Long-Term Multiple-Dose Pharmacokinetics of Human Monoclonal Antibodies (MAbs) against Human Immunodeficiency Virus Type 1 Envelope gp120 (MAb 2G12) and gp41 (MAbs 4E10 and 2F5)

Beda Joos,1* Alexandra Trkola,1 Herbert Kuster,1 Leonardo Aceto,1 Marek Fischer,1 Gabriela Stiegler,2 Christine Armbruster,3 Brigitta Vcelar,2 Hermann Katinger,2 and Huldrych F. Günthard1

Division of Infectious Diseases and Hospital Epidemiology, University Hospital, Zürich, Switzerland

Received 19 December 2005/Returned for modification 3 February 2006/Accepted 22 February 2006

While certain antibodies directed against the human immunodeficiency virus (HIV) envelope have the potential to suppress virus replication in vitro, the impact of neutralizing antibodies in vivo remains unclear. In a recent proof-of-concept study, the broadly neutralizing monoclonal antibodies 2G12, 4E10, and 2F5 exhibited inhibitory activities in vivo, as exemplified by a delay of the viral rebound following the interruption of antiretroviral therapy. Unexpectedly, the antiviral effect seen was most prominently due to 2G12 activity. To further investigate whether differential HIV-inhibitory activity was due to different pharmacokinetic properties of the antibodies, we performed a formal pharmacokinetic analysis with 14 patients. Repeated infusions at high dose levels were well tolerated by the patients and did not elicit an endogenous immune response against the monoclonal antibodies. The pharmacokinetic parameters of all three antibodies correlated with each other. Mean estimates were 0.047, 0.035, and 0.044 liter/kg for the central volume of distribution of 2G12, 4E10, and 2F5, respectively, and 0.0018, 0.0058, and 0.0077 liter/kg · day for the systemic clearance of 2G12, 4E10, and 2F5, respectively. Monoclonal antibody 2G12 had a significantly longer elimination half-life (21.8 ± 7.2 days [P < 0.0001]) than monoclonal antibodies 4E10 (5.5 ± 2.2 days) and 2F5 (4.3 ± 1.1 days). The comprehensive pharmacokinetic data from this long-term multiple-dose phase II study were coherent with those from previous short-term phase I studies, as assessed by compartmental and noncompartmental techniques. The anti-HIV type 1 antibodies studied showed distribution and elimination kinetics similar to those seen for other human-like antibodies. Further studies examining tissue concentrations to explain the differential in vivo activity of the anti-gp120 antibody compared with those of the two anti-gp41 antibodies are warranted.

Potent antiretroviral drug treatment regimens are capable of suppressing human immunodeficiency virus (HIV) in plasma to levels below the limit of detection of the most sensitive assays. However, latent reservoirs of infected cells and low-level virus replication persist and prevent its complete elimination (8, 9, 12, 13, 17, 19, 47). Various vaccination strategies aimed at provoking or supporting the cellular and humoral anti-HIV immune responses of infected individuals have been pursued so far (27). However, they have all failed to efficiently control the infection, and it remains unclear whether this is attributable to the shortcomings of the vaccines used or to the lack of the HIV-infected host’s immune system to mount an adequate response. Studies with patients with long-term non-progressing HIV infection (32) as well as passive immunization trials performed with animal models (3, 20, 31, 35) indicate that neutralizing antibodies can contribute to the control of viremia in vivo.

In a recent proof-of-concept passive immunization trial with humans, we have demonstrated that a cocktail of the three broadly neutralizing monoclonal antibodies (MAbs) 2G12, 4E10, and 2F5 was able to delay viral rebound in patients whose infections were fully suppressed by antiretroviral treatment before administration of the antibodies. Unexpectedly, the inhibitory effect was primarily due to antibody 2G12, as evidenced by phenotypic and genotypic escape studies (42). While MAbs 4E10 and 2F5 recognize two adjacent highly conserved epitopes on the membrane-proximal ectodomain of the HIV type 1 (HIV-1) envelope protein gp41, monoclonal antibody 2G12 binds to a noncontinuous epitope composed of glycosylation residues distributed over the envelope protein gp120 (36, 39, 43, 44). The antibodies were highly active against HIV-1 in vitro (4, 36, 39, 43, 44) as well as in animal studies (3, 30, 31). Safety and tolerability were demonstrated in earlier phase I clinical trials (1, 2).

During the phase II clinical trial, high doses of the three neutralizing antibodies were given in combination to 14 HIV-1-infected individuals at weekly intervals over 3 months. Plasma concentrations were monitored longitudinally over an extended period of time comprising the accumulation phase and steady state as well as the terminal washout phase. Here, we were primarily interested in determining the disposition kinetics of the antibodies and specifically addressed the question of whether potential differences in distribution kinetics might help to explain the differential biological activities of the three monoclonal antibodies in vivo (42). Furthermore, exact longitudinal pharmacokinetic data will inevitably be needed for planning for future passive immunization trials. In addition, such a long-term pharmacokinetic analysis may be of general
interest in the rapidly growing field of therapeutic applications of monoclonal antibodies in a variety of diseases in humans.

MATERIALS AND METHODS

Subjects. Fourteen HIV-1-infected patients (8 chronically infected patients and 6 acutely infected patients; 10 men and 4 women) aged from 21 to 57 years (mean age, 40 years) with a mean weight of 75 kg (range, 49.5 to 85 kg) were entered into a single-site, prospective, open-label, nonrandomized, phase II trial of passive immunization. They were selected from a total of 58 individuals (27 acutely infected patients and 31 chronically infected patients) based on the sensitivities of their autologous virus isolates to monoclonal antibodies 2G12, 4E10, and 2F5 (see reference 42 for details of subject demographics and the criteria used for enrollment). The study complied with the principles of the Declaration of Helsinki, and informed written consent was obtained from each volunteer in accordance with the guidelines of the local ethical committee.

Antibodies. The three different recombinant anti-HIV-1 monoclonal antibodies 2G12 (lot nos. T531002-A and TS93004-A), 4E10 (lot no. T531002-A) and 2F5 (lot nos. T361002-A and TS80703-A) were obtained ready for intravenous infusion as 12-mg/mL aqueous solutions acidified with acetic acid (final concentration, 2 mM; pH 4.7) and 10% maltose from Pseudomonas Scientific, Vienna, Austria, and were stored at 4°C. They were produced by recombinant expression in Chinese hamster ovary (CHO) cells as immunoglobulin G1 (IgG1)]. The generation, production, and characterization of the Mabs were described previously (5, 23, 24, 43). All three antibodies contain identical constant regions and differ only in their variable regions, which were derived from the original hybridoma cells. MAb 4E10 and 2F5 recognize two contiguous, highly conserved epitopes on the external domain of the HIV-1 envelope glycoprotein gp120. MAb 2G12 is specific for a conformational epitope on a variable-domain of N-linked glycan residues distributed over the C2 to C4 domains of envelope gp120. All three antibodies potently neutralize primary HIV-1 isolates of different HIV-1 subtypes (36, 44).

Dosing. The study medication was given to the selected six acutely infected patients and the eight chronically infected patients a total of 13 times over a period of 11 weeks. On days 0, 3 or 4, 7, 14, 21, 28, 35, 42, 49, 56, 63, 70, and 77 the monoclonal antibodies were administered as a series of three consecutive intravenous infusions, each of which consisted of 1 g of 2G12, followed by 1 g of 4E10 and, finally, 1.3 g of 2F5. On day 0 the antibodies were administered by slow intravenous infusion (1 h per antibody) through the same 0.2-μm-pore-size in-line filter. Since this infusion was tolerated without any problems, in all subsequent infusions the infusion rate was increased to 30 min per antibody. The antibody solutions were adapted to room temperature, and at the end of each series the infusion tubes were flushed with physiological NaCl solution. Antiretroviral therapy was stopped on day 1. The protocol specified that antibody treatment be continued with loading doses at day 3 or day 4, followed by further infusions at weekly intervals. Two patients received the loading dose on the fourth day of the study.

Safety evaluations. The subjects were observed for adverse events, and vital signs (body temperature, pulse, blood pressure) were measured before and 0.5 to 1 h after the infusion. A physical examination was performed at the screening visit and at weeks 0, 4, 8, 12, 16, 20, and 24. Aspartate aminotransferase (AST), alanine aminotransferase, bilirubin, blood urea nitrogen, creatinine, lactate dehydrogenase, and alkaline phosphatase levels were measured at the screening visit and at weeks 2, 6, 10, 12, 18, and 24. Urine was analyzed (dip test strip, sediment, microalbumin) at all visits. Additionally, the subjects were closely monitored immunologically and virologically. In one case (subject 6) antiretroviral therapy with zidovudine, lamivudine, and lopinavir had to be reintroduced at week 13 due to a drop in CD4+ T-cell counts below 250 cells per μL.

The most common adverse symptoms possibly related to the repeated infusions of the antibodies were myalgia (n = 5) and joint pain (n = 4). Shivering (n = 1). transiently elevated alanine aminotransferase levels (n = 2), and transient microalbuminuria (n = 2) also occurred. Subject 4 experienced fluctuating skin rashes following the first and second infusions (day 0 and day 3, respectively), and passive immunization was stopped. The patient underwent allergic skin testing (prick test) for each of the three monoclonal antibodies infused as well as for the buffer solution. None of the tests revealed any evidence for an allergic type I reaction, and 3 weeks after the infusion was stopped the patient could be reimmunized from the beginning of the infusion sequence. Subject 13 received 13 further infusion series (a total of 15 infusions) without adverse events.

Specimen collection. Blood samples were drawn during the antibody administration phase before infusion and 30 min after the end of infusion of the last antibody on days 0, 3 or 4, 7, 14, 21, 28, 35, 42, 49, 56, 63, 70, and 77. Subsequently, blood was collected at biweekly intervals on days 84, 98, 112, 124, 154, and 168. Additional samples were randomly obtained from 11 of the 14 patients at various times during the follow-up (days 185 to 312, one for four specimens per patient). Plasma was separated from anticoagulated blood and was stored at −80°C before analysis. Overall, 476 samples (34 per subject) were obtained over a period of 45 weeks and were used to measure the levels of monoclonal antibodies 2G12, 4E10, and 2F5.

Pharmacokinetic analyses. The pharmacokinetics of the antibodies administered seemed to be best described by a two-compartment model since the plasma concentrations of Mabs 2G12, 4E10, and 2F5 showed a biphasic decline. Comparison with a one-compartment model was performed by fitting both models to the time course of the plasma levels observed during the postadministration phase (day 77 to day 168). Booster simulation and modeling software, version 3 (SimulStat, A. Bourne, Coaching Capstone, Oak Park, IL). High likelihood estimates and P values as well as lower values of the Akaike information criterion and the Schwartz criteria were all in favor of the two-compartment model.

The pharmacokinetic parameters were estimated by fitting the model to the plasma concentrations of the samples collected during the first dosage interval, as well as to the trough levels during steady state (days 21 to 77) and the concentrations after the last and 2F5 over a period of 4 weeks. In the second clinical trial, single doses of 1 g of MAB 4E10 were administered to eight volunteers; and after 1 week seven of them received three weekly treatments consisting of Mabs 4E10 (1 g), 2F5 (1 g), and 2G12 (0.5 g). To compare the results with the estimates obtained from the present long-term study, we reanalyzed the data from the present studies according to a two-compartment model by fitting the model to individual plasma concentration-time data.

Assessment of immune responses against the monoclonal antibodies. The immunogegecies of Mabs 2G12, 4E10, and 2F5 were analyzed by determination of anti-2G12, anti-4E10, and anti-2F5 IgM and IgG levels in plasma at weeks 0 (predose), 6, and 12 by a μ- and λ-specific double-sandwich ELISA. The λ-specific ELISA for the determination of IgG was chosen in order not to detect coated 2G12, 4E10, and 2F5 antibodies, which contain κ chains. Ninety-six-well microtiter plates were coated overnight with 2G12, 4E10, or 2F5 as the capture antigen. The plates were then washed and incubated with serial twofold dilutions of plasma samples for 1 h. After the plates were washed, goat anti-human IgG (λ-specific) conjugated with alkaline phosphatase or goat anti-human IgG (μ-specific) conjugated with horseradish peroxidase was added and the plates were incubated for another hour. After the plates were washed, horseradish peroxidase- or alkaline phosphatase substrate was added; and the enzymatic reaction, which led to a colored product, was measured spectrophotometrically. The development of IgM and IgG responses against the antibodies was measured in the plasma samples taken before and 42 and 84 days after the first infusion. A positive sample was defined by a greater than threefold increased ELISA readout compared with that for the predose sample.

Statistical analyses. Arithmetic means and standard deviations were calculated. The Kruskal-Wallis test with Dunn’s posttest was applied to evaluate multiple comparisons. The significance level was a P value of <0.05. Statistical
analyses were performed by using GraphPad Prism, version 4.03 (GraphPad Software Inc.).

RESULTS

Fourteen patients with acute or chronic HIV infection were enrolled in this phase II clinical trial. They received the three human recombinant monoclonal antibodies 2G12, 4E10, and 2F5 intravenously at high doses before antiretroviral treatment was stopped; and subsequently, the administration of antibodies was continued for a total of 3 months. Thereafter, the patients were closely monitored for another 3 months. The plasma levels of the passively administered antibodies were measured throughout the trial. The trough concentrations of 2G12, 4E10, and 2F5 found in plasma (mean concentrations during multiple dosing intervals) averaged 485 ± 163, 130 ± 49, and 68 ± 22 mg/liter, respectively. The mean peak levels (individual maximum) were 920, 163, 130 mg/liter, respectively.

The plasma concentration-versus-time profiles showed biexponential declines when they were plotted on a semilogarithmic scale and were consistent with a two-compartment pharmacokinetic model. Each antibody had a rapid distribution phase, followed by a log-linear terminal elimination phase. The model chosen fit the available data well, as demonstrated by median goodness-of-fit $r^2$ values of 0.9839, 0.9866, and 0.9982 for 2G12, 4E10, and 2F5, respectively (range, 0.9515 to 0.9995 for the 42 individual fits).

The resulting pharmacokinetic parameters that were derived from individual fittings for each patient are summarized in Table 1. The estimated $V_c$ varied little across the different antibodies. Normalization to body weight did not improve the variability of the pharmacokinetic parameter estimates, and the estimates found in male and female patients were not significantly different. The first-order distribution rate constants between vascular and extravascular compartments ($k_{12}$ and $k_{21}$) and, accordingly, also the equilibrium (steady-state) volumes of distribution ($V_{ss}$) were similar among the three antibodies. The average $V_{ss}$ was 3.2 times the average $V_c$: the $V_{ss}$-to-$V_c$ ratios were comparable (2.9 ± 0.7, 3.1 ± 1.2, and 3.6 ± 1.2 for MAbs 2G12, 4E10, and 2F5, respectively). In contrast, 2G12 had a significantly slower total systemic clearance than 4E10 or 2F5 ($P < 0.01$ and $P < 0.001$, respectively) and accumulated over time (weeks 0 to 11). Even at week 24, 13 weeks after the administration of the last dose, the plasma levels of 2G12 averaged 60 mg/liter. The mean elimination half-life of 2G12 was 21.8 ± 7.2 days, which was substantially higher than the 5.5 ± 2.2 and 4.3 ± 1.1 days found for 4E10 and 2F5, respectively. Figure 1 shows the plasma concentrations of the three antibodies measured (mean ± standard deviation) together with the calculated mean concentration profiles constructed from time zero to week 24 by use of the respective infusion rates and the estimates obtained for the parameters $V_c$, $k_{10}$, $k_{12}$, and $k_{21}$.

The pharmacokinetic parameters of all three antibodies were significantly correlated with each other. The absolute

---

**TABLE 1. Pharmacokinetic parameters obtained with 13 intravenous doses of monoclonal antibodies 2G12, 4E10, and 2F5 over 11 weeks**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>2G12</th>
<th>4E10</th>
<th>2F5</th>
</tr>
</thead>
<tbody>
<tr>
<td>$k_{10}$ (day$^{-1}$)</td>
<td>0.037 ± 0.017</td>
<td>0.151 ± 0.068</td>
<td>0.171 ± 0.043</td>
</tr>
<tr>
<td>$V_c$ (liter · kg$^{-1}$)</td>
<td>0.047 ± 0.011</td>
<td>0.035 ± 0.010</td>
<td>0.044 ± 0.010</td>
</tr>
<tr>
<td>$k_{12}$ (day$^{-1}$)</td>
<td>1.006 ± 0.121</td>
<td>1.197 ± 0.267</td>
<td>1.216 ± 0.142</td>
</tr>
<tr>
<td>$k_{21}$ (day$^{-1}$)</td>
<td>0.594 ± 0.149</td>
<td>0.682 ± 0.323</td>
<td>0.511 ± 0.143</td>
</tr>
<tr>
<td>CL (liter · kg$^{-1}$ · day$^{-1}$)</td>
<td>0.0018 ± 0.0012</td>
<td>0.0058 ± 0.0038</td>
<td>0.00077 ± 0.0028</td>
</tr>
</tbody>
</table>

---

$^a$ 2G12 was administered at 1 g (13.7 ± 2.2 mg/kg), 4E10 was administered at 1 g (13.7 ± 2.2 mg/kg), and 2F5 was administered at 1.3 g (17.8 ± 2.8 mg/kg).

$^b$ $k_{10}$, first-order elimination rate constant from the blood compartment; $V_c$, central volume of distribution; $k_{12}$ and $k_{21}$, first-order distribution rate constants between blood and tissue compartments; CL, systemic clearance.
distribution volumes showed the strongest correlations ($P = 0.0005$, $P = 0.0007$, and $P = 0.0001$ for the comparisons of 2G12 with 4E10, 2G12 with 2F5, and 4E10 with 2F5, respectively), followed by clearance ($P = 0.0001$, $P = 0.004$, and $P = 0.0006$, respectively), elimination rate constants ($P = 0.0006$, $P = 0.0346$, and $P = 0.0107$, respectively), and distribution rate constants ($P = 0.0006$, $P = 0.0061$, and $P = 0.0208$, respectively). No strong correlations were found for $k_{21}$ ($P = 0.0592$, $P = 0.0691$, and $P = 0.0382$, respectively).

There were no serious adverse effects as a result of the administration of the antibodies during this study, and the hematological and biochemical parameters did not change significantly between the pretreatment and the posttreatment periods. Minor adverse symptoms possibly related to the infusions included myalgia ($n = 5$), arthralgia ($n = 4$), and exanthema ($n = 1$). No significant induction of humoral immune responses against the monoclonal antibodies was detected by a $\mu$- and $\lambda$-specific double-sandwich ELISA (Fig. 2). A minor anti-4E10 IgM response was observed by week 12 in only one patient (patient 10; 3.6 times above the background response) in the absence of any anti-4E10 IgG response. However, the presence of this low level of anti-4E10 IgM did not appear to accelerate the rate of clearance of 4E10.

In addition to the long-term multiple-dose phase II study described above, we also assessed the pharmacokinetics of MAbs 2G12, 4E10, and 2F5 by two-compartment analysis of data obtained from two previous phase I studies. The results of noncompartmental analyses have already been published (1, 2) and are presented in Table 2, along with those of our two-compartment analysis of the monoclonal antibodies in all patients participating in the three different studies. Overall, the average central volumes of distribution ranged from 2.6 to 3.7 liters, which corresponds to a range of 0.036 to 0.049 liters per kg of body weight. The terminal elimination half-lives of 2G12, 4E10, and 2F5 were 18.5, 5.0, and 4.8 days, respectively.

### DISCUSSION

MAbs 2G12, 4E10 and 2F5 are broadly neutralizing human IgG1 antibodies which bind to the surface gp120 or transmem-

![FIG. 2. Development of IgM (top) and IgG (bottom) immune responses against the monoclonal antibodies. Data are presented as the mean ± standard deviation change in anti-2G12 (circles), anti-4E10 (diamonds), and anti-2F5 (squares) concentrations compared with the pretreatment values.](http://aac.asm.org/)

### TABLE 2. Summary of the values of the pharmacokinetic parameters for antibodies 2G12, 4E10, and 2F5

<table>
<thead>
<tr>
<th>MAb</th>
<th>No. of patients</th>
<th>Dose (mg)</th>
<th>$t_{1/2a}$ (days)</th>
<th>$t_{1/2b}$ (days)</th>
<th>$V_c$ (liter/kg)</th>
<th>Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>2G12</td>
<td>7</td>
<td>500</td>
<td>ND$^b$</td>
<td>15.17 (5.60)</td>
<td>ND</td>
<td>Noncompartmental (1)</td>
</tr>
<tr>
<td>7</td>
<td>500</td>
<td>1.24 (0.98)</td>
<td>13.84 (3.19)</td>
<td>0.047 (0.004)</td>
<td>Noncompartmental (2)</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>1,000</td>
<td>2.84 (1.56)</td>
<td>16.62 (4.13)</td>
<td>ND</td>
<td>Two-compartment$^e$</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>1,000</td>
<td>1.22 (0.59)</td>
<td>16.97 (8.09)</td>
<td>0.054 (0.009)</td>
<td>Two-compartment$^e$</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>1,000</td>
<td>0.70 (0.08)</td>
<td>21.80 (7.21)</td>
<td>0.047 (0.011)</td>
<td>Two-compartment$^e$</td>
<td></td>
</tr>
<tr>
<td>28</td>
<td>0.97 (0.61)</td>
<td>18.45 (7.50)</td>
<td>0.049 (0.009)</td>
<td>Two-compartment$^e$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4E10</td>
<td>7</td>
<td>1,000</td>
<td>ND</td>
<td>7.73 (5.42)</td>
<td>ND</td>
<td>Noncompartmental (1)</td>
</tr>
<tr>
<td>8</td>
<td>1,000</td>
<td>0.54 (0.45)</td>
<td>4.15 (1.31)</td>
<td>0.038 (0.006)</td>
<td>Two-compartment$^e$</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>1,000</td>
<td>0.61 (0.15)</td>
<td>5.49 (2.22)</td>
<td>0.035 (0.010)</td>
<td>Two-compartment$^e$</td>
<td></td>
</tr>
<tr>
<td>22</td>
<td>0.59 (0.29)</td>
<td>5.01 (2.07)</td>
<td>0.036 (0.009)</td>
<td>Two-compartment$^e$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2F5</td>
<td>7</td>
<td>1,000</td>
<td>ND</td>
<td>6.09 (6.03)</td>
<td>ND</td>
<td>Noncompartmental (1)</td>
</tr>
<tr>
<td>7</td>
<td>1,000</td>
<td>1.70 (0.28)</td>
<td>5.79 (1.18)</td>
<td>0.050 (0.008)</td>
<td>Two-compartment$^e$</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>1,000</td>
<td>1.04 (0.22)</td>
<td>6.54 (2.06)</td>
<td>ND</td>
<td>Noncompartmental (2)</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>1,000</td>
<td>0.88 (0.25)</td>
<td>4.60 (3.22)</td>
<td>0.059 (0.010)</td>
<td>Two-compartment$^e$</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>1,300</td>
<td>0.58 (0.07)</td>
<td>4.31 (1.12)</td>
<td>0.044 (0.010)</td>
<td>Two-compartment$^e$</td>
<td></td>
</tr>
<tr>
<td>28</td>
<td>0.93 (0.50)</td>
<td>4.75 (1.91)</td>
<td>0.049 (0.011)</td>
<td>Two-compartment$^e$</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$^a$ Comparison of mean values from three clinical studies. Data represent means (standard deviations).

$^b$ ND, not determined.

$^c$ Second phase I study.

$^d$ First phase I study.

$^e$ Phase II study.

$^f$ Summary of three studies.
brane gp41 envelope glycoproteins of HIV-1. In a recent proof-of-concept study we have demonstrated that at high doses a cocktail of these three monoclonal antibodies exhibits in vivo inhibitory activity against virus replication in some patients with established HIV-1 infection (42). Interestingly, the main antiviral effect seen was primarily attributable to the 2G12 antibody, as evidenced by unambiguous phenotypic and genotypic escape studies. In contrast, no in vivo escape against 4E10 and 2F5 was detected. Here we present a formal pharmacokinetic analysis of a clinical trial which was used to explore whether the differential pharmacokinetic properties between the two anti-gp41 antibodies and anti-gp120 antibody 2G12 might partially be responsible for the unexpected differences in the activities observed in vivo. The objective of the present study was to evaluate the pharmacokinetics of the three antibodies and to explore whether they induce the development of an endogenous antibody response. Despite the use of 30% higher doses of 2F5, the peak and trough plasma concentrations of 2G12 achieved were substantially higher than those of 2F5 and 4E10 achieved. The higher 2F5 dosage was chosen based on the results of the preceding phase I studies, where a shorter half-life of 2F5 compared to those of 2G12 and 4E10 was found (1, 2).

The distribution and elimination pharmacokinetics of the three antibodies presented in this report indicate that they have kinetics which are similar to those of other antibodies, with small volumes of distribution and relatively long elimination half-lives (15, 28). The central volumes of distribution observed in the present study were 0.035 to 0.047 liter/kg, which is equivalent to the assumed standard plasma volume (3 to 5% of total body volume). This suggests that the disposition of the antibodies is initially limited to the vascular compartment. However, the steady-state distribution volumes attained during the postdistributive phase were approximately three times greater, which indicates that a significant quantity of the antibodies is delivered to the tissue compartment. It is important that by using a two-compartment model, the apparent distribution volumes are strongly underestimated in cases where antibody binds with a high affinity to extravascular sites because the assumption of a rapid equilibrium between both compartments is not fulfilled. In addition, this approach assumes that elimination occurs exclusively from the central compartment, which is probably also not correct. Elimination of antibodies is very likely taking place in the central and peripheral compartments simultaneously. However, our experimental data did not allow the differentiation of this. In analogy to other therapeutic antibodies with kinetics comparable to those of the antibodies used in this study, it may be expected that at least 50 to 80% of the given dose was distributed to tissues. Nevertheless, determination of tissue concentrations would be needed to confirm this. In previous studies tissue concentrations ranging from 10 to 50% of the simultaneous plasma levels were found for most antibodies, and in some studies even much greater tissue concentration-to-blood concentration ratios of 2 to 13 were reported (28). Although the clearance values and the half-lives were highly variable, they were in the same range as those reported for other humanized IgG antibodies (28). As in the earlier studies, 2G12 was found to have a significantly longer elimination half-life than 4E10 and 2F5 (1, 2). All three antibodies are of the same IgG1 subtype and differ only in their variable regions. However, minor changes in structures or glycosylation patterns may affect the pharmacokinetics of antibodies and may be possible reasons for these alterations.

The repeated antibody infusions at a high dose level of 3.3 g weekly were well tolerated by the patients. No severe side effects or toxicities were reported during the study. We specifically checked whether a humoral immune response against the passively administered human antibodies developed, since this could have safety implications for the patients and as such responses could be associated with an allergic reaction or could result in the more rapid clearance of other humanized antibodies possibly needed for therapy in the future. Importantly, no significant induction of an immune response against the monoclonal antibodies was found. The slight increase in the anti-4E10 IgM response, which was detected in one sample at week 12, is probably meaningless, as an endogenous antibody response would typically be observed within 7 to 10 days following administration (28) and no transition to an anti-4E10 IgG response was found. Of note, an endogenous immune response directed against the monoclonal antibodies would most likely elicit both κ- and λ-specific IgG simultaneously as well as significant levels of IgM. Thus, there is a minor chance that some potential κ-specific response was missed.

Recently, it has been suggested that 4E10 and 2F5 have polyspecific autoantibody reactivities, and therefore, these antibodies might potentially predispose individuals to autoimmunity if they were infused into patients (21, 34). In our trial, despite the high and sustained levels of these two antibodies, we have not found a clinical correlate for the in vitro findings reported by Haynes et al. (21). In particular, no clinical symptoms or signs compatible with any autoimmune disease appeared during or after the study (poststudy follow-up, 13 to 18 months). Thus, we conclude that despite these in vitro findings by Haynes et al. (21), further clinical investigation with these antibodies is possible. Yet, if such a clinical investigation is undertaken, systematic investigations for laboratory parameters suggestive of autoimmune disease should be undertaken.

The multiple-dose phase II study described here established the long-term kinetics of MAbs 2G12, 4E10, and 2F5 following the administration of high doses. The three antibodies have plasma kinetics that correspond to a two-compartment model. Comparison with the kinetics of other therapeutic antibodies indicate that 2G12, 4E10, and 2F5 have distribution and elimination kinetics that are similar to those of the other compounds of this rapidly growing class of therapeutic agents. At present, approximately 20 monoclonal antibody preparations (antibodies, antibody fragments, or antibody fusion proteins) are on the market. These drugs are in use for a wide range of therapeutic applications in oncology as well as because of their activities against arthritis and other inflammatory disorders. Owing to their large molecular sizes, they are often associated with more complex pharmacokinetic and pharmacodynamic properties than small-molecule drugs. A saturable interaction with target antigen may influence antibody disposition and may potentially lead to nonlinear distribution and elimination. Moreover, independent of antigen, saturable binding to the intracellular receptor FcRn protects IgG from catabolism. Consequently, IgG antibodies often exhibit concentration-dependent elimination. A detailed over-
Numerous trials have evaluated the pharmacokinetics of antibodies used clinically, but the results of relatively few long-term studies have been published. In most instances only a small number of dosing intervals was observed and sample collection was completed within a few weeks. Some exceptions of studies with antibody preparations that were administered by the intravenous route (10, 16, 22, 29, 40, 45) or the extravascular route (6, 26, 33, 41) with sample collection periods ranging from 5 to 27 weeks can be found. In the same way, only a few reports addressing the clinical pharmacokinetics of antibodies directed against HIV have been published. The first trial assessed a chimeric monoclonal antibody to the V3 loop of the HIV-1 envelope gp120 over 21 weeks (18, 38). Subsequent studies evaluated the kinetics of monoclonal antibody F105 directed to the CD4-binding site of gp120 (7, 46), a humanized antibody binding to the V3 epitope GPGRAF (11), and a humanized anti-CD4 antibody (25). Overall, a single study with HIV-infected patients (18, 38) and only few studies in other disease settings (6, 10, 16, 22, 26, 29, 33, 40, 41, 45) have so far established the pharmacokinetics of antibodies after long-term multiple-dose administration in humans.

The comprehensive data presented here indicate that the anti-HIV-1 envelope monoclonal antibodies examined show distribution and elimination kinetics similar to those seen for other human-like antibodies and are thus expected to be largely distributed to the tissue compartment. Notably, although MAbs 2F5 and 4E10 have the shortest half-lives, we could not identify a distribution phenomenon that would explain the apparently lower in vivo activities of the two anti-gp41 antibodies compared to that of the gp120 antibody 2G12. While our clinical trial suggested that 2G12 had a dominant effect on the study outcome, the in-depth analysis of the pharmacokinetic data and evaluation of the distribution parameters revealed no differences between these antibodies that could reflect on their in vivo activities.

ACKNOWLEDGMENTS
We thank our patients for their commitment; M. Winniger, U. Berberat, R. Hafner, B. Hasse, U. Karrer, R. Oberholzer, C. Schnei- der, and C. Grube for patient care; F. Burgener and C. Leemann for technical help; M. Schlumpfi for infrastructural help; E. Gremlich for study monitoring; I. Nievergelt and C. Vogtli for administrative assis- tance; and R. Weber for general support of the project.

Financial support was provided by the Swiss National Science Foundation (grant PP00B-102647 to A.T. and grant 3100A0-103748 to H.G.; and a T.) research grants from the Union Bank of Switzerland, the Gebert-Ru¨f Foundation (P.041/02), and the FAIR Foundation to A.T. and R. F. Siliciano. 1997. Quantifi- cation of latent tissue reservoirs and total body viral load in HIV-1 infec- tion. Nature 387:183–188.


22. Kovarik, J. M., B. Nashan, P. Neuhaus, P. A. Clavien, C. Gerbeau, M. L,


