Comparative Pharmacodynamics of Keliximab and Clenoliximab in Transgenic Mice Bearing Human CD4

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Accepted for publication December 10, 1999 This paper is available online at http://www.jpet.org

ABSTRACT

Keliximab and clenoliximab are monkey/human chimeric CD4 monoclonal antibodies (mAbs) of the IgG1 and IgG4 isotypes, respectively. The pharmacokinetics (PK) and pharmacodynamics (PD) of these mAbs were evaluated in transgenic mice bearing human CD4 molecules on their T cells after a single i.v. administration at three dose levels (5–125 mg/kg). The PK of keliximab and clenoliximab were similar, dose-dependent, and adequately described by a two-compartment model with saturable elimination from both compartments. The enumeration of circulating CD4+ T cells and density of CD4 on their surface were determined as the PD effects. An indirect response model was proposed to characterize the PD effects. With the increase in mAb dose, the maximum intensity (R_max) of PD effects was increased, and the time to reach R_max shifted to later times. At all three dose levels, keliximab caused a relatively rapid decline in the number of circulating CD4+ T cells, which then recovered gradually. In contrast, clenoliximab at the lowest dose (5 mg/kg) did not produce a significant effect on CD4+ T cell counts compared with the placebo group. At high doses, clenoliximab caused a significant decrease in the number of CD4+ T cells. Keliximab appeared to be more potent and efficient in depleting CD4+ T cells. Both mAbs produced similar down-modulation of CD4 at corresponding dose levels. The findings of this study are consistent with the results of a recent clinical trial that emphasize the importance of this transgenic mouse model for evaluating PK/PD to support clinical development of anti-human CD4 mAbs.

Monoclonal antibodies (mAbs) are in development and clinical use for diagnosis and treatment of numerous diseases. mAbs directed against T cells have been studied for their potential to modify pathological conditions in which T cells are involved, including autoimmune disorders such as rheumatoid arthritis (RA), transplant rejection, or graft versus host disease (Hafler and Weiner, 1988; Horneff et al., 1993; Vincenti et al., 1998). One of the most promising targets for mAbs against T cells has been the CD4, a membrane-bound glycoprotein expressed on T lineage cells, including a subset of peripheral T cells. CD4 is critical for interaction with antigen-presenting cells bearing class II major histocompatibility complex (MHC II), which results in an enhancement of the immune response (Veillette et al., 1988). Antibodies to CD4 have been shown to inhibit T cell proliferation and cytokine production in vitro (Takeuchi et al., 1987; Schrenzemeier and Fleisher, 1988).

Keliximab (SB-210396) and clenoliximab (SB-217969) are monkey/human chimeric anti-human CD4 mAbs. Clenoliximab is an IgG4 derivative of the IgG1 mAb, keliximab, with heavy- and light-chain-variable regions from cynomolgus macaque and human lambda light-chain (λ) and γ4 heavy-chain constant domains. Both keliximab and clenoliximab bind to the same epitope on domain 1 of human CD4, with identical specificity and affinity, and are very effective in blocking the interaction of CD4 with MHC II on antigen-presenting cells and B lymphocytes. Keliximab exhibits no binding to complement component C1q and thus does not mediate complement-dependent cytotoxicity. However, as expected of an IgG1 mAb, keliximab shows efficient binding to human IgG Fc receptors, and thus causes depletion of CD4+ T cells. In contrast, clenoliximab is expected to cause little or no depletion of CD4+ T cells due to the reduction of the Fc-dependent effector functions. Recently, clinical studies of these IgG1 and IgG4 mAbs were conducted in RA patients (Yocum et al., 1998; Mould et al., 1999).

Preclinical investigation of the pharmacology of these mAbs has been complicated by their specificity for human
CD4 antigen; these mAbs cross react only with chimpanzee CD4 antigen. The development of transgenic mice bearing human CD4 in place of mouse CD4 (HuCD4/Tg) made it feasible to evaluate preclinical pharmacokinetics (PK) and pharmacodynamics (PD) of these mAbs. Keliximab and clenoliximab are pharmacologically active in the HuCD4/Tg mice and thus are expected to produce PD effects similar to those in humans. The main objectives of this study were to evaluate PK of keliximab and clenoliximab after a single i.v. administration at three dose levels (5–125 mg/kg) and to characterize their effects on circulating CD4⁺ T cells in HuCD4/Tg mice. A PK/PD model is proposed to describe the time course of these PD effects.

Materials and Methods

Chemicals. Keliximab, clenoliximab, and recombinant soluble CD4 were expressed in Chinese hamster ovary cells and purified to homogeneity at SmithKline Beecham Pharmaceuticals. Mouse anti-human IgG1 (clone HP 6069) and IgG4 (clone HP 6023) mAbs were purchased from Zymed Laboratories Inc. (San Francisco, CA) and CALTAG (Burlingame, CA), respectively. All other chemicals were of reagent grade or better.

Animal Husbandry. Male HuCD4/Tg mice were obtained from Charles River Laboratories (Raleigh, NC). These animals express physiological levels of human CD4 and the transgene is appropriately regulated during T cell development. Furthermore, the human CD4 gene restores normal helper cell functions relative to animals with no endogenous CD4 gene (Killeen et al., 1993). The mice were approximately 9 to 17 weeks of age (approximately 30–44 g) at the initiation of dosing. Mice were individually housed in stainless steel cages in a controlled environment (72 ± 4°F; 40–70% relative humidity) with a 12-h light/dark cycle. Mice were offered 5002 Certified Rodent Diet (PMI Feeds, Inc., St Louis, MO) ad libitum. Filtered tap water was available ad libitum from an automatic watering system. This study was conducted in accordance with the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the National Institutes of Health.

Administration of Anti-CD4 mAbs. HuCD4/Tg mice were given a single i.v. dose of vehicle or drug (5, 25, or 125 mg/kg) via a tail vein at a dose volume of 10 ml/kg on day 1 (n = 10–13/group). Five mice per group were bled (approximately 50 μl) from a tail vein into heparinized capillary tubes at approximately the same time of day on days −4, 2, 4, 8, and 15 and then every 2 weeks until the CD4⁺ T cell count returned to baseline values (serial bleeding). Each blood sample was divided for lymphocyte subset analysis (whole blood) and to prepare plasma for the analysis of mAbs.

mAb Assays. Plasma concentrations of unbound keliximab and clenoliximab were determined using electrochemiluminescent immunoassays based on the binding of the anti-CD4 mAbs to recombinant soluble CD4. Plasma was incubated with biotinylated soluble CD4, streptavidin-conjugated paramagnetic beads, and a ruthenium-labeled mouse anti-human mAb specific for the C₄₂ domain of human IgG1 (for keliximab) or the C₃₂ domain of human IgG4 (for clenoliximab) (Hamilton and Morrison, 1993). Electrochemiluminescent responses were recorded with an Origen analyzer (Igen Inc., Gaithersburg, MD). The lower limits of quantification for the immunoassays were 500 and 50 ng/ml using a 50-μl aliquot of sample (plasma diluted 1:100, 0.5-μl equivalents neat plasma) for keliximab and clenoliximab, respectively. For both assays, inter- and intra-run precision was ±8.0% over the assay calibration range and average bias was ±4.1%. A positive response in these assays is indicative of a species containing an antigen-combining site and a remote epitope of the constant region of the heavy chain. The presence of these two features would suggest that the analyte measured is primarily active, intact, and unbound monoclonal antibody.

Flow Cytometry. Peripheral blood lymphocyte populations were analyzed using a flow cytometer (CytoronAbsolute, Ortho Diagnostics, Westwood, MA). For characterization of T cell subclones and anti-CD4 mAb coating, cells were stained with the three-color panels, CD2/OKT4/CD8 and CD3/OKT4/OKT4A, respectively. After a 40-min incubation period, the red blood cells were lysed, leaving the antibody-coated white cells intact. Immunophenotyping analysis included a determination of the absolute number of CD4⁺ cells in CD2⁺ T-lymphocyte population using OKT4 (Ortho Pharmaceuticals, Raritan, NJ) as well as the OKT4 mean fluorescence intensity (CD4 MFI) on these cells. The latter value (CD4 MFI) is proportional to the number of CD4 epitopes on the surface of CD4⁺ T-lymphocytes. The OKT4 antibody used in these analyses binds to an epitope on the CD4 molecule that is distinct from the epitope recognized by clenoliximab and keliximab. These measurements, therefore, were not expected to be affected by treatment with either drug. This was supported by a relatively constant OKT4 CD4 lymphocyte count before and after drug administration in clinical studies of clenoliximab (data not shown).

The percentage of T cell coating by mAb (keliximab or clenoliximab) was assessed by counting the CD4⁺ cells using OKT4 and a second reagent, OKT4A (Leu3a; Ortho Pharmaceuticals), which binds to the same epitope as mAbs. Percentage of T cell coating was derived from the absolute cell counts (OKT4 and OKT4A) using the following equation:

\[
\text{Percentage of CD4 coating} = 100 - \left( \frac{100 \times \text{Leu3a}}{\text{OKT4}} \right)
\]

Pharmacokinetics. A two-compartment model with saturable elimination from both compartments was proposed to characterize PK of keliximab and clenoliximab (Fig. 1). A one-compartment milliary model was not able to describe the plasma concentration-time data simultaneously at all three dose levels because Vₘₐₓ alone was not sufficient to characterize a relatively rapid initial fall in concentrations at high doses when the plasma concentrations were significantly higher than the estimated Kₘ value. The fitting of concentration-time data to a two-compartment model with bi-directional intercompartment rate constants resulted in a negligibly small value for the intercompartment rate constant (kᵢₚₑᵢₜ), which describes the distribution from tissue to the plasma compartment.

Pharmacokinetic model with nonlinear elimination

Indirect response PD model for anti-CD4 antibodies

Fig. 1. PK and PD models for keliximab and clenoliximab. The PD model represents the effect of anti-CD4 antibodies on number of circulating CD4⁺ T cells and cell surface CD4. Symbols are defined in the text.
The proposed two-compartment PK model is based on the understanding of distribution and metabolism of these anti-human CD4 mAbs and assumes that 1) anti-CD4 mAbs permeate into the tissue compartment unidirectionally from plasma across capillaries; 2) these mAbs bind to CD4 on T cells in both circulation (plasma compartment) and tissues; and 3) the major pathway for the disposition of these mAbs is mediated through their binding to CD4 in both plasma and tissue compartments. Thus, the disposition of keliximab and clenoliximab (after a single i.v. administration) at three dose levels can be described as:

$$\frac{dC_p}{dt} = \frac{V_{max} \cdot C_p}{K_m + C_p} - k_{PT} \cdot C_p \quad (2)$$

$$\frac{dC_T}{dt} = k_{PT} \cdot C_p - \frac{V_{max} \cdot C_T}{K_m + C_T} \quad (3)$$

Initial conditions: $C_p = \text{dose} / V_c$; $C_p = 0$;

where $C_p$ and $C_T$ are concentration of unbound mAb in the plasma and tissue compartments, $V_{max}$ represents the maximal elimination rate, $K_m$ is the Michaelis-Menten constant, $V_c$ is the central volume of distribution, and $k_{PT}$ describes the distribution of mAb from plasma to tissue compartment.

The plasma concentration versus time data for keliximab and clenoliximab were sparse around the half-life, as shown previously in humans (Mould et al., 1999). The mean pooled plasma concentration-time data for these two mAbs were pooled to estimate PK parameters because they were similar. This was also observed in the previous PK studies in both HuCD4 transgenic mice and in monkeys (Gobburu et al., 1998) and clenoliximab (Mould et al., 1999). The mean pooled plasma concentration versus time data for all three dose levels were simultaneously fitted to the proposed PK model using a nonlinear regression program, Adapt II (D’Argenio and Schumitzky, 1997).

**Pharmacodynamics**

**Descriptive Analysis.** PD descriptors [area under the baseline and effect curve (ABEC), $R_{max}$ and $T_{Rmax}$] were calculated to summarize the effect of treatment on the number of circulating CD4$^+$ T cells and CD4 density on their surface (CD4 MFI). The ABEC was determined by the linear trapezoidal method to characterize the overall effect of treatment. The intensity of the drug effect was assessed by calculating the maximum inhibitory response (%$R_{max}$) as:

$$\% \ R_{max} = \left( \frac{R_0 - R_{min}}{R_0} \right) \times 100 \quad (4)$$

where $R_0$ is the pretreatment baseline and $R_{min}$ is the nadir of the measured PD determinant after treatment.

**PD Modeling.** The number of circulating CD4$^+$ T cells, density of CD4 molecule on the surface of T cells (CD4 MFI), and the fraction of CD4 occupied by mAb (%coating) were determined as the PD effects of keliximab and clenoliximab.

**Number of circulating CD4$^+$ T cells.** The effect of keliximab and clenoliximab on circulating CD4$^+$ T cell counts was characterized by an indirect PD response model (Daynaka et al., 1993; Sharma and Jusko, 1996; Sharma and Jusko, 1998). Recently, similar models were used for PK/PD analysis of anti-CD40 ligand in monkeys (Gobburu et al., 1998) and clenoliximab in RA patients (Mould et al., 1999).

The proposed model assumes that production of circulating CD4$^+$ T cells is an apparent zero-order process ($k_{in}$) and their loss from circulation is controlled by a first order rate constant ($k_{out}$), as shown in Fig. 1. It is postulated that these mAbs cause stimulation of the loss of CD4$^+$ T cells ($k_{out}$), which fully describes the decrease in number of circulating CD4$^+$ T cells. Thus, the effect ($R$) of keliximab and clenoliximab can be described as:

$$\frac{dR}{dt} = k_{in} - k_{out} \times S(t) \times R \quad (5)$$

and

$$S(t) = 1 + \frac{S_{max} \times C_p}{\Delta C + C_p} \quad (6)$$

where $S(t)$ is the stimulation function, $C_p$ is the plasma concentration of unbound mAb, $S_{max}$ is the maximum stimulation of $k_{out}$ attributed to mAb, $\Delta C$ is the plasma concentration of unbound mAb producing 50% of the maximum stimulation of $k_{out}$, and $R_0$ is the pretreatment baseline. The value of $k_{out}$ was not estimated and cannot be calculated as $k_{out}/R_0$.

The number of CD4$^+$ T cells versus time data for the active treatment groups were not adjusted for the placebo effect because: 1) placebo effect was significant only at the first time point; 2) the contribution of placebo effect to the PD response of high doses of mAbs may be minimal; and 3) it is difficult to adjust for inhibitory placebo effects when the active treatment also produces inhibition.

**Surface density of CD4.** An indirect PD model similar to the one described above was used to characterize the down-modulation of CD4 on the surface of circulating T cells after treatment with anti-human CD4 mAbs. The proposed model assumes that the production and expression of CD4 on the surface of the T cell is an apparent zero-order process ($k_{in}$) and the loss of CD4 is controlled by a first order rate constant ($k_{out}$). The model assumes that mAbs cause stimulation of $k_{out}$, which fully describes the down-modulation of CD4 density.

Pharmacodynamic data were analyzed using the nonlinear mixed effects modeling program NONMEM (version V) (Sheiner and Beal, 1981). The distribution of random residual errors and interanimal variability were described by the exponential error models. The first-order estimation method was applied in NONMEM for the parameter estimates. The PD data (CD4$^+$ T cell number and CD4 density) for each drug at all three dose levels were fitted simultaneously, except for the effect of 5 mg/kg clenoliximab on the number of circulating CD4$^+$ T cells. This dose group was not included in modeling because it showed no significant PD effect compared with placebo. PK parameters estimated from the mean concentration-time data were used to assign free concentration ($C_p$) of keliximab and clenoliximab for eq. 6.

**Percent CD4 coating.** The %coating value as a function of time was described by the Langmuir adsorption isotherm equation (Sung et al., 1992):

$$\% \ CD4 \ coating = \left( \frac{K_a \cdot C_p}{1 + K_a \cdot C_p} \right) \times 100 \quad (7)$$

where $C_p$ is the concentration of unbound mAb as a function of time, and $K_a$ is the in vitro mAb affinity constant. PK parameters estimated from mean concentration-time data were used to assign plasma concentration ($C_p$) of unbound keliximab and clenoliximab for eq. 7.

**Results**

Pharmacokinetics. The plasma concentration-time profiles of keliximab and clenoliximab were described by a mechanism-based PK model with saturable elimination from plasma and tissue compartments (Fig. 1). The individual plasma concentration-time data along with the fitted curves are shown in Fig. 2. There was good agreement between the predicted and observed concentrations. The estimated PK
parameters are presented in Table 1. The estimated tissue volume of distribution was 10-fold higher than the plasma volume of distribution, which may be explained by the significantly higher number (approximately 5-fold) of CD4 T cells in the extravascular compartments, e.g., spleen, thymus, and lymph nodes, compared with the same number of components in circulation. Although the fitted line described the concentration versus time data well, the estimation of $K_m$ may only be an approximation because limited data were available around the estimated $K_m$.

Pharmacodynamics

**Descriptive Analysis.** CD4$^+$ T cells. All animals had a pronounced decrease in numbers of circulating CD4$^+$ T cells after a single i.v. dose of keliximab or clenoliximab as indicated by a significantly lower nadir for all dose groups than that for placebo (Fig. 3). The placebo group also exhibited a significant decrease ($R_{\text{max}}$; $\geq 30\%$) in CD4$^+$ T cell number at the first sampling time point (2 day) compared with the pretreatment baseline. The mean $R_{\text{max}}$ for CD4$^+$ T cells increased and the time of occurrence of $R_{\text{max}}$ ($T_{\text{Rmax}}$) shifted to later times with the increase in dose: 2, 4, and 9 days for 5, 25, and 125 mg/kg dose groups for both mAbs, a signature response pattern for drugs producing indirect PD responses. With a 25-fold change in the dose of mAbs from 5 to 125 mg/kg, the 1.3-fold increase in $R_{\text{max}}$ value was not significant, indicating that the maximum intensity of the response was achieved at low doses (Fig. 3).

The ABEC for circulating CD4$^+$ T cells increased with the increase in dose for both mAbs. For keliximab, mean ABEC values for CD4$^+$ T cells at all three dose levels were significantly higher than those for placebo (Fig. 4). In contrast, the mean ABEC value at 5 mg/kg clenoliximab was not significantly different from placebo. At high doses (25 and 125 mg/kg), the effects on CD4$^+$ T cell numbers were similar for both clenoliximab and keliximab (Fig. 4). The ABEC value for CD4$^+$ T cells increased nonproportionately with a 25-fold increase in dose. The degree of nonlinearity was more pronounced at the higher doses (between 25 and 125 mg/kg) for both mAbs, especially for keliximab.

The maximum intensity of the effect ($\%R_{\text{max}}$) on CD4$^+$ T cell number was similar for keliximab and clenoliximab at corresponding doses. It is interesting to note that, although the $\%R_{\text{max}}$ at 5 mg/kg clenoliximab was significantly higher than that for placebo, the overall response (ABEC) was not. This is due to the transient and minimal effect of clenoliximab at 5 mg/kg dose on circulating CD4$^+$ T cells. A similar

**TABLE 1**

<table>
<thead>
<tr>
<th>Parameter (unit)</th>
<th>Estimate</th>
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<tr>
<td>$V_{\text{max}}$ (ng/ml/h)</td>
<td>890</td>
</tr>
<tr>
<td>$K_m$ (ng/ml)</td>
<td>5249</td>
</tr>
<tr>
<td>$V_C$ (ml)</td>
<td>2.5</td>
</tr>
<tr>
<td>$V_T$ (ml)</td>
<td>25.6</td>
</tr>
<tr>
<td>$\kappa_{PT}$ (day$^{-1}$)</td>
<td>0.15</td>
</tr>
</tbody>
</table>

![Fig. 2. Plasma concentrations of keliximab and clenoliximab for all animals at three dose levels. Symbols represent individual experimental data points. The lines represent the results of least-square regression fitting of the model to the mean pooled data for both mAbs at all three dose levels simultaneously to a two-compartment model with a saturable elimination from both compartments.](image)

![Fig. 3. The intensity of the effect of keliximab and clenoliximab on the number of circulating CD4$^+$ T cells and cell surface CD4 as measured by the nadir of these PD determinants after treatment.](image)
transient effect on CD4$^+$ T cell count was observed after treatment with placebo as shown in Fig. 5. In contrast, keliximab at 5 mg/kg dose produced a relatively prolonged effect on CD4$^+$ T cell count (Fig. 5).

**CD4 density.** A significant down-modulation of CD4 density (CD4 MFI) on the surface of circulating T cells was evident in all animals receiving active treatment as indicated by a lower nadir for all dose groups compared with that for placebo (Fig. 3). There was no change in CD4 MFI in animals receiving placebo. With the increase in mAbs dose, mean CD4 MFI $R_{max}$ remained almost constant, indicating that the maximum intensity of the response was achieved at the lowest dose used in this study (Fig. 3). In contrast, mean CD4 MFI value for ABEC increased with the increase in dose (Fig. 4). There was an almost proportional increase in CD4 MFI ABEC between the 5- and 25-mg/kg dose groups for both mAbs: 4- to 5-fold increase in mean CD4 MFI ABEC value with a 5-fold increase in the dose (Fig. 4). However, there was a $<2.5$-fold increase in mean CD4 MFI ABEC with a further 5-fold increase in the dose from 25 to 125 mg/kg. Overall, both keliximab and clenoliximab produced similar effects on the CD4 density at the dose range evaluated.

**CD8$^+$ T cells.** Keliximab and clenoliximab have no effect on the CD8$^+$ T cells (data not shown) as expected because these mAbs bind to the epitope on CD4 antigen and exhibit no cross-binding to CD8 antigen.

**PD Modeling.** CD4$^+$ T cells. A rapid decline and slow recovery in the number of circulating CD4$^+$ T cells was evident at all three dose levels for keliximab. In contrast, clenoliximab at a 5-mg/kg dose exhibited a transient effect on CD4$^+$ T cell number similar to what was observed in placebo-treated animals (Fig. 5). The highest dose of mAbs used (125 mg/kg) was high enough to produce full inhibition of the system as evident by almost complete depletion of circulating CD4$^+$ T cells (Fig. 6). PD data at all three dose levels of keliximab were fitted simultaneously to the proposed PK/PD model. However, for clenoliximab, the 5-mg/kg dose was not included in modeling and data for the 25- and 125-mg/kg dose were fitted simultaneously. The fitted lines show that the effect versus time data for both mAbs were adequately described by the indirect response model (Fig. 6). The fitted PD parameters for both keliximab and clenoliximab are listed in Table 2. The estimated values of zero-order ($k_{in}$) and first-order ($k_{out}$) rate constants describing the production and loss of CD4$^+$ T cells were similar for both mAbs, as expected. Furthermore, the overall CD4$^+$ T cell turnover calculated from the estimated parameters of the proposed PD model [$t_{1/2} = \ln(2)/k_{out} = 4.1$ weeks] in this study is consistent with the literature values (Sprent and Tough, 1994; Tough and Sprent, 1994). In general, the naive T cells have a relatively slow turnover ($t_{1/2} \geq 5$ weeks), whereas the memory T cells exhibit rapid turnover ($t_{1/2} \sim 2$ weeks). The HuCD4/Tg mice used in this study have an approximately 7:3 ratio of naive to memory phenotype.

Keliximab appeared to be more potent than clenoliximab in depleting circulating CD4$^+$ T cells as indicated by 10-fold lower SC$_{50}$ values for keliximab (Table 2). These findings are supported by the results of the in vitro mechanistic studies that showed that, compared with keliximab, clenoliximab...
has a 10- to 100-fold lower affinity for Fc receptor, a critical step involved in the depletion of CD4+ T cells by anti-CD4 mAbs. Moreover, clenoliximab does not cause any significant lysis or apoptosis of CD4+ cells in vitro up to a concentration of 25 µg/ml (Reddy et al., 2000). The joint fitting of PD data described the CD4+ T cell number versus time profiles well for keliximab (Fig. 6). However, the model underpredicted the effect of 25 mg/kg clenoliximab especially at the early time points. The observed higher suppression of CD4+ T cell number at the early time points may be due to the contribution of a placebo effect because clenoliximab has low potency. For the effect of clenoliximab on CD4+ T cells, the standard error of the estimates (S.E.E.) of PD parameters was not estimated by the NONMEM program because variability in the data was high and the population size was limited.

**CD4 Density.** A down-modulation of CD4 density on the surface of T cells (CD4 MFI) occurred after a single i.v. administration of keliximab or clenoliximab at all three dose levels (Fig. 7). The recovery of CD4 density was relatively rapid after a single i.v. dose for both mAbs. The maximal down-modulation of CD4 density (CD4 MFI $R_{\text{max}}$) was <50% and achieved at 25 mg/kg dose of mAbs. Individual animal CD4 MFI versus time data for all three doses of each mAb were fitted simultaneously to the proposed PD model. The estimated PD parameters for keliximab and clenoliximab were similar (Table 3).

The values of maximum stimulatory factor attributed to mAbs were estimated $[S_{\text{max}} = (R_0 - R_{\text{max}})/R_{\text{max}}]$ from CD4 MFI versus time data obtained at the highest dose (125 mg/kg) because this dose produced full inhibition of the pharmacological system (Sharma and Jusko, 1998). The values of $S_{\text{max}}$ were then fixed to estimate other PD parameters for keliximab and clenoliximab separately by nonlinear mixed effect modeling. PD parameters for both mAbs were estimated with a relatively reasonable precision. The fitted lines show that the CD4 MFI profiles for keliximab were adequately described by the proposed PD model (Fig. 7). However, the joint fittings were only approximate for clenoliximab CD4 MFI profiles especially for the 125 mg/kg dose group which exhibited high variability (Fig. 7).

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**TABLE 2**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Keliximab</th>
<th>Clenoliximab</th>
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<tbody>
<tr>
<td>$k_{\text{in}}$ (% lymphocytes/h)</td>
<td>0.035 (11%)</td>
<td>0.032</td>
</tr>
<tr>
<td>$S_{\text{max}}$</td>
<td>28.2 (18%)</td>
<td>16.2</td>
</tr>
<tr>
<td>$S_{\text{max}}$ (ng/ml)</td>
<td>37500 (54%)</td>
<td>419000</td>
</tr>
<tr>
<td>$R_0$ (% lymphocytes)</td>
<td>34.1 (7.9%)</td>
<td>34.1</td>
</tr>
</tbody>
</table>

| Variances | Between-animal variability in $R_0$ | 13% (48%) | 13% |
| Proportional residual error | 29% (20%) | 26% |

*Typical values (%S.E.E.) listed for keliximab and clenoliximab were estimated from simultaneous fitting of all three doses and fitting of two doses (25 and 125 mg/kg), respectively.*
Coating of the CD4 on the surface of circulating T cells by keliximab or clenoliximab was evident in all animals receiving active treatment (Fig. 8). The duration of saturated (100%) coating increased significantly with the increase in dose for both mAbs. The coating persisted for at least 2, 4, and 9 days in all animals after a single i.v. administration of 5, 25, and 125 mg/kg dose of mAb, respectively (Fig. 8). Coating of CD4 by the mAbs was not apparent when plasma concentrations of keliximab and clenoliximab became nonquantifiable. As expected from the high affinity of these anti-CD4 mAbs and the relatively small amount of CD4 antigen in circulation, relatively low blood concentrations of

**TABLE 3**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Keliximab</th>
<th>Clenoliximab</th>
</tr>
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<tbody>
<tr>
<td>$h_{in}$ (CD4 MFI/h)</td>
<td>3.58 (14%)</td>
<td>8.62 (65%)</td>
</tr>
<tr>
<td>$S_{max}$</td>
<td>0.54 (Fixed)</td>
<td>0.40 (Fixed)</td>
</tr>
<tr>
<td>$SC_{50}$ (ng/ml)</td>
<td>54100 (33%)</td>
<td>59400 (19%)</td>
</tr>
<tr>
<td>$R_0$ (CD4 MFI)</td>
<td>137 (0.9%)</td>
<td>136 (1.1%)</td>
</tr>
<tr>
<td>Variances</td>
<td>3.1 (29%)</td>
<td>5.3 (39%)</td>
</tr>
<tr>
<td>Between-animal variability in $R_0$</td>
<td>4.4 (16%)</td>
<td>5.4 (12%)</td>
</tr>
</tbody>
</table>

*Typical values (%S.E.E.) listed were estimated from simultaneous fitting of all three doses.

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Fig. 8. Time course of CD4 occupancy on the surface of circulating T cells for indicated treatment. Symbols are the individual experimental data points for all three doses ($n = 5$), and lines represent the results of simulation using in vitro affinity constant ($K_a$) and plasma concentrations of keliximab and clenoliximab predicted from typical values of the PK parameters.
mAbs resulted in complete antigen coating. The %coating profiles for all three dose levels were simulated by the Langmuir adsorption isotherm (eq. 7) using in vitro affinity constant ($K_A = 10^8 \text{M}^{-1} = 0.0067 \text{liter/µg}$) and concentrations predicted by the PK model. These simulated profiles based on the in vitro affinity constant adequately describe the observed time course of %coating in vivo (Fig. 8).

Discussion

Pharmacokinetics. The disposition profiles of both keliximab and clenoliximab in transgenic mice bearing human CD4 in place of murine CD4 were similar. The plasma concentrations of both mAbs declined rapidly after a duration that was dependent on the dose. The marked nonlinearity of the PK of keliximab and clenoliximab may be attributed to saturable binding of these mAbs to the cell surface CD4. The steady-state volume of distribution estimated in this study was higher ($V_{ss} = V_C + V_T = 800 \text{ml/kg}$) than that reported for other antibodies. This relatively high $V_{ss}$ may be due to the specific binding of these mAbs to CD4 in highly perfused organs such as spleen as well as nonspecific uptake in the liver (Davis et al., 1996). These findings are supported by the previous studies in which it was observed that the disposition of keliximab was highly dependent on the presence and distribution of the CD4 molecule (Davis et al., 1996; Davis and Bugelski, 1998). It is plausible that the main pathway for the disposition of these mAbs is mediated through binding to the human CD4 because murine CD4 knockout mice had substantially prolonged and higher plasma concentrations of these mAbs(s) relative to those in transgenic mice expressing human CD4 (Davis et al., 1996).

Pharmacodynamics. Keliximab and clenoliximab mediate their in vivo immunomodulatory effects via indirect response mechanisms such as: 1) removal of CD4$^+$ T cells from the peripheral lymphoid organs, systemic circulation, and/or sites of inflammation; 2) down-modulation of cell surface CD4; and/or 3) inhibition of CD4-MHC II interactions resulting in the inhibition of T cell activation. The removal of CD4$^+$ T cells can occur via antibody effector mechanisms such as induction of antigen-specific lysis or apoptosis of CD4$^+$ cells, and down-modulation of cell surface CD4 may be due to internalization or stripping of CD4 from the surface of T cell as has been shown in humans (Hepburn et al., 1998).

In this study, the PD endpoints such as number of circulating CD4$^+$ T cells, density of CD4, and %coating of CD4 on T cells were determined as surrogate markers for the immunomodulatory effect of keliximab and clenoliximab in transgenic mice expressing human CD4. The design of this study was suitable for estimating parameters of the indirect PD model due to the following features: 1) i.v. bolus doses were used and 2) the highest dose used (125 mg/kg) was sufficient to produce full inhibition of the pharmacological system as indicated by near complete depletion of circulating CD4$^+$ T cells and maximal down-modulation of CD4 density (CD4 MFI $R_{max}$) at the 125-mg/kg dose.

An integrated PK/PD model was proposed to characterize the time course of the effect of keliximab and clenoliximab on the surrogate markers based on the mechanism of action of these mAbs. The model used is based on the indirect-response concept that accounts for mechanistic delay in production of responses and shift of the time of occurrence of maximum response to the later times with the increase in dose (Dayneca et al., 1993; Sharma and Jusko, 1996, 1998). Because of the indirect nature of these responses, even as plasma concentrations of keliximab and clenoliximab approached nonquantifiable levels and CD4 coating returned to the baseline value, effects on the circulating CD4$^+$ T cells (depletion of T cells and down-modulation of CD4 density) apparently persisted. The gradual return to baseline can be explained by the magnitude of the rate controlling input of new CD4$^+$ T cells into the blood and the expression of CD4 molecule on the surface of T cells.

Clenoliximab was designed to attenuate the CD4$^+$ cell-depleting potential of keliximab but to retain immunomodulatory effects. The depletion of CD4$^+$ cells, a concern in treatment of diseases requiring chronic administration, may occur through binding of the Fc domain of keliximab to Fc receptors on the effector cells and the F(ab') domain to CD4$^+$ cells. This study showed that, unlike keliximab, clenoliximab had a 10- to 100-fold lower affinity for Fc receptor in vitro than does keliximab (Reddy et al., 2000). The percentage-specific lysis for clenoliximab (<10%) was significantly lower than that for keliximab (~30%) in the concentration range evaluated (0–25 µg/ml). Furthermore, clenoliximab has 10- to 100-fold lower affinity for Fc receptor in vitro than does keliximab (Reddy et al., 2000). However, in this PK/PD study, clenoliximab at doses of 25 and 125 mg/kg caused significant depletion of CD4$^+$ T cells. This may be explained by the differences in the concentrations achieved in vivo in this study and those used in the in vitro study. The plasma concentrations of clenoliximab at 25 and 125 mg/kg were >20-fold higher (>500 µg/ml) than the maximum in vitro concentration evaluated in the antibody-dependent cellular cytotoxicity assay. Although human effector cells were used in the in vitro study, it is possible that at these high concentrations clenoliximab can cause antigen-specific lysis of CD4$^+$ cells via binding to Fc receptors on the mouse effector cells in this transgenic model in a manner similar to keliximab.

Down-modulation of cell surface CD4, evident in all active treatment groups after a single i.v. dose of keliximab or clenoliximab, may be caused in part by antibody-mediated stripping as observed in humans. It is important to recognize that these mAbs cannot strip the majority of CD4 from the surface of T cells even at very high doses, and thus the estimated value of the maximum stimulatory factor was low (0.40–0.54) for both mAbs. It is plausible that receptor-receptor interactions are involved in down-modulation and, below 60 to 70% residual density, the frequency of these interactions is reduced. Similar observations were made in the clinical data from RA patients (Mould et al., 1999).

In conclusion, keliximab and clenoliximab cause a dose-dependent decrease in the number of circulating CD4$^+$ T cells and down-modulation of CD4 on the surface of T cells. We have presented an indirect response model to characterize the time course of effects of two anti-CD4 mAbs on CD4$^+$
T cells in transgenic mice expressing human CD4. The findings of this study are similar to the results from clinical trials at comparable doses. The results of this study emphasize the importance of this transgenic model for preclinical studies evaluating PK/PD of anti-human CD4 mAbs before clinical development.

References


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