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The effects of an anti-CD4 monoclonal antibody, keliximab, on peripheral blood CD4+ T-cells in asthma

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The effects of an anti-CD4 monoclonal antibody, keliximab, on peripheral blood CD4+ T-cells in asthma. O.M. Kon, B.S. Sihra, L.C. Loh, J. Barkans, C.H. Compton, N.C. Barnes, M. Larché, A.B. Kay. ©ERS Journals Ltd 2001.

ABSTRACT: CD4+ T-cells are likely to be involved as a source of pro-inflammatory cytokines in asthma. This study assessed the effects of an infusion of keliximab (IDEC CE9.1), an anti-CD4+ monoclonal antibody, on peripheral blood CD4+ T-cells in corticosteroid-dependent asthmatics.

Three cohorts of patients (termed $C_{0.5}$: n=6, $C_{1.5}$: n=5, and $C_{3.0}$: n=5) received a single infusion of 0.5, 1.5 or 3.0 mg·kg^{-1}, respectively, with a fourth receiving placebo (C_{pl}: n=6), and were followed-up for 4 weeks. By flow cytometry in peripheral blood, pre- and postinfusion assessment was made of: a) CD4 and CD8 counts and mean fluorescence; b) CD25, human leukocyte antigen-DR (HLA-DR), CD45RO and CD45RA expression on CD4+ T-cells; and c) interferon (IFN)-γ, interleukin (IL)-4 and IL-5 expression in CD4+ T-cells. Keliximab's *in vitro* effects on allergen-specific peripheral blood mononuclear cells (PBMC) proliferation in atopic asthmatics were also evaluated.

There was a significant increase in lung function (peak expiratory flow rate) in the $C_{3.0}$ group. Following infusion in $C_{0.5}$, $C_{1.5}$ and $C_{3.0}$ but not C_{pl}: 1) the CD4, but not CD8 count was significantly decreased; 2) there was total loss of Leu3a staining; 3) there were significant reductions in the mean fluorescence of OKT4 binding; and 4) there were significant reductions in the numbers of CD25, HLA-DR, CD45RO and CD45RA/CD4+ cells. There were no changes in CD4+ cell expression of IFN-γ, IL-4 or IL-5. Keliximab caused a significant reduction in T-cell proliferation as compared to a control monoclonal antibody.

Keliximab, as an anti-CD4 monoclonal antibody, leads to a transient reduction in the number of CD4+ T-cells and modulation of CD4+ receptor expression in severe asthmatics. The effects of keliximab may be mediated through a decrease in CD4+ surface expression and T-lymphocyte numbers, in addition to a reduction in allergen-induced proliferation.

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The mucosal damage and bronchial hyperresponsiveness characteristic of asthmatic airways is believed to result from lipid mediators and granule-derived basic proteins from eosinophils [1]. Eosinophil differentiation, maturation, endothelial adherence, activation and degranulation are enhanced by the cytokines interleukin (IL)-5, IL-3 and granulocyte macrophage-colony stimulating factor (GM-CSF). CD4+ T-cells are an important source of these cytokines [2] and furthermore, are increased in bronchoalveolar lavage fluid and bronchial biopsies from patients with asthma [3, 4], are activated in acute and severe asthma [5] and correlate with eosinophil numbers and activation [2, 6].

Keliximab (IDEC CE9.1) is a chimeric immunoglobulin (Ig)G1 λ monoclonal antibody (mAb) [7] that binds specifically to human CD4 antigen. A randomized double-blind, placebo-controlled trial has recently been performed, evaluating the efficacy of a single infusion of keliximab in severe chronic corticosteroid dependent asthmatics [8]. Significant increases in morning and evening peak expiratory flow rates (PEFR) were observed in the highest dose (3.0 mg·kg^{-1}) cohort.

To investigate the possible mode(s) of action of keliximab in asthma, the present study evaluated the *in vivo* effects of this mAb on peripheral CD4+ T-cells and their *ex vivo* expression of cytokines in these patients. It was hypothesized that keliximab's clinical effect related to the: 1) degree of coating of CD4; 2) reduced expression of CD4 antigen on the T-cell; 3) circulating concentrations of the antibody; 4) changes in activation markers; and 5) alteration of the T-helper cell (Th)1/Th2 cytokine profile. In addition the *in vitro* effects of the antibody on the allergen-specific proliferation of peripheral blood mononuclear cells (PBMC) was evaluated from house-dust mite sensitive asthmatics. It was hypothesized that these would be decreased in a dose-dependent manner.

Study design

Clinical study

Twenty-two severe oral corticosteroid-dependent asthmatics were recruited from the London Chest Hospital and Royal Brompton Hospital, London. Full patient characteristics have been previously described [8]. Following a baseline period of >5 days, a single intravenous infusion of keliximab or placebo was administered and patients followed-up for 4 weeks. Three successive cohorts of patients were studied. The first cohort (n=6) received 0.5 $mg \cdot kg^{-1}$ of keliximab ($C_{0.5}$), the second cohort (n=5) 1.5 $mg \cdot kg^{-1}$ ($C_{1.5}$), and the third cohort (n=5) received 3.0 $mg \cdot kg^{-1}$ ($C_{3.0}$). In randomized order, two patients per cohort (six patients overall) received a placebo infusion (C_{pl}). All patients gave written, informed consent and the study was approved by the ethics committees of the East London City and Hackney Health Authority, and Royal Brompton Hospital.

Peripheral blood was collected at preinfusion and at 48 h and 14 days postinfusion for intracellular flow-cytometry. Blood was also collected for whole blood differential and three colour flow-cytometric analysis at baseline and 24 h, 48 h, 7 days, 14 days and 28 days following infusion. All samples were taken at 09:00 h (±2 h) and prior to the patients receiving their daily oral corticosteroid dose. Pharmacokinetic assessments were made prior to infusion (0 h), and at 0.5, 1.0, 2.0 (end of infusion), 2.08, 2.25, 2.5, 3.0, 4.0, 6.0, 8.0, 12 and 24 h after the start of the infusion. Further pharmacokinetic assessments were made on days 2, 7, 14 and 28 postdosing.

Allergen specific proliferation studies

Peripheral venous blood was obtained from a separate group of six atopic subjects with mild asthma, who were skin prick test-positive to *Dermatophagoides pteronyssinus* (*Der-p*). They all had demonstrable serum radioallergosorbent test (RAST) immunoglobulin E (IgE) to *Der-p* (table 1). They required short-acting β_2-agonist inhalers intermittently to control mild asthmatic symptoms, were not using inhaled corticosteroids and had not required systemic corticosteroids in the last 6 months.

Table 1. – Patient characteristics for allergen-specific stimulation experiments assessing proliferation by T-cells

Patient	Sex	Age yrs	*der p* RAST $KU \cdot L^{-1}$	Serum IgE $KU \cdot L^{-1}$
1	F	29	52.5	180
2	M	29	28.3	127
3	F	22	16.3	79
4	F	33	25.9	114
5	F	41	25.0	72
6	F	25	92.6	291

der p RAST: *Dermatophagoides pteronyssinus* radioallergosorbent assay; IgE: immunoglobulin-E; M: male; F: female. All subjects had a decrease in forced expiratory volume in one second of $\geq 20\%$ to either allergen or histamine provocation concentration of <3 $mg \cdot mL^{-1}$.

Materials and methods

Antibodies and reagents

The following directly conjugated mAbs were used for flow-cytometric analysis of the peripheral blood: OKT4 phycoerythrin (PE) (Ortho Diagnostics, Bucks, UK), OKT8 fluorescein isothiocyanate (FITC), OKT3 energy coupled dye (ECD), CD3 ECD, CD8 ECD (Coulter, Bedfordshire, UK), IgG1 FITC, Leu3a PE, CD2 FITC, CD25 FITC, CD45RA FITC, IgG2a FITC, HLA-DR FITC (Becton Dickinson, Oxford, UK), IgG2 FITC, CD45RO FITC (Dako, Bucks, UK). The following PE conjugated antibodies from Pharmingen (San Diego, CA, USA) were used for intracellular cytokine estimations: MOPC-1 (IgG1), 4S.B3 (IFN-γ), 8D48 (IL-4), R35-95 (IgG2a) and JES1-39D10 (IL-5). Phorbol 12-myristate 13-acetate (PMA), foetal calf serum (FCS), Histopaque, human AB (blood group) serum, calcium ionophore, monensin and saponin were obtained from Sigma (Poole, UK). Glutamine, penicillin, streptomycin and Roswell Park Memorial Institute (RPMI) 1640 were purchased from Gibco (Paisley, UK). N-2-hydroxyethylpiperazine-N-2-ethanesulphonic acid (HEPES)-buffered RPMI medium was purchased from Chester Beatty Laboratories (London, UK). "Multiprep" cell lysis and fixation system and Isoton II isotonic buffer were purchased from Coulter (Hialeah, FL, USA). Paraformaldehyde (PFA) and dimethyl sulphoxide (DMSO) were obtained from BDH (Essex, UK). *Dermatophagoides pteronyssinus* (*Der-p*) (Aquagen extract) was purchased from ALK (Horshølm, Denmark). Tritiated methylthymidine was purchased from Amersham (Buckinghamshire, UK).

Three colour surface flow-cytometry

The flow-cytometric cell surface parameters (±their alternative antibody epitopes) selected for analysis were: CD2: this receptor is found on virtually all T-cells, natural killer (NK) cells and thymocytes. CD3 (OKT3): the common T-cell receptor complex (TCR). CD4 (Leu3a/OKT4): the major histocompatibility complex class (MHC) II receptor. CD8 (OKT8): the MHC class I receptor. CD25: the IL-2 receptor β chain. CD45RA: expressed in resting and naive T-memory cells. CD45RO: expressed in activated T-memory cells. HLA-DR: one of the MHC class II molecules (human leukocyte antigen DR).

At each visit, a whole blood differential count was performed using a Technicon H*1 (Coulter). One hundred-microlitre aliquots of ethylenediamine tetra-acetic acid (EDTA) peripheral whole blood were stained with the antibodies and incubated in the dark for 30 min. Leu3a, which competes with keliximab was used to identify "coated" cells and OKT4, which does not compete with keliximab, was used to identify CD4+ cells still in peripheral blood despite binding

with keliximab. Red blood cells were then lysed and the remaining cells fixed using the "Multiprep" system. The cells were resuspended in 250 μL of 0.5% formaldehyde in an isotonic buffer (Isoton II, Coulter) for analysis (performed within 48 h) by a "blinded" flow-cytometrist on an Elite Epics cell-sorter (Coulter) equipped with a 488 nm argon laser. Ten thousand events were analysed for each parameter.

Intracellular flow-cytometry

Heparinized peripheral venous blood was obtained from each subject. PBMCs were isolated by density gradient centrifugation over Histopaque. Cells were washed twice in phosphate-buffered saline (PBS) and then suspended at 1×10^6 cells·mL^{-1} in culture medium RPMI 1640, 10% foetal calf serum (FCS), 2 mM L-glutamine, penicillin (100 units·mL^{-1}), streptomycin (100 μg·mL^{-1}) and stimulated with phorbol 12-myristate 13-acetate (PMA) (10 ng·mL^{-1}) and calcium ionophore (1 μM) in the presence of 3 μM monensin. Cells were incubated for 5 h at 37°C in humidified air containing 5% CO_2. After washing twice in PBS, PBMCs were fixed by suspending them at 4°C for 10 min in 4% paraformaldehyde dissolved in PBS. After one wash in PBS, cells were resuspended in a solution of 10% dimethylsulphoxide (DMSO), 50% FCS in RPMI 1640 at a concentration of 10×10^6 cells·mL^{-1} and stored at -80°C until processed and analysed in a batched and "blinded" fashion at the end of the clinical study analysis.

Cells were defrosted in a water-bath for 30 s followed by the addition of 5 mL of PBS at room temperature. Cells were washed twice in PBS and then permeabilized by suspending them in a 0.1% saponin solution in PBS containing 10% mixed human serum ("saponin buffer"). Aliquots of 10^6 permeabilized cells were incubated with the PE conjugated cytokine-specific mAb or isotype control in saponin buffer (at a final antibody concentration of 5 μg·mL^{-1}) and OKT4 FITC and CD3 ECD for 30 min in the dark. Cells were washed once in saponin-buffer then once in PAB (PBS containing 0.5% weight/volume bovine serum albumin and 0.1% sodium azide) prior to resuspension in 250 μL 0.5% formaldehyde solution in isoton and analysis by flow cytometry. The lymphocyte population was gated by characteristic forward and side-scatter and then subgated by both CD4 and CD3 positivity. Two thousand events were analysed for each cytokine.

Pharmacokinetic assays

Blood samples were collected into heparinized tubes and chilled in an ice-bath. Plasma was separated by centrifugation stored in polypropylene tubes at -70°C until analysis. These were assayed for keliximab using a electrochemiluminescent immunoassay by Tektagen Incorporated (Malvern, PA, USA) with a lower detection limit of 40.0 ng·mL^{-1}.

Cell proliferation studies

PBMCs were isolated from heparinized blood samples by density gradient centrifugation, washed twice in HEPES-buffered RPMI medium and resuspended in RPMI medium supplemented with 5% human AB serum, 2 mM L-glutamine, penicillin (100 units·mL^{-1}) and streptomycin (100 μg·mL^{-1}). A short-term culture technique requiring a single antigenic stimulation of PBMC was used, as described previously [9]. PBMCs were cultured at 1×10^6 cells·mL^{-1} with 25 μg·mL^{-1} *Der-p* for proliferation assays. Keliximab or an irrelevant chimeric human/macaque IgG1 monoclonal antibody (anti-respiratory syncitial virus) was added to culture medium to achieve a concentration of 0.5, 1, 10, 50 and 100 μg·mL^{-1} to mirror the concentrations achieved in the clinical study. Proliferation was measured on day 7 by adding 37 kBq of tritiated methylthymidine·well^{-1} at the last 16 h of culture and assaying label incorporation by liquid scintillation spectroscopy.

Statistical methods

Data were analysed by an independent statistician.

Surface and intracellular flow-cytometry. Differences in the recorded values between the four groups at baseline were tested using a one-way analysis of variance. The significance of variation over time within a group was tested with a 2-way analysis of variance (ANOVA). Where significant variation was found, it was investigated by testing the change from baseline using a paired t-test. Differences in the changes from baseline between the four groups on each day were tested using a one-way ANOVA. Where significant variation was found this was further investigated using all pairwise comparisons of the least squared means at the 5% level (statistical significance at $p \leqslant 0.05$).

Proliferation responses. The distribution of the data was tested and significant deviations from normality were found. Variation across doses within each group was tested using a nonparametric 2-way ANOVA and any significant variation further investigated using a Wilcoxon signed-rank test. Differences in the changes between the isotype control and keliximab group were tested using a Wilcoxon rank-sum test.

Results

Pharmacokinetics

In all active treatment cohorts, the concentration of keliximab increased during the 2-h infusion period with mean maximum concentrations (Cmax) of 11.5 μg mL^{-1} for $C_{0.5}$, 42.9 μg mL^{-1} for $C_{1.5}$ and 107 μg mL^{-1} for $C_{3.0}$. This then remained constant for ~6 h for $C_{0.5}$, 10 h for $C_{1.5}$ and 22 h for $C_{3.0}$. Nonquantifiable concentrations were found in two of six subjects at day 1, two of six subjects at day 2 and two of six subjects at day 7 in $C_{0.5}$. In the $C_{1.5}$ group, nonquantifiable concentrations were found in one of

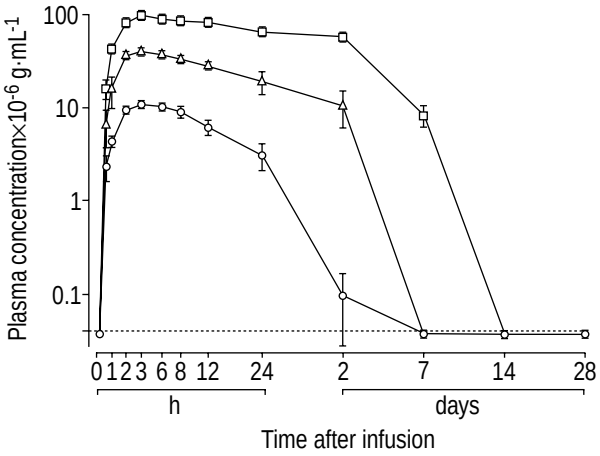

Fig. 1.–Pharmacokinetic data (mean maximum concentrations) from all cohorts following infusion of keliximab. Data presented as mean±SEM. □: 3.0 mg·kg^{-1} group; △: 1.5 mg·kg^{-1}; ○: 0.5 mg·kg^{-1}.

five patients at day 2 but with the remaining four patients having no detectable antibody by day 7. In the C3.0 group, one of five patients had nonquantifiable concentrations at day 7, two had nonquantifiable concentrations at 2-weeks and the remaining two had nonquantifiable concentrations at 4 weeks, although no samples had been analysed on these last two subjects for their 2 week visit (therefore raising the possibility that they may have had undetectable concentrations at 2 weeks). These data are summarized in figure 1. Anti-idiotypic antibodies were not detectable at day 28 postinfusion.

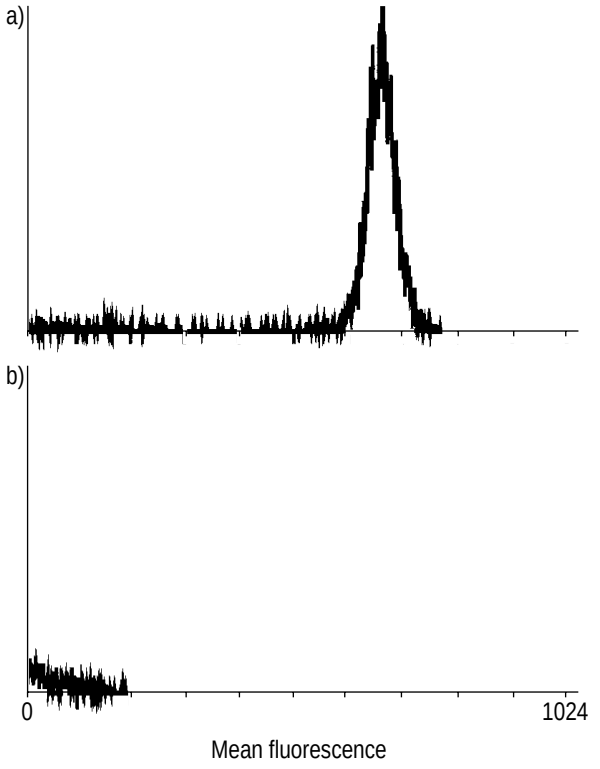

Fig. 2.–Sample histogram of Leu3a staining a) pre- and b) postinfusion with keliximab in a patient from the C0.5 cohort with complete loss of fluorescence postinfusion (sample gated on CD3+/OKT4+ cells).

Clinical study immunophenotypes

Rapid and efficient coating of the CD4+ lymphocyte was achieved in all subjects who received keliximab as shown by complete loss of mean fluorescence (mf) in Leu3a staining (fig. 2). The mf had returned to baseline values in C0.5 and C1.5, but was still apparent in C3.0 at day 7 (fig. 3). Modulation of OKT4 staining

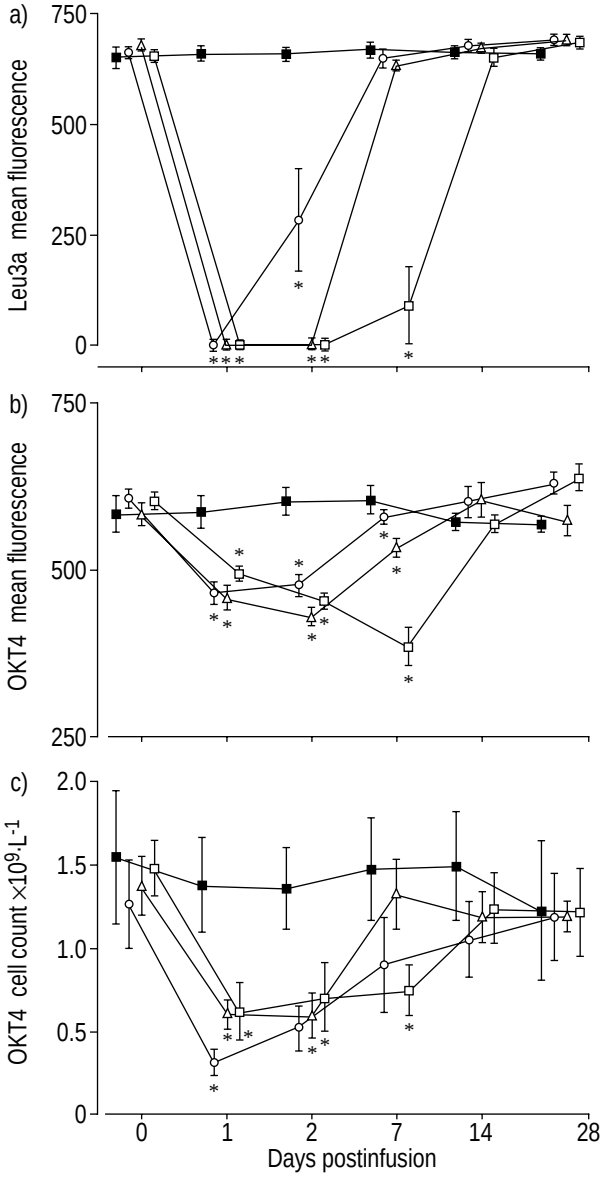

Fig. 3.–Changes in: a) the mean fluorescence of Leu3a staining; b) the mean fluorescence of OKT4 staining; and c) numbers of OKT4+ cells following keliximab. Data represents mean±SEM. ■: placebo group; ○: 0.5 mg·kg^{-1} keliximab group; △: 1.5 mg·kg^{-1} group; □: 3.0 mg·kg^{-1} group. *: significantly decreased ($p<0.05$) from baseline values.

Table 2. – Effects of keliximab on mean CD2, CD3 and CD8 cell counts ($\times 10^9 \cdot L^{-1}$)

Cell Type	Dosing cohort	Baseline	Day 1	Day 2	Day 7	Day 14	Day 28	2-way ANOVA p-value
CD2	C_{pl}	1.952	1.898	1.920	1.948	1.952	1.436	NS
	$C_{0.5}$	1.906	0.818*	1.126	1.480	1.718	1.890	0.012
	$C_{1.5}$	2.026	1.224*	1.050	0.904	1.758	1.746	0.016
	$C_{3.0}$	2.592	1.484*	1.534*	1.516*	2.204	2.338	0.015
CD3	C_{pl}	2.140	1.863	1.888	2.022	2.082	1.648	NS
	$C_{0.5}$	1.724	0.670*	0.852	1.298	1.494	1.740	0.004
	$C_{1.5}$	1.874	0.998*	0.874*	1.756	1.538	1.538	0.001
	$C_{3.0}$	2.360	1.288*	1.388*	1.352*	1.946	2.150	0.012
CD8	C_{pl}	0.512	0.465	0.465	0.497	0.547	0.377	NS
	$C_{0.5}$	0.374	0.288	0.300	0.336	0.380	0.470	NS
	$C_{1.5}$	0.460	0.342	0.228	0.376	0.383	0.340	NS
	$C_{3.0}$	0.786	0.544	0.560	0.504	0.644	0.830	NS

ANOYA: analysis of variance; NS: not significant. $C_{0.5}$, $C_{1.5}$, $C_{3.0}$ refer to the concentrations of keliximab received ($mg \cdot kg^{-1}$). C_{pl}: refers to placebo group. *: significantly decreased when compared to baseline values.

was also noted in all subjects, but of less magnitude than that of the changes with Leu3a. The modulation in OKT4 mf was of greatest magnitude in the $C_{3.0}$ group (fig. 3).

The absolute counts of CD4+ T-lymphocytes decreased by day 1, but had returned to baseline values by day 7 in $C_{0.5}$ and $C_{1.5}$ although they were significantly decreased until day 14 in $C_{3.0}$ (fig. 3). Although this effect was most prolonged in the $C_{3.0}$ group, there was no dose effect in terms of the maximal absolute decrease in CD4 counts between any of the active treatment groups when evaluated at 24 h postinfusion (fig. 3). No effect was seen on CD8 counts although there were similar changes in both CD2 and CD3 counts (table 2).

The numbers of CD4+ cells expressing CD25, HLA-DR, CD45RO and CD45RA were decreased in all three active treatment (but not placebo) cohorts (table 3). These returned to baseline values in $C_{0.5}$ and $C_{1.5}$ by day 7, and in $C_{3.0}$ by day 14. In the remaining circulating CD4+ lymphocytes, there was a significant decrease from baseline in CD25 expression in $C_{0.5}$ at day 1, day 2 and day 7 postinfusion (fig. 4) and an increase in expression of HLA-DR in both $C_{0.5}$ (day 1, 2 and 7 postinfusion) and $C_{1.5}$ (day 1 postinfusion) (fig. 4). There was no significant variability in CD45RO expression but a significant decrease in CD45RA expression was found in all three active cohorts ($C_{0.5}$ until day 2 postinfusion, $C_{1.5}$ until day 1 postinfusion and $C_{3.0}$ until day 2 postinfusion) (fig. 4).

Intracellular flow-cytometry. There were no significant changes from baseline in the expression of IL-4, IL-5 and IFN-γ in OKT4+/CD3+ T-lymphocytes in any of the three cohorts.

Monocyte and eosinophil counts. There were no significant reductions in peripheral blood monocyte or eosinophil counts from baseline in the 7 and 14 day measurements in any of the three cohorts.

Proliferation results. Keliximab inhibited *Der p*-stimulated T-cell proliferation from all six sensitized

Table 3. – Effects of keliximab on mean CD25, HLA-DR, CD45RO and CD45RA/CD+cell counts ($\times 10^9 \cdot L^{-1}$)

Cell type	Dosing Cohort	Day 0	Day 1	Day 2	Day 7	Day 14	Day 28	2-way ANOVA p-value
CD25	C_{pl}	0.289	0.268	0.277	0.389	0.395	0.307	NS
	$C_{0.5}$	0.349	0.071*	0.098*	0.141*	0.297	0.342	0.001
	$C_{1.5}$	0.414	0.136*	0.122*	0.356	0.278	0.400	0.001
	$C_{3.0}$	0.333	0.122*	0.132*	0.138*	0.260	0.271	0.008
HLA-DR	C_{pl}	0.176	0.094	0.099	0.154	0.134	0.086	NS
	$C_{0.5}$	0.087	0.036*	0.051*	0.089	0.094	0.097	0.033
	$C_{1.5}$	0.149	0.094*	0.082*	0.116	0.090*	0.124	0.01
	$C_{3.0}$	0.123	0.067	0.062*	0.067*	0.094	0.135	0.009
CD45RO	C_{pl}	0.734	0.707	0.659	0.692	0.758	0.553	NS
	$C_{0.5}$	0.646	0.172*	0.301*	0.469	0.566	0.681	0.001
	$C_{1.5}$	0.848	0.398*	0.367*	0.800	0.744	0.727	0.001
	$C_{3.0}$	0.969	0.369*	0.434*	0.447*	0.718	0.810	0.001
CD45RA	C_{pl}	0.903	0.752	0.758	0.834	0.798	0.718	NS
	$C_{0.5}$	0.751	0.160*	0.243	0.485	0.582	0.668	0.002
	$C_{1.5}$	0.630	0.219*	0.267*	0.628	0.458	0.510	0.002
	$C_{3.0}$	0.654	0.173*	0.186*	0.218*	0.434	0.568	0.001

ANOVA: analysis of variance; NS: not significant. $C_{0.5}$, $C_{1.5}$, $C_{3.0}$ refer to the concentrations of keliximab received ($mg \cdot kg^{-1}$); C_{pl}: refers to placebo group. HLA-DR: human leukocyte antigen DR. *: significantly decreased when compared to baseline values.

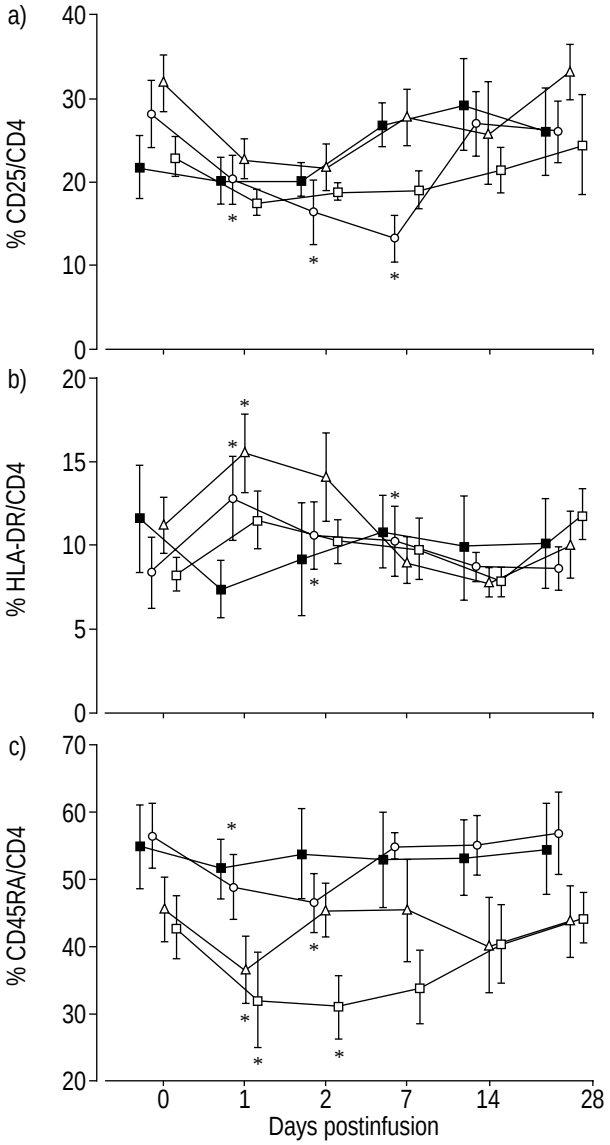

Fig. 4.–Effect of keliximab on relative expression of a) CD25; b) HLA-DR; and c) CD45RA on remaining circulating CD4+ cells. Data represents mean±SEM. ■: placebo group; ○: 0.5 mg·kg^{-1} keliximab group; △: 1.5 mg·kg^{-1} group; □: 3.0 mg·kg^{-1} group. *: significantly decreased ($p<0.05$) from baseline values.

asthmatics *in vitro* (fig. 5). There appeared to be a trend to increased inhibition with ascending concentrations but this was not statistically significant.

Discussion

The CD4 protein is a 55 kD molecule with four extracellular domains, a single transmembrane domain and a short intracytoplasmic tail. It is expressed on the cell-membrane of mature, thymus derived lymphocytes. It binds to a nonpolymorphic region on class II MHC molecules and behaves like an adhesion molecule by stabilizing the T-cell receptor-antigen interaction and thereby facilitates T-cell activation *via*

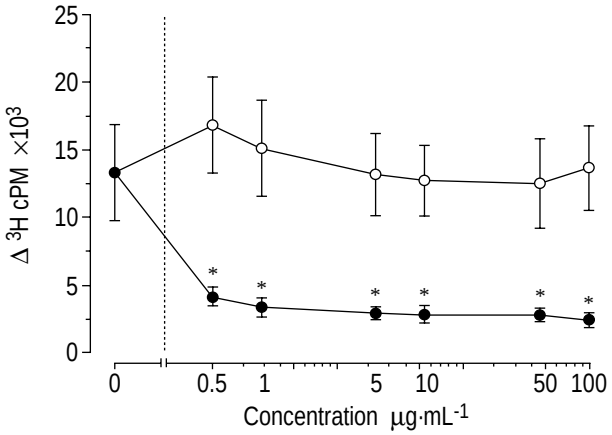

Fig. 5.–The inhibition of proliferation to *Dermatophagoides pteronyssinus* stimulation in six house dust mite sensitive subjects at concentrations of 0.5, 1, 10, 50 and 100 μg·mL^{-1} of keliximab (expressed as thymidine incorporation). ●: keliximab; ○: isotype antibody. *: $p<0.05$ *versus* baseline (0.0 μg mL^{-1}) proliferation.

the T-cell receptor (TCR). The CD4 molecule also physically associates with the TCR upon TCR cross-linking as part of the antigen recognition process and may play a direct part in T-cell activation as a coreceptor *via* the tyrosine kinase p56^{lck}.

Keliximab [7], a chimeric human/macaque anti-CD4 mAb, is an IgG1 λ antibody with macaque variable regions and human constant regions. The clinical improvements in a group of severe asthmatics following an infusion of keliximab has previously been described [8], but its precise mechanism of action in this group of patients has not been elucidated.

There was effective coating of all CD4+ T-cells as demonstrated by the complete loss of Leu3a staining which competes directly for the keliximab binding site. Because of its binding to D1, which is adjacent to the MHC binding site, stereotactic inhibition of the binding of MHC to CD4 may be a possible mechanism. The decrease of absolute numbers of CD2+ and CD3+ T-cells in addition to that of OKT4+ (which binds to domain D4 and is therefore not competitive with keliximab) T-cells indicates that there was also a transient removal from circulation of ~50% of such cells. It is thought that the mechanism of cell removal by anti-CD4 mAbs is through the Fc portion, C3b receptor mediated phagocytosis, complement mediated killing or apoptosis [10, 11]. Although keliximab is able to bind to Fc receptors, it does not bind to C1q nor mediates complement dependent cell cytotoxicity [12]. There was rapid repopulation of these cells by 7 days in $C_{0.5}$ and $C_{1.5}$ but only by 14 days in $C_{3.0}$. The maximum decrease of CD4+ cells in circulation was not concentration dependent and there were no significant differences between all active cohorts at 24 h. This reflects the effectiveness of keliximab binding as confirmed by the complete loss of Leu3a staining at 24 h postinfusion, irrespective of the dose administered (therefore implying the complete saturation of CD4 receptor by even the lowest dosing cohort at this time point). However, the decrease in $C_{3.0}$ was most prolonged and it is possible that the period of modulation of CD4 expression on

T-cells is the critical factor for the clinical effect of improved PEFR noted in this cohort [8]. In addition, previous studies have shown that circulating CD4+ counts may not be a good indicator of the anti-inflammatory effect on target organs [13] and it is possible that only the highest dosing cohort achieved adequate local concentrations in bronchial tissue. The changes in peripheral blood are mirrored by the pharmacokinetic data (fig. 1) and the repopulation noted may reflect the release of cells from lymphoid or nonlymphoid tissue on clearance of keliximab. The mf and immunophenotype of the repopulating cells was identical to those at baseline by day 14 and suggest that these may be the original cells returning into peripheral circulation rather than a naive population of T-cells. In addition there were no incidents of cytokine-release syndrome. It is therefore unlikely that there was any significant cell-death.

Downregulation/modulation of the CD4 molecule was also noted with receptor density being modulated until the 14 day assessment. This is in keeping with previously studied effects of such anti-CD4 antibodies in other patient groups [14, 15] and such a reduction may decrease its coreceptor efficacy.

The changes in absolute CD4+ cells were also mirrored by decreases in absolute numbers of CD25/CD4+, HLA-DR/CD4+, CD45RO/CD4+ and CD45RA/CD4+ cells but these are likely to merely reflect the mAb′s effect on the total CD4 cell count. With the exception of a decrease in CD45RA, there were variable effects on the percentage expression of these markers in the remaining circulating cells, with no correlation to its clinical effect. It is possible that CD45RA+/CD4+ cells are more sensitive to the effects of keliximab, but it should be noted that there was no concomitant increase in the proportion of CD45RO+ cells. This is in keeping with a previous *in vivo* study in rheumatoid arthritis of the effects of an anti-CD4 mAb (cM T412) on CD45RA+ cells [16] although other *in vitro* studies of anti-CD4 mAb′s have shown differing effects [17–19]. There therefore does not appear to be any selective sequestration of activated or memory T-cells by keliximab. Similarly the relative expression of IFN-γ, IL-4 and IL-5 were unaltered in the remaining circulating CD4+ T-cells. This indicates that keliximab has no apparent differential effect on the Th1 and Th2 type cytokine profile. Anti-CD4 mAbs are capable of inducing tolerance, irrespective of their ability to cause depletion [15, 20] and may also deliver a negative signal *via* their interaction with the CD4 molecule [21]. The results of the allergen-specific stimulation studies have demonstrated inhibition of proliferative responses, but without clear evidence of a dose-response.

CD4 is also expressed on monocytes [22] and eosinophils [23]. Although it is possible that keliximab may have exerted its clinical effects through an action on these pro-inflammatory cells, the peripheral blood eosinophil and monocyte counts were not decreased in the present study.

Given the evidence of possible clinical efficacy of an anti-CD4 mAb in asthma, it may be useful to consider the incidence of asthma in human immunodeficiency virus (HIV). Although it is well recognized that patients with acquired immune deficiency syndrome (AIDS) or HIV infection have increased serum concentrations of IgE, there is no evidence to suggest a greater or lesser prevalence of asthma, atopic disease or airway hyperresponsiveness in HIV [24–26]. There is, in fact, evidence that patients with more severe depletion of CD4 counts have a lesser prevalence of asthma [27]. The presentation of airway disease in HIV disease is complicated, and may relate to direct airway infection by HIV, secondary infection, or even concurrent treatment. Given that the exact effects of an anti-CD4 mAb on the T-cell vary significantly, dependent on the specific mAb studied, it is difficult to extrapolate the effects of all anti-CD4 mAb′s on asthma by using HIV as a clinical model of CD4 depletion.

In summary, this study has evaluated the immunological effects of an anti-CD4 monoclonal antibody in chronic severe asthmatics and has demonstrated that there was rapid and effective binding to all CD4+ T-cells with a transient reduction in numbers of circulating CD4+ T-cells, in addition to modulation of CD4+ receptor expression. Anti-idiotype antibodies were not detectable at day 28 postinfusion. This study also suggests that therapy aimed at the CD4 T-cell may be a useful adjunctive therapy in corticosteroid-dependent asthmatics.

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