthma, interleukin-4 monoclonal antibody preclinical safety toxicology

INTRODUCTION

Although the cause of asthma has not been defined completely, it is clear that asthma is the result of a series of cellular and cytokine-mediated events that induce chronic airway inflammation. Interleukin (IL)-4 is thought to be a key cytokine in the early stages of asthma because of its role in regulating B-cell isotype switching to immunoglobulin (Ig)E production, eosinophil chemotaxis and the development of effector T-cell responses [1,2], Interleukin-4 is produced by T lymphocytes, activated mast cells and basophils. Along with other cytokines (including IL-5 and IL-13), IL-4 can induce the development of allergic inflammatory diseases and can promote the differentiation of undifferentiated helper T cells (T_H0) into type 2 helper T cells (T_H2) [3,4]. These T_H2 cells, in turn, secrete a pattern of cytokines including IL-3, IL-4, IL-5, IL-13 and granulocyte-macrophage colony-stimulating factor [5] that initiate and perpetuate the asthmatic inflammatory response leading to airway inflammation, obstruction and hyperresponsiveness characteristic of chronic asthma [6]. Both IL-4 and IL-5 mRNA and protein are elevated in asthmatic airway tissues [7, 8].

Inhibition of IL-4 activity could potentially reduce the pulmonary inflammation and remodelling that define chronic persistent asthma. Because T_H2 differentiation is IL-4-dependent, IL-4 neutralization may inhibit the development of T_H2 cells and the subsequent events that lead to allergic inflammation [9]. Also, as IL-4 induces IL-5 synthesis, IL-4 neutralization may also block IL-5-dependent pulmonary eosinophilia. Inhibition of IL-4 may also reduce aberrant IgE production and subsequent IgE-mediated, mast cell-dependent inflammation. Furthermore, because IL-4 up-regulates collagen and fibronectin synthesis in subepithelial fibroblasts (leading to airway remodelling), inhibiting IL-4 may prevent long-term reduction in pulmonary function [10]. Finally, animal studies have revealed that IL-4 knockout mice

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sensitized to antigen were unable to develop allergic eosinophilic airway infiltration and did not produce antigen-specific IgE following exposure to aerosolized antigen [11]. Additionally, these mice failed to develop airway hyperresponsiveness following chronic aerosol exposure to antigen.

Interleukin-13 is related closely to IL-4 and has similar downstream functions. Produced by activated T_H2 cells, T_H0 cells, mast cells and dendritic cells, IL-13 can also stimulate IgE production by B cells [12]. However, because T cells do not express IL-13 receptors, IL-13 does not promote T_H2 responses or suppress T_H1 cell differentiation as does IL-4 [13]. Because of this difference in function, it is possible that suppressing IL-4 will prevent IL-13 up-regulation. Thus, although other cytokines are involved in the development of asthma, the neutralization of IL-4 alone may be sufficient to decrease eosinophil accumulation in the airways and to reduce lung airway remodeling in asthmatic patients.

Studies in murine models of asthma have demonstrated that both anti-IL-4 antibodies and soluble IL-4 receptors can block the downstream events associated with asthma. The administration of an aerosolized, soluble, murine recombinant IL-4 receptor inhibited IL-4 activity in mice and prevented the development of allergen-induced and allergen-dependent immediate hypersensitivity responses [14]. Similarly, in a murine model of atopic asthma, mice treated with anti-IL-4 monoclonal antibody (MoAb) prior to active sensitization with ovalbumin showed lower serum IgE levels compared with saline-treated controls [15].

Pascolizumab (SB 240683) is a humanized anti-IL-4 MoAb developed originally by GlaxoSmithKline (Philadelphia, PA, USA) and currently in development at Protein Design Laboratories, Inc. (Fremont, CA, USA). Pascolizumab blocks the interaction of IL-4 with its receptor (Fig. 1), thereby inhibiting the early events of asthma including T_H2 cell differentiation, eosinophilia and IgE up-regulation. Preclinical data indicate that blocking these events *in vivo* may prevent airway inflammatory cell infiltration and remodelling in asthmatic patients. In the current study, the biological activity and preclinical pharmacology of pascolizumab and its parent antibody, 3B9, were evaluated. As a prelude to clinical trials, the safety and toxicity profile of

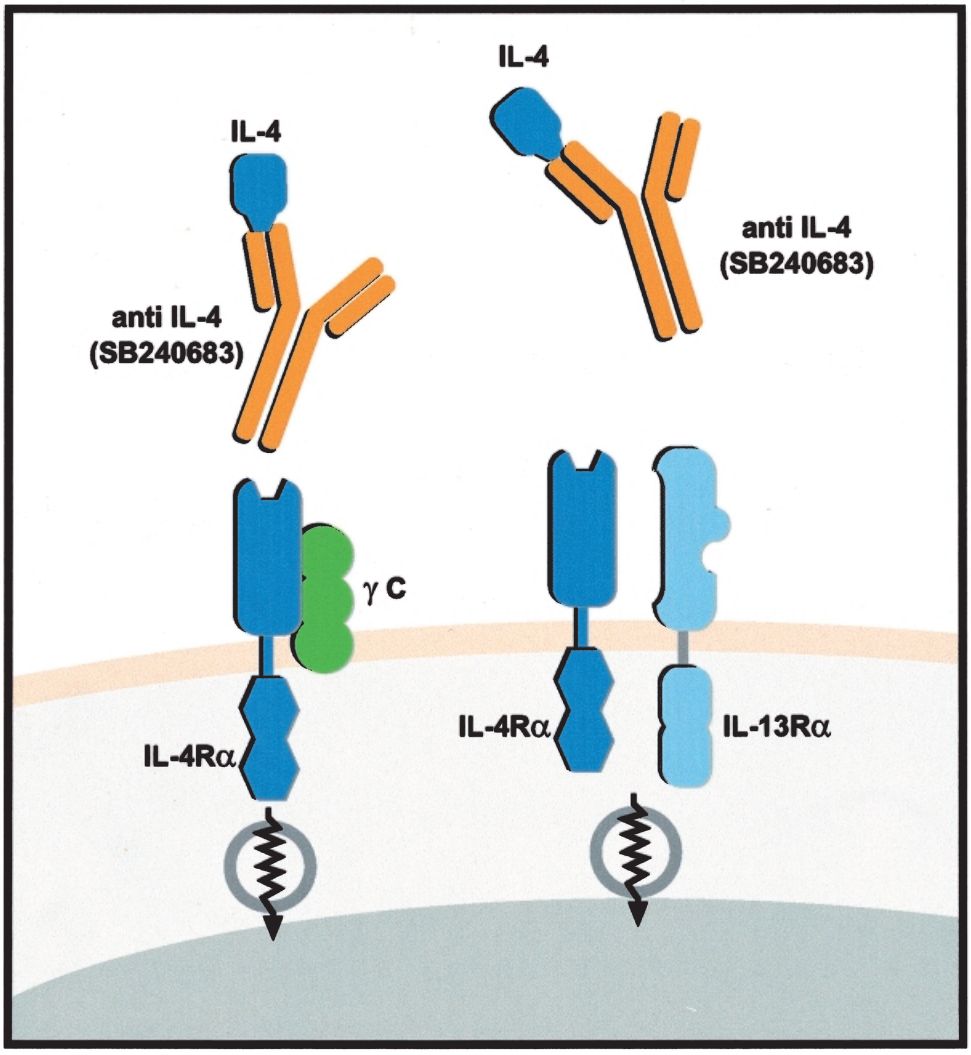

Fig. 1. Effect of pascolizumab binding of IL-4 inhibits IL-4 interaction with the alpha chain of the IL-4 receptor. IL = interleukin; anti-IL-4 = antibody against IL-4; γC = gamma signalling chain of the IL-4 receptor; IL-4Rα = IL-4 receptor alpha; IL-13Rα = IL-13 receptor alpha.

pascolizumab was evaluated in cynomolgus monkeys, the only species in which antibody cross-reactivity was demonstrated.

MATERIALS AND METHODS

Humanization of 3B9

Pascolizumab (SB 240683) is a fully humanized MoAb constructed using conventional molecular techniques to graft complementarity-determining regions from the parent murine antihuman IL-4 antibody (3B9) into human IgG1 kappa heavy- and light-chain frameworks. Substitutions were made to retain antibody activity and to reflect human Ig group/subgroup preferences (SMART™ technology; Protein Design Laboratories, Inc., Fremont, CA, USA).

Activity of pascolizumab in vitro

Affinity Studies. The kinetics of antibody binding to IL-4 were analysed at 25°C in a BIAcore biosensor. Pascolizumab was attached to the solid phase with protein A. Binding of IL-4 in solution was determined by change in refractive index. On and off rates of the antibody binding as well as the dissociation constant (K_d) were calculated. The interaction between IL-4 and pascolizumab was characterized by isothermal titration microcalorimetry and biosensor analysis.

IL-4-dependent T-cell proliferation. Human peripheral blood mononuclear cells (PBMCs) were isolated by Ficoll-paque centrifugation and incubated in 4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid-buffered RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS), 1% L-glutamine and 1% penicillin-streptomycin plus 10 μg/ml phytohaemagglutinin (PHA) for 72 h at 37°C. The PHA-activated blasts were cultured (10^5 cells/ml) in round-bottom microtitre plate wells with human IL-4 (2 ng/ml) and pascolizumab for 3 days. Samples were pulsed with 0·5 μCi [^{3}H]-thymidine and processed for scintillation counting in a 1205 Betaplate™ (EG & G Wallac, Inc., Gaithersburg, MD, USA).

IL-4-dependent TF-1 cell proliferation. TF-1 cells (American Type Culture Collection, a human erythroleukaemic cell line responsive to IL-4) were cultured in 4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid-buffered RPMI-1640 medium supplemented with FBS, L-glutamine, and antibiotics. Round-bottom 96-well microtitre plates were seeded with $2{\cdot}5 \times 10^3$ cells/well and incubated for 48 h in the presence of serially diluted antibody plus IL-4 (1 ng/ml). Cultures were pulsed with [^{3}H]-thymidine and processed for scintillation counting.

IL-4-dependent IgE synthesis

Human PBMCs were separated by centrifugation over Ficoll-paque, washed, and resuspended to $1{\cdot}25 \times 10^6$ cells/ml in supplemented RPMI 1640 medium. Cell suspensions (800 μl) were aliquoted into a 48-well plate along with IL-4 (3 ng/ml), hydrocortisone succinate (1 μM) and pascolizumab. Samples were incubated for 14 days at 37°C in an atmosphere of 5% CO_2. At the end of the culture period, supernatants were removed and IgE concentrations measured by enzyme-linked immunosorbent assay (ELISA).

IL-4-dependent human spleen B-cell proliferation. B cells were purified from human tonsils or spleens using standard techniques and frozen in liquid nitrogen until required. Cells were resuspended to 2×10^6 viable cells per ml and incubated with phorbol 12,13-dibutyrate (10^{-8} M) and anti-IgM antibody (1 μg/ml) for 45 min at 37°C. Cells were washed, aliquoted into microtitre plate wells and incubated with IL-4 (2 ng/ml) plus pascolizumab for 72 h. B-cell proliferation was measured by [^{3}H]-thymidine incorporation.

IL-4-dependent CD23 expression on B cells. Purified tonsil B cells were incubated with IL-4 (1 ng/ml) plus pascolizumab for 48 h in RPMI-1640 medium with 15% FBS. Membrane-expressed CD23 Fc receptor type epsilon (FcεRII; The Binding Site, Birmingham, UK) was determined by flow cytometric analysis.

Inhibition of IL-4–dependent T_H2 cell differentiation by 3B9

Peripheral blood mononuclear cells from tetanus toxoid (TT)-sensitive donors were purified on Ficoll-paque and resuspended to 3×10^6 cells/ml in AIM-V medium with 15% FBS, 1 mM sodium pyruvate, and 40 nM 2-mercaptoethanol. Cells were cultured for 5 days at 37°C with adsorbed tetanus vaccine (1 : 375 dilution), IL-4 (1–2 ng/ml) and MoAb 3B9. Supernatants were removed and frozen prior to testing for IL-5. Cell proliferation was determined by [^{3}H]-thymidine incorporation. Interleukin-5 and interferon gamma (IFN-γ) were measured with commercial ELISA kits (R&D Systems, Minneapolis, MN, USA).

Inhibition of in vivo *human IgE production by MoAb 3B9*

Six–8-week-old severe combined immunodeficient (SCID) mice received intraperitoneal (i.p.) injection of human PBMCs ($3{\cdot}5 \times 10^7$ cells/animal) from house dust mite-sensitive donors. Each mouse received five daily i.p. injections of recombinant human interleukin (rhIL)-4 (10 μg/mouse/day) starting on the day after reconstitution. Blood samples were obtained from the orbital plexus weekly (weeks 2–10). Sera were assayed for human IgE and IgG. Monoclonal antibody 3B9 (0·5–10 mg per mouse) was given as a single i.p. injection at the time of reconstitution.

Species specificity of 3B9 and pascolizumab. Species specificity was confirmed by 3B9 and pascolizumab inhibition of native and recombinant human IL-4 activity and recombinant murine, bovine or rat IL-4 in the T-cell proliferation assay. Monoclonal antibody 3B9 and pascolizumab were assessed for their recognition of supernatant components from PHA-activated guinea pig and dog spleen cells. Monoclonal antibody 3B9 binding to human and monkey IL-4 was analysed by standard ELISA. Pascolizumab inhibition of human and monkey T-cell responses to recombinant monkey IL-4 was measured.

Immunochemistry. Immunohistochemical analysis was performed to detect pascolizumab binding to normal adult male and female human tissue (31 tissue types; 1–3 donors/tissue type). Cryostat sections of surgical and autopsy specimens (fixed at 4°C in methanol/acetone) were incubated with biotinylated-pascolizumab followed by peroxidase-conjugated streptavidin. Following peroxidase development, sections were counterstained with haematoxylin. Controls included IL-4–coated sepharose beads and an isotype-matched, biotinylated-humanized IgG1 MoAb.

Single- and repeat-dose toxicity studies in cynomolgus monkeys

Cynomolgus monkeys received one intravenous (i.v.) dose of 0, 5, 50 or 500 mg/kg (three male and three female monkeys/group) of pascolizumab, followed by a second equal dose 1 month later (two male and two female monkeys/group). Changes in laboratory parameters (chemistry, urinalysis, haematological parameters including lymphocyte subsets and lymphocyte mitogen responses), electrocardiography, ophthalmological examination, histological parameters and body weight were noted in addition to adverse clinical responses for 1 month following each dose.

Chronic toxicity in cynomolgus monkeys

Three female and three male monkeys (including two female and two male monkeys per group from the previous study approximately 6 months after the last pascolizumab treatment) were administered monthly doses of pascolizumab (10 mg/kg subcutaneous [s.c.], 10 mg/kg i.v. or 100 mg/kg i.v.) or placebo for 9 months. Changes in laboratory and physical parameters (listed above) and adverse events were noted.

Assessment of anti-pascolizumab antibodies in monkey sera. Serum samples (diluted 1 : 5 in phosphate-buffered saline/0·1% bovine serum albumin) were incubated with TAG-pascolizumab (ruthenium-labelled), affinity-purified rabbit antipascolizumab antibody standard or unknown sera, and biotin–pascolizumab or biotin–3B9 for 1 h, followed (without washing) by streptavidin-coated paramagnetic microbeads for 30 min. Biotin-labelled pascolizumab would allow detection of anti-isotypic and anti-idiotypic antibody responses and biotin-labelled 3B9 would allow detection of anti-idiotypic responses. ORIGEN® Assay Buffer was added and electrochemiluminescent responses recorded on an ORIGEN® Analyser (IGEN International, Gaithersburg, MD, USA). The dynamic range for both assays was 47–6000 ng/ml.

Determination of pascolizumab in monkey plasma. The presence of pascolizumab in monkey plasma was determined using a selective electrogenerated chemiluminescence immunoassay similar to that described previously [16]. The lower limit of detection for pascolizumab was 50 ng/ml. Pharmacokinetic parameters, including the antibody half-life, peak and trough concentrations, area under the curve and maximal drug concentration, were calculated.

Animal care and use and human tissue use. All study designs were reviewed and approved by the Institutional Animal Care and Use Committees. Definitive toxicity studies were conducted in accordance with Good Laboratory Practices for Nonclinical Laboratory Studies (21 CFR, part 58). Human blood was collected from volunteer donors in the laboratory following approved consenting procedures, and unused samples were discarded.

RESULTS

In vitro *characterization of the IL-4 epitope bound by 3B9 and pascolizumab*

Cynomolgus monkey IL-4 has an amino acid sequence identity with human IL-4 of 93%. The IL-4 amino acid sequence in other species, including mouse, rat, cow, goat and horse, shows less homology to the human sequence. Additionally, the amino acid sequence of the IL-4 epitope bound by 3B9 and SB 240683, encompassing residues Ala70-Trp81 present in the C helix of IL-4, is 100% identical between human and monkey. Neither 3B9 nor pascolizumab recognizes IL-4 from mouse, rat, cow, goat or horse with low homology in this region.

Affinity studies. The affinity of the interactions between pascolizumab and IL-4, measured by titration microcalorimetry, indicated that binding of pascolizumab to IL-4 occurred at a molar ratio of 2 IL-4 molecules per 1 antibody molecule, with a K_d of 45 pM at 25°C. Pascolizumab was stable against thermal unfolding to at least 65°C. Biosensor studies showed rapid binding to IL-4 by pascolizumab and a slow dissociation phase. The k_{on} and k_{off} values were $3{\cdot}4 \times 10^6$ $M^{-1}s^{-1}$ and $2 \times 10^{-4}s^{-1}$, respectively, and the K_d was 59 pM. BIAcore biosensor studies were also performed using immobilized IL-4 and soluble IL-4 receptor alpha chain-Fc. Pascolizumab inhibited the binding of IL-4 to its receptor with a 50% inhibitory concentration (IC_{50}) of approximately 10 nM. Greater than 90% inhibition was achieved with 250 nM pascolizumab.

In vitro *pharmacology of 3B9 and pascolizumab*

A series of *in vitro* studies were conducted with the parent MoAb (3B9) and pascolizumab to determine their effects on IL-4–dependent downstream events and to confirm that the humanization process did not alter significantly the binding affinity of the antibody.

IL-5 synthesis. In vitro studies demonstrated that IL-5 synthesis by human PBMCs in response to the recall antigen TT was significantly increased in the presence of exogenous rhIL-4 (1–2 ng/ml; Table 1). In contrast, rhIL-4 did not influence cell proliferation or the production of the T_H1 cytokine, IFN-γ. These results confirm that IL-4 is capable of preferentially stimulating the production of T_H2 cytokines (e.g. IL-5) over T_H1 cytokines (e.g. IFN-γ) in response to antigen. Monoclonal antibody 3B9 was tested in this model for its ability to inhibit IL-4-dependent IL-5 synthesis by PBMCs in the presence of TT (Fig. 2). Antigen-driven, IL-4-dependent IL-5 synthesis was inhibited by MoAb 3B9 in a dose-dependent manner with an IC_{50} = 730 pM, providing

Table 1. Effect of IL-4 on tetanus toxoid PBMC proliferation and cytokine production

Treatment	Proliferation, cpm $\times 10^3$ (s.e.m.)	IL-5, pg/ml (s.e.m.)	IFN-γ, pg/ml (s.e.m.)
Medium alone	46·5 (3·3)	122 (15)	1148 (264)
IL-4	48·8 (3·0)	298 (20)*	1983 (258)

IL = interleukin; PBMC = peripheral blood mononuclear cell; cpm = counts per minute; SEM = standard error of the mean; IFN-γ= interferon gamma.

*$P < 0{\cdot}001$ *versus* medium control.

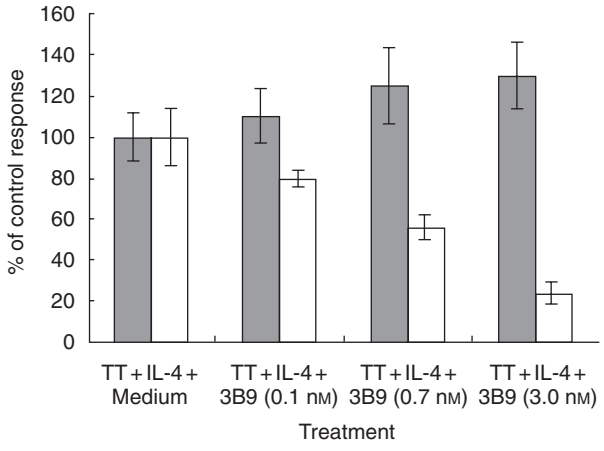

Fig. 2. Effect of MoAb 3B9 on antigen-driven IL-4-dependent T-cell proliferation and IL-5 synthesis. Results are expressed as percentage of the control response obtained with TT-stimulated cells in the presence of IL-4. IL = interleukin; TT = tetanus toxoid. ■, Cell proliferation; □, IL-5 production.

evidence that antibody neutralization of IL-4 can down-regulate T_H2 cell cytokine production.

T-cell proliferation. To confirm that the humanized antibody retained the ability to inhibit IL-4-dependent functions and to confirm the cross-species specificity of the antibody, pascolizumab was tested for its ability to inhibit IL-4-dependent T-cell proliferation using human or monkey T cells (Fig. 3a). Pascolizumab inhibited the response of human and monkey T cells to recombinant monkey IL-4 with mean IC_{50} values of 268 pM and 587 pM, respectively. Although the mean IC_{50} values were lower for inhibition of human and monkey T cells to recombinant human IL-4 (157 pM and 234 pM, respectively), pascolizumab was able to inhibit IL-4-induced activation in both species.

Neutralization of IL-4 bioactivity. Pascolizumab and 3B9 inhibition of IL-4 bioactivity is summarized in Table 2. Both antibody treatments inhibited the proliferation of human T cells (Fig. 3a), human B cells and TF-1 cells (Fig. 3b). Interleukin-4-dependent IgE production by human PBMCs (Fig. 4a) and up-regulation of Fc∈RII (CD23; Fig. 4b) on human B cells were also blocked by both antibodies. These in vitro analyses indicate that the antigen-binding affinity and pharmacodynamic properties were retained during the humanization of 3B9 to pascolizumab.

Inhibition of IgE production by 3B9. Because IgE production is up-regulated following B-cell stimulation by IL-4, the inhibition of IL-4-dependent IgE production by MoAb 3B9 was examined in a SCID mouse model. In this model, SCID mice that had been administered immune cells from house dust mite-sensitive human donors were administered IL-4 (10 μg/mouse/day), causing a >twofold increase in IgE production within 4 weeks. Treatment of mice with MoAb 3B9 at the time of reconstitution inhibited IgE production in a dose-dependent manner (Fig. 5). The highest dose of MoAb 3B9 (10 mg) rendered IgE levels undetectable at all time-points. Serum IgG levels were unaffected by 3B9 treatment (data not shown).

Tissue cross-reactivity. Immunohistochemistry studies demonstrated no pascolizumab staining in frozen sections from 37

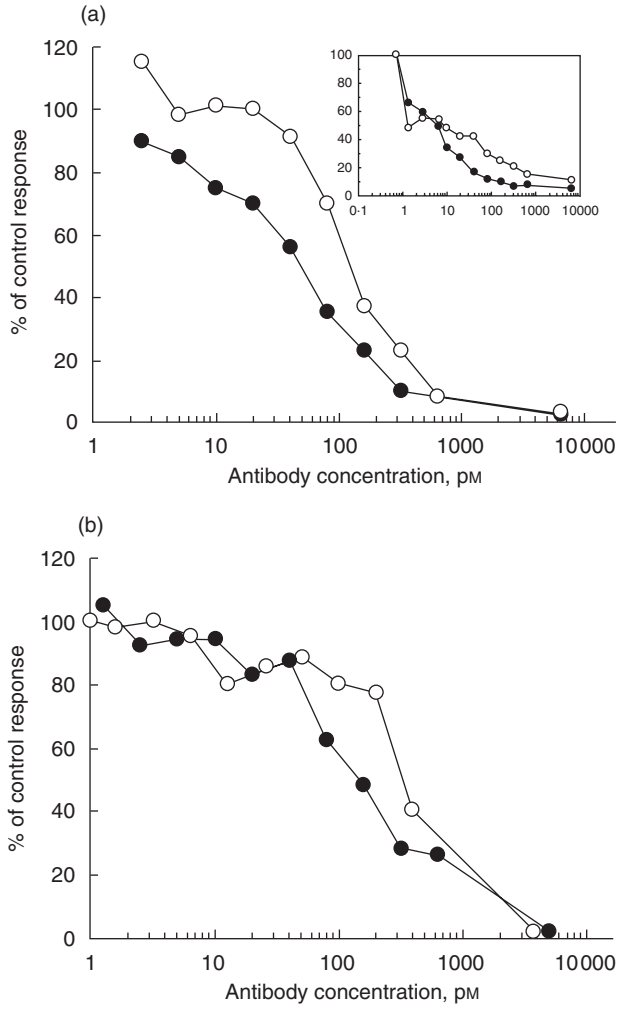

Fig. 3. Effect of pascolizumab on human (a), cynomolgus monkey (inset) and TF-1 cell (b) IL-4-dependent T-cell proliferation. Results are expressed as percentage of the control response obtained with IL-4-stimulated cells. ●, MoAb 3B9; ○, pascolizumab.

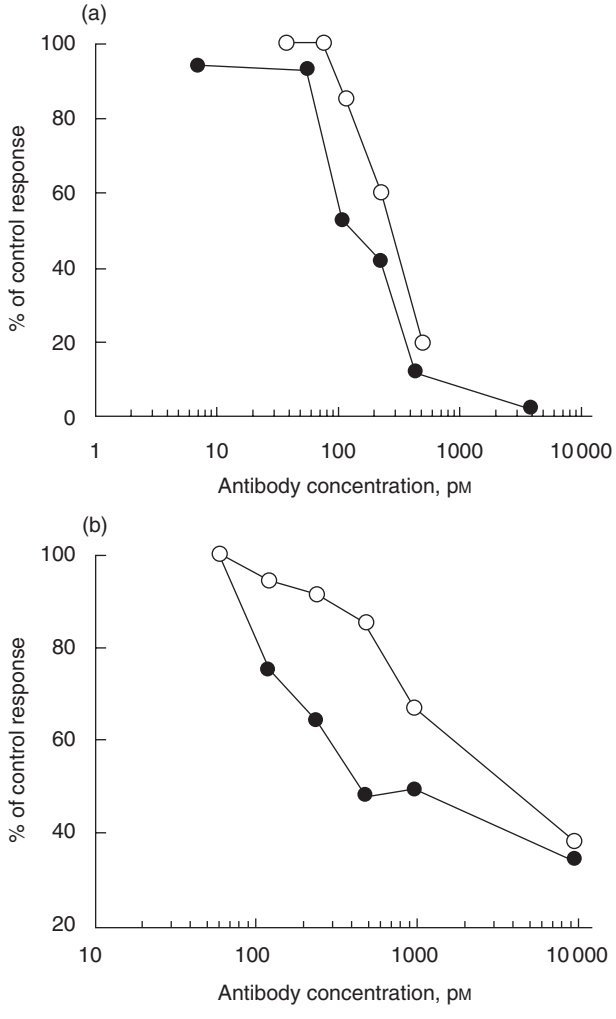

Fig. 4. Effect of MoAb 3B9 and pascolizumab on IL-4-dependent IgE synthesis from human PBMCs (a) and surface expression of CD23 on purified human tonsillar B cells (b). Results are expressed as percentage of the control response obtained with IL-4-stimulated cells. ●, MoAb 3B9; ○, pascolizumab.

Table 2. Pharmacological activity of pascolizumab *in vitro**

Antibody	T-cell proliferation IC_{50}, pM	TF-1 cell proliferation IC_{25}, pM	IgE production IC_{50}, pM	CD23 expression IC_{50}, pM	Tonsil B-cell proliferation IC_{50}, pM
Pascolizumab	157	694	278	950	377
	$(113–262)_5$	$(334–1000)_3$	$(240–311)_2$	$(600–1300)_2$	3/4
MoAb 3B9	31	87	612	147	134
	$(11–60)_{10}$	$(27–139)_3$	$(370–1070)_6$	$(53–272)_3$	$(80–231)_4$

IC_{50} = 50% inhibitory concentration; IC_{25} = 25% inhibitory concentration. *Mean $(range)_n$.

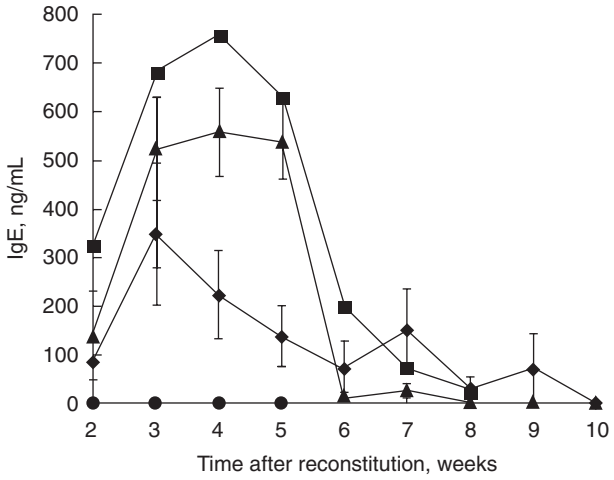

Fig. 5. Effect of MoAb 3B9 on the production of IgE from SCID mice reconstituted with immune cells from atopic volunteers. Six–8-week-old SCID mice received purified PBMCs from house dust mite-sensitive donors, dosed with rhIL-4 for 4 days and a single IP MoAb 3B9 injection at the time of PBMC administration. ▲, IL-4; ■, IL-4 + MoAb 3B9 (0·5 mg); ◆, IL-4 + MoAb 3B9 (1.0 mg); ●, IL-4 + 3B9 (10 mg).

normal human tissue types. These results suggest that pascolizumab may not be appropriate for immunohistochemistry on frozen tissue, or that the IL-4 concentrations in the tissues were below the level of sensitivity necessary for detection of pascolizumab binding.

Pharmacokinetics and safety of pascolizumab in cynomolgus monkeys

The pharmacokinetics and safety of pascolizumab were evaluated in cynomolgus monkeys as a prelude to testing in humans. Following the first dose of pascolizumab, a transient hyperemia, facial flushing lasting less than 1 h, was observed one of six cynomolgus monkeys that received 50 mg/kg and in two of six monkeys that received 500 mg/kg. No other adverse events were observed during this period. One monkey of each sex from each treatment group was necropsied at the end of the observation period. There were no drug-related histopathological findings in these monkeys. The remaining monkeys were administered a second dose of antibody and monitored for an additional month. Both antibody doses were well tolerated. Following the first dose of pascolizumab, one monkey developed anti-idiotypic antipascolizumab antibodies that resulted in enhanced plasma clearance of pascolizumab. No additional monkeys developed antipascolizumab antibodies.

In a chronic dosing study to assess toxicity and pharmacokinetics of i.v. and s.c. dosing, cynomolgus monkeys were administered pascolizumab monthly for 9 months. There were no significant changes in laboratory analyses, electrocardiogram measurements, ophthalmic observations, histology or body weight parameters (data not shown). There were no effects on lymphocyte numbers or subsets or responsiveness to mitogenic stimulation. There was no effect on circulating IgE levels in these non-allergic monkeys. A transient antibody response (including anti-idiotypic antibodies) was detected in two female monkeys after receiving the first 10-mg/kg i.v. dose, which was not evident following the second, third or fourth monthly doses. There were no adverse clinical responses in these antibody-positive monkeys; however, rapid serum elimination of pascolizumab was observed in the two monkeys expressing anti-idiotypic antibody (Fig. 6). A similar effect of rapid pascolizumab clearance was observed in one monkey, dosed with 10 mg/kg s.c., that did not test positive for anti-idiotypic antibodies, due presumably to residual levels of circulating pascolizumab that would compete in the assay. By the fifth dose, plasma pascolizumab concentration profiles in these monkeys were indistinguishable from the other monkeys in the same dose groups.

After chronic dosing, the terminal half-life of the antibody was 8·9 ± 1·4 days. The highest trough concentrations of antibody were reached after approximately 4 monthly doses. There was a slight accumulation of the drug at the 100-mg/kg dose level (Table 3; Fig. 6). The area under the curve following both the 10-mg/kg s.c. and i.v. dosing was similar, indicating 100% bioavailability of antibody after s.c. delivery. Pascolizumab was detected successfully in bronchoalveolar lavage (methods described in Hart *et al*. [17]) from four of 12 monkeys that received 10 mg/kg s.c. or i.v. (range 40–80 ng/ml) and in all the monkeys that received 100 mg/kg i.v. (range 350–900 ng/ml).

DISCUSSION

In the present study, we have characterized the *in vitro* activity, pharmacokinetics and safety of a humanized anti-IL-4 MoAb, pascolizumab (SB 240683) and its parent murine MoAb, 3B9. Monoclonal antibody 3B9 inhibited IL-4-dependent events, including IL-5 synthesis, T_H2 cell activation and IgE upregulation. The humanized pascolizumab MoAb retained the

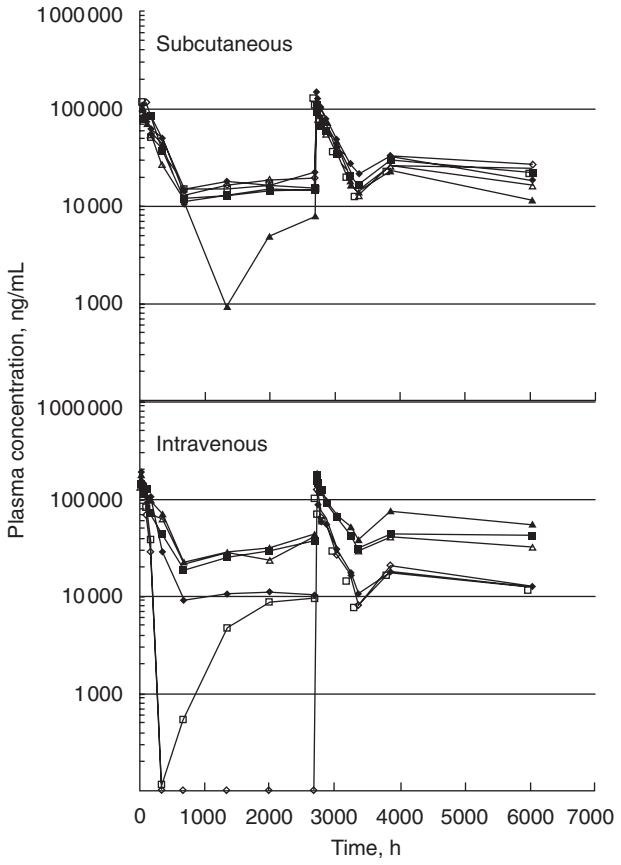

Fig. 6. Plasma concentration *versus* time profiles for pascolizumab on the 9-month toxicity study. The peaks present between 0 and 600 h and again between 2700 and 3500 h represent full pharmacokinetic profiles after the first and fifth doses. The remaining time-points represent the trough plasma concentrations of pascolizumab present in plasma samples collected immediately prior to each monthly dose. There was no change in the clearance profile after repeat dosing. A slight accumulation was observed in the trough plasma concentrations between the first and fifth doses. Symbols represents individual monkeys: filled symbols represent males; open symbols represent females.

species specificity, avidity and IL-4 neutralizing activity of 3B9. Titration microcalorimetry and BIAcore biosensor studies demonstrated rapid IL-4 binding by pascolizumab and slow dissociation. Taken together, the *in vitro* data suggest that pascolizumab specifically inhibits IL-4; therefore, *in vivo* testing was performed to define the preliminary safety of pascolizumab.

Because the *in vitro* studies demonstrated specificity of pascolizumab for cynomolgus monkey IL-4, the pharmacokinetics and safety of pascolizumab were assessed in cynomolgus monkeys. These *in vivo* studies demonstrated that pascolizumab treatment was well tolerated with slight antibody accumulation achieved after chronic dosing due to the long plasma half-life. There were no drug-related immunotoxicological or histopathological findings. There was no effect on circulating IgE levels in monkeys treated with pascolizumab; however, the monkeys had not been sensitized prior to initiation of the safety studies and thus did not have elevated levels of IgE. These *in vivo* and *in vitro* studies provide the rationale for testing the safety and efficacy of pascolizumab in humans.

Existing treatments for asthma provide indirect evidence that IL-4 inhibition may decrease the asthmatic response. Theophylline and other phosphodiesterase inhibitors may decrease IL-4 production in inflammatory cells, due possibly to increasing intracellular 3′,5′-adenosine monophosphate [18]. Additionally, allergen-specific immunotherapy reduces IL-4 expression in circulating lymphocytes [19], and prednisolone reduces the number of IL-4 and IL-5 mRNA-expressing cells and increases IFN-γ mRNA [20], suggesting an immunological shift to a T_H1 response. Therefore, it is hypothesized that direct inhibition of IL-4 with a specific antibody may prevent T-cell maturation to the T_H2 phenotype, which would in turn prevent secretion of T_H2 cytokines including IL-4 and IL-5 in asthmatic patients. Furthermore, anti-IL-4 therapy might also reduce IgE production, inhibit mast cell growth, reduce eosinophil chemotaxis to the airway epithelium and decrease inflammatory destruction of the airways. Inhibition of IL-4 with a soluble form of the IL-4 receptor is currently being evaluated in the clinic with promising early results, including effects on forced expiratory volume in 1 s and on asthma symptom scores [21].

A major obstacle to the successful use of antibody therapy is the host immune response to treatment. Humanization of murine

Table 3. Preclinical pharmacokinetics of pascolizumab following chronic monthly dosing for 9 months in cynomolgus monkeys

	Males ($n = 3$)				Females ($n = 3$)			
	Observed mean C_{max},* μg/ml		Mean $AUC_{(0–4\ week)}$, μg h/ml		Observed mean C_{max},* μg/ml		Mean $AUC_{(0–4\ week)}$, μg h/ml	
Dose group, mg/kg (route)	Dose 1	Dose 5	Dose 1	Dose 5	Dose 1	Dose 5	Dose 1	Dose 5
10 (s.c.)	105	117	30 669	32 043	113	119	30 136	33 382
10 (i.v.)	164	174	38 921	43 178	152	155	40 588	33 850
100 (i.v.)	1351	1771	286 688	384 808	1175	1879	257 995	364 559

C_{max} = maximal drug concentration; AUC = area under the curve; s.c. = subcutaneous; i.v.= intravenous. *C_{max} values were generally observed at the first sampling time, approximately 24 h post-dosing.

MoAbs for clinical use dramatically reduces their immunogenicity, thereby reducing the immune response in humans [22]. However, as occurred in this study, administration of humanized antibodies to monkeys can induce antibody responses [23]. The antibody response can vary from neutralizing antibodies that enhance clearance of the MoAb from circulation, to more severe anaphylactic responses [24]. In the current study, an antibody response occurred in only 12·5% of the monkeys (one in the two-dose regimen and two in the 9-month regimen) with no adverse events apparently related to the antipascolizumab response. In the 9-month dosing regimen, the antipascolizumab response was transient and was not detectable following the second, third or fourth doses. It is possible that the anti-idiotypic immune response was modulated via the pharmacological activity of the anti-IL-4 antibody on humoral immune responses [1]. Although the antipascolizumab antibodies did cause rapid serum elimination of pascolizumab, the pharmacokinetic profile of pascolizumab following the disappearance of antipascolizumab was comparable to that in the monkeys that did not develop an anti-antibody response. It is predicted that pascolizumab will be less immunogenic in humans than it was in monkeys; however, anti-idiotypic responses may still occur. As in the primate study, it is believed that such transient antibody responses will not interfere with long-term treatment of humans with therapeutic levels of pascolizumab.

In summary, *in vitro* studies confirm the specificity of pascolizumab for human and primate IL-4. *In vitro*, the antibody inhibits the downstream events associated with IL-4 stimulation, including IgE and CD23 up-regulation and T-cell proliferation, and may therefore be of potential benefit in the treatment of chronic asthma. Toxicology studies in cynomolgus monkeys confirm that the antibody is well tolerated during acute and chronic dosing. Furthermore, the high binding affinity, long half-life and low immunogenicity of the humanized anti-IL-4 antibody suggest that further evaluation in human clinical trials is warranted for pascolizumab.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the assistance and support of Rodd Polsky, Charles Hottenstein, Carol Silverman, Elisabeth Gore, Donna Williams and Mike Semanik in the conduct of this work. The authors also thank Drs Charles Davis and Peter Bugelski for their helpful discussions over the course of the studies reported here.

REFERENCES

1 Ricci M. IL-4: a key cytokine in atopy. Clin Exp Allergy 1994; **24**:801–12.
2 Pauwels RA, Brusselle GG, Tournoy KG, Lambrecht BN, Kips JC. Cytokines and their receptors as therapeutic targets in asthma. Clin Exp Allergy 1998; **28** (Suppl. 3):1–5.
3 Coyle AJ, Bertrand C, Tsuyuki S *et al.* IL-4 differentiates naive $CD8^+$ T cells to a 'Th2-like' phenotype. a link between viral infections and bronchial asthma. Ann NY Acad Sci 1996; **796**:97–103.
4 Coyle AJ, Le Gros G, Bertrand C *et al.* Interleukin-4 is required for the induction of lung Th2 mucosal immunity. Am J Respir Cell Mol Biol 1995; **13**:54–9.
5 Parronchi P, Macchia D, Piccinni MP *et al.* Allergen- and bacterial antigen-specific T-cell clones established from atopic donors show a different profile of cytokine production. Proc Natl Acad Sci USA 1991; **88**:4538–42.
6 Ricci M, Matucci A, Rossi O. IL-4 as a key factor influencing the development of allergen-specific Th2-like cells in atopic individuals. J Invest Allergol Clin Immunol 1997; **7**:144–50.
7 Ying S, Humbert M, Barkans J *et al.* Expression of IL-4 and IL-5 mRNA and protein product by $CD4^+$ and $CD8^+$ T cells, eosinophils, and mast cells in bronchial biopsies obtained from atopic and non-atopic (intrinsic) asthmatics. J Immunol 1997; **158**:3539–44.
8 Humbert M, Durham SR, Ying S *et al.* IL-4 and IL-5 mRNA and protein in bronchial biopsies from patients with atopic and nonatopic asthma: evidence against 'intrinsic' asthma being a distinct immunopathologic entity. Am J Respir Crit Care Med 1996; **154**:1497–504.
9 Garlisi CG, Kung TT, Wang P *et al.* Effects of chronic anti-interleukin-5 monoclonal antibody treatment in a murine model of pulmonary inflammation. Am J Respir Cell Mol Biol 1999; **20**:248–55.
10 Buttner C, Skupin A, Reimann T *et al.* Local production of interleukin-4 during radiation-induced pneumonitis and pulmonary fibrosis in rats: macrophages as a prominent source of interleukin-4. Am J Respir Cell Mol Biol 1997; **17**:315–25.
11 Pauwels RA, Brusselle GJ, Kips JC. Cytokine manipulation in animal models of asthma. Am J Respir Crit Care Med 1997; **156**:S78–S81.
12 Wills-Karp M, Luyimbazi J, Xu X *et al.* Interleukin-13. central mediator of allergic asthma. Science 1998; **282**:2258–61.
13 Oettgen HC, Geha RS. IgE in asthma and atopy: cellular and molecular connections. J Clin Invest 1999; **104**:829–35.
14 Nasert S, Millner M, Herz U *et al.* Therapeutic interference with interferon-γ(IFN-γ) and soluble IL-4 receptor (sIL-4R) in allergic diseases. Behring Inst Mitt 1995; **96**:118–30.
15 Zhou CY, Crocker IC, Koenig G, Romero FA, Townley RG. Anti-interleukin-4 inhibits immunoglobulin E production in a murine model of atopic asthma. J Asthma 1997; **34**:195–201.
16 Zia-Amirhosseini P, Minthorn E, Benincosa LJ *et al.* Pharmacokinetics and pharmacodynamics of SB-240563, a humanized monoclonal antibody directed to human interleukin-5, in monkeys. J Pharmacol Exp Ther 1999; **291**:1060–7.
17 Hart TK, Cook RM, Zia-Amirhosseini P *et al.* Preclinical efficacy and safety of mepolizuMoAb (SB-240563), a humanized monoclonal antibody to IL-5, in cynomolgus monkeys. J Allergy Clin Immunol 2001; **108**:250–7.
18 Tohda Y, Nakahara H, Kubo H, Muraki M, Fukuoka M, Nakajima S. Theophylline suppresses the release of interleukin-4 by peripheral blood mononuclear cells. Int Arch Allergy Immunol 1998; **115**:42–6.
19 O'Brien RM, Byron KA, Varigos GA, Thomas WR. House dust mite immunotherapy results in a decrease in Der p 2-specific IFN-gamma and IL-4 expression by circulating T lymphocytes. Clin Exp Allergy 1997; **27**:46–51.
20 Bentley AM, Hamid Q, Robinson DS *et al.* Prednisolone treatment in asthma. Reduction in the numbers of eosinophils, T cells, tryptase-only positive mast cells, and modulation of IL-4, IL-5, and interferon-gamma cytokine gene expression within the bronchial mucosa. Am J Respir Crit Care Med 1996; **153**:551–6.
21 Steinke JW, Borish L. Th2 cytokines and asthma. Interleukin-4: its role in the pathogenesis of asthma and targeting it for asthma treatment with interleukin-4 receptor antagonists. Respir Res 2001; **2**:66–70.
22 Vaswani SK, Hamilton RG. Humanized antibodies as potential therapeutic drugs. Ann Allergy Asthma Immunol 1998; **81**:105–15.
23 Newman R, Alberts J, Anderson D *et al.* 'Primatization' of recombinant antibodies for immunotherapy of human diseases: a macaque/human chimeric antibody against human CD4. Biotechnology (NY) 1992; **10**:1455–60.
24 Hakimi J, Chizzonite R, Luke DR *et al.* Reduced immunogenicity and improved pharmacokinetics of humanized anti-Tac in cynomolgus monkeys. J Immunol 1991; **147**:1352–9.