Pharmacokinetics of Murine Anti-Human CD3 Antibodies in Man Are Determined by the Disappearance of Target Antigen

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ABSTRACT
Therapy with monoclonal antibodies (mAbs) is characterized by a molar ratio of receptor to drug that is higher than usual in pharmacotherapy. As a consequence, changes in the amount of receptors induced by the therapy may have important consequences for pharmacokinetics. We therefore analyzed the pharmacokinetics and pharmacodynamics of an experimental therapeutic CD3 antibody, CLB-T3/4.A (murine IgA), which was given as a rejection treatment to renal transplant patients. Patients were treated with 5 mg of the mAb, as a daily bolus injection, during 10 days. Mean trough levels of mAbs increased during the 1st week, and decreased thereafter. However, about one-third of the patients had continuously rising trough levels and about one-third displayed a steady state, that was reached only after 4 days. On the first day of treatment, mAb concentrations showed a biphasic plasma disappearance curve. On subsequent days, monophasic plasma disappearance curves were observed with mean half-lives of 6 to 8 h. Administration of the mAb induced disappearance of target antigen from the peripheral blood, which could explain the difference in kinetics between day 1 and subsequent days shown by a simulation of the multidose curve of plasma concentrations, based on target antigen depletion. We conclude that at this dose the pharmacokinetics of CLB-T3/4.A were to a great extent determined by antibody-induced changes in antigen in peripheral blood. Moreover, determinations of pharmacokinetic and pharmacodynamic parameters based on single-dose data and traditional compartment models were inadequate for the purpose of prediction and extrapolation.

For almost all common drugs, binding of the drug to its receptor does not change the pharmacokinetics in a significant way. However, in the case of mAbs, the molar ratio of receptor (the antigen) to drug (the antibody) is high compared with most other pharmacokinetic settings. Therefore, pharmacokinetics of xenogeneic monoclonal antibodies (mAbs) are difficult to model with the commonly used compartment models. Modulation of the amount of antigen, as may occur after interaction with antibody, is expected to have measurable consequences on the pharmacokinetics. Moreover, formation of xenospecific antibodies may lead to increased elimination of the xenogeneic antibody.

We present a pharmacokinetic and pharmacodynamic analysis of CLB-T3/4.A (hereafter IgA-CD3), a murine nonmitogenic CD3 mAb, directed against the CD3 antigen, which is part of the T cell receptor/CD3 (TCR/CD3) complex and present on all T lymphocytes. The TCR/CD3 complex recognizes antigen and subsequently delivers activation signals to the interior of the T cells. Clinically, CD3 mAbs have been used mainly as immunosuppressive drugs in transplantation. In this study, IgA-CD3 was used for treatment of acute rejection episodes in renal transplant patients. In patients treated with CD3 mAbs, T lymphocytes disappear quickly from the circulation and, in addition, lose their TCR/CD3 complex almost entirely (Chatenoud et al., 1982). As mentioned, an extra difficulty may be the formation of human anti-mouse antibodies (HAMAs). However, the reported incidence and clinical significance of these HAMAs vary considerably (Goldmann et al., 1986; Schroeder et al., 1990).

Originally, T cell depletion was supposed to be the central mechanism by which the murine CD3 mAb OKT3 exerted its action. However, as there is no evidence of massive T cell death, interference with T cell receptor function by antigenic modulation is more likely to be important (Hirsch et al., 1988). We show that blockade of T cell proliferation in vitro by a nonmitogenic CD3 monoclonal antibody correlates with down modulation of the TCR/CD3 complex. Therefore, for the pharmacodynamic analysis we used in vivo TCR/CD3 down modulation as the central effect mechanism.

Experimental Procedures

Patients and Treatment Protocol. A total of 18 renal transplant patients with a first acute rejection episode were treated with a 10-day course of IgA-CD3, 5 mg/day, injected each day within 5 min. IgA-CD3 (CLB-T3/4.A) was produced by the CLB (Amsterdam, Netherlands).
The Netherlands) and has been described before (Van Lier et al., 1987, Parlevliet et al., 1994). All patients received maintenance immunosuppression consisting of prednisolone (10 mg/day) and cyclosporin (Neoral, target trough levels between 100 and 200 ng/ml), which was continued during the rejection treatment. The first administration of the mAb was preceded by 500 mg of methylprednisolone i.v. and 25 mg of promethazine p.o. Fifteen of the patients received a complete course and three were treated for 5, 6, and 7 days, respectively, and switched to a different treatment because of treatment failure. In one additional patient, treatment failure was evident after completion of the course. Therefore, we had 14 patients who had a satisfactory response to the treatment.

**Blood Sampling.** Peripheral blood was collected at regular intervals before, during, and after the treatment. Serum was collected and stored at -80°C until further use in ELISAs. PBMC were isolated from heparinized blood by Ficoll density centrifugation and used for flow cytometry. Absolute lymphocyte counts were determined by routine hematological procedures on blood anticoagulated with EDTA.

**ELISAs.** CD3 mAb levels in serum were measured in a sandwich ELISA for murine IgA. Microtiter plates were coated with rat anti-mouse IgA mAb (CLB). Standards (IgA-CD3) and samples were diluted in phosphate-buffered saline (PBS) supplemented with 10% (v/v) pooled human serum (CLB), 1% (v/v) cow’s milk, 0.02% (v/v) Tween 20 and 1 mM EDTA. Binding was detected with horseradish peroxidase-labeled rat anti-mouse α mAb (CLB). The plates were developed with tetramethylbenzidine as a substrate and read at 540 and 450 nm. The lower limit of detection was 10 ng/ml.

HAMAs were measured by ELISA. Microtiter plates were coated with IgA-CD3 and blocked subsequently with PBS/0.2% gelatin/0.02% Tween 20. The second layer consisted of serial dilutions of patient sera in PBS/0.02% Tween 201 mM EDTA. Binding was detected with horseradish peroxidase-labeled antibodies to human IgG (Dako, Glostrup, Denmark). Plates were developed and read as described above. Post-treatment sample dilutions with an optical density at least twice the optical density of the corresponding pre-treatment sample dilution were labeled positive.

In a small number of patients, the specificity of the HAMAs was analyzed. Known positive serum samples were incubated with either TEPC15 (mouse IgA; Sigma, St. Louis, MO) or CLB-T3/4.2a (a murine IgG2a switch variant of IgA-CD3; Van Lier et al., 1987) preceding the ELISA. HAMAs still present after exhaustive TEPC15 absorption were labeled anti-idiotypic antibodies, and HAMAs still present after exhaustive CLB-T3/4.2a absorption were labeled anti-idiotypic antibodies. In all tested samples both types of antibodies were found. Finally, specificity of the assay was confirmed by showing that preincubation with IgA-CD3 rendered positive samples negative.

**Flow Cytometry.** Flow cytometry was performed on a FACScan flow cytometer (Becton Dickinson, Erembodegem, Belgium). Analysis of flow cytometry data was performed with PC-LYSSY software (Becton Dickinson). The mAbs used were CD4-PECy5 and CD8-PECy5 from Dako; CD19-PE, CD3-FITC + CD16-PE + CD56-PE (NK-simultest), CD45-FITC + CD14-PE (Lymphogate), CD3-FITC (Leu-4), and irrelevant mouse-IgG1-FITC from Becton Dickinson; TEPC15 (mouse IgA) from Sigma; anti-mouse κ rat-IgG1-FITC and anti-TCRαβ-FITC Immunotech (Marseille, France); irrelevant rat-IgG1-FITC from Pharmingen (San Diego, CA).

Lymphocyte subsets (CD4<sup>+</sup> T cells, CD8<sup>+</sup> T cells, CD19<sup>+</sup> B cells; CD3<sup>+</sup> and CD16/56<sup>+</sup> NK cells) were determined by FACS analysis of fresh PBMC. A lymphocyte gate was drawn, based on forward scatter and side scatter. The total percentage of lymphocytes (CD4<sup>+</sup>, CD8<sup>+</sup>, CD19<sup>+</sup>) within the lymphocyte gate was determined. Subset percentages were divided by the total lymphocyte percentage in the gate and multiplied with the absolute lymphocyte count to obtain absolute subset numbers.

**Drug Concentration Determination.** For determination of drug concentration in serum, serial dilutions of serum were performed and labeled with mAb IgA-CD3. In a limited number of patients, free TCR/CD3 was detected with an anti-TCRαβ-PE mAb. Mean fluorescence intensities (MFI) of green (FITC) and red (PE) fluorescence for CD4<sup>+</sup> and CD8<sup>+</sup> cells were calculated and MFI for irrelevant control antibodies were subtracted. The resulting values were expressed as a percentage of baseline MFI, measured preceding the mAb treatment. Instrument settings were kept constant per patient.

**In Vitro Experiments.** For inhibition studies, PBMC from healthy individuals were cultured in triplicate for 6 days in round bottom 96-well culture plates (Costar, Cambridge, MA) at 40,000 lymphocytes in a final volume of 170 µl per well. The cultures were stimulated with irradiated allogeneic PBMC (3000 rad, 40,000 lymphocytes per well), in the presence or absence of graded amounts of IgA-CD3 or isotype control. The medium consisted of Iscove’s modified Dulbecco’s medium supplemented with 5% heat-inactivated (45°C, 56°C) freshly drawn autologous serum, 50 µM 2-ME, penicillin (100 IU/ml) and streptomycin (100 µg/ml). The culture plates were incubated at 37°C in a humidified atmosphere of 5% carbon dioxide. Proliferation was measured by addition of [³H]thyminedin (0.2 µCi/well) for the final 8 h, and determination of thymidine incorporation on a scintillation counter. The same PBMC were used to study TCR/CD3 expression in vitro, after incubation with graded amounts of IgA-CD3. Flow cytometry was performed as described above.

**Data Analysis.** To analyze concentration versus time data after single administration of IgA-CD3, we used standard 1- and 2-compartment models with first-order output (Gibaldi and Perrier, 1975). Plasma half-life of IgA-CD3 was calculated with log-linear regression. Simulation of the pharmacokinetic consequences of target antigen depletion was performed with Berkeley-Madonna software (Berkeley-Madonna 8.0; R. Macey and G. Oster, University of California at Berkeley), for simulation of differential equation models. Differential equations used for simulation:

\[
\frac{d mAb}{dt} = \text{input} - K_{bind} \cdot \text{mAb} \cdot CD3_{ag} - K_{out} \cdot \text{mAb} \cdot \text{mAb}
\]

\[
\frac{d CD3_{ag}}{dt} = \text{production} - K_{bind} \cdot \text{mAb} \cdot CD3_{ag} - K_{out} \cdot CD3_{ag} \cdot CD3_{ag}
\]

\[
\frac{d \text{complex}}{dt} = K_{bind} \cdot \text{mAb} \cdot CD3_{ag} - K_{out} \cdot \text{complex} \cdot \text{complex}
\]

In these equations mAb is IgA-CD3, input is the daily bolus injection, \(K_{bind}\) is the rate constant for IgA-CD3/CD3 antigen complex formation, \(CD3_{ag}\) is CD3 antigen as present in peripheral blood, \(K_{out}\) is the rate constant for the spontaneous elimination of IgA-CD3 (i.e., not through complexification), production is the spontaneous production and/or reappearance of CD3 antigen, \(K_{out} \cdot CD3_{ag}\) is the rate constant for the spontaneous elimination of CD3 antigen (i.e., not through complexification), complex is the antigen-antibody complex, and \(K_{out} \cdot \text{complex}\) is the elimination rate constant of complex.

The following parameter values were used for simulation in Fig. 7:

\(K_{bind} = 3; K_{out} \cdot \text{mAb} = 1.2; K_{out} \cdot CD3 = 0.5; \text{production} = 1; K_{out} \cdot \text{complex} = 2.\)
Results

Murine IgA Serum Levels

Figure 1A shows the geometric mean serum trough levels of the 15 patients who completed the course. The highest mean trough levels were reached after 6 and 7 days of treatment, followed by a gradual decrease. Figure 1B shows the mean decay curves after a single dose of IgA-CD3, on days 1, 2, 4, and 10 for the same 15 patients. On days 2, 4, and 10, monoeponential elimination kinetics were observed. The mean increases in mAb level (peak minus preceding trough levels) on these days were 1476, 1422, and 1301 ng/ml, respectively. On the first day of treatment, a lower mean peak concentration of 997 ng/ml was observed. The concentrations during the first 3 h suggested a distribution phase (half-life 2.4 h) not seen on days 2, 4, and 10. After this phase, elimination kinetics similar to that already described above, were observed. The calculated mean plasma half-lives for this phase on days 1, 2, 4, and 10 were 6, 6, 8.3, and 8.2 h, respectively.

The mAb serum levels on days 2, 4, and 10 could be described with a 1-compartment model with a distribution volume of approximately 3.5 liters (corresponding to the plasma volume). The mAb serum levels on day 1 fitted best with a 2-compartment model. The difference between the later days and day 1 was probably caused by the binding of the mAb to its receptor on T cells in peripheral blood. On the first day, normal T cell numbers with normal CD3 antigen expression were present in the peripheral blood. These cells rapidly disappeared during that first day, so that on subsequent days binding of the mAb to its receptor was strongly reduced.

However, a one-compartment model could not be used to describe the mean multidose curve because no steady state was reached (Fig. 1A). Individual patients differed with respect to the moment at which the maximal trough levels were reached. We observed three patterns (Fig. 2): group A had initially increasing trough levels (until days 4–6), followed by a decrease (n = 5), group B had initially increasing trough levels until day 4, followed by a steady state (n = 6) and group C had continuously increasing trough levels (n = 4). Figure 3 shows the variation in plasma half-lives for the three groups identified in Fig. 2. In all patients, serum half-lives increased from day 2 to day 4. From day 4 to day 10 a decrease was observed in group A (increase-decrease), no change in group B (steady state), and, with one exception, an increase in group C (continuous increase).

Amount of CD3 Antigen

T Cell Numbers. Mean peripheral blood T cells numbers in time are depicted in Fig. 4A. T cells were defined as CD4-positive or CD8-bright-positive lymphocytes, irrespective of CD3 antigen expression, which may become undetectable because of the IgA-CD3 treatment. This way, T cell numbers could reliably be estimated, as the four lymphocyte subsets of CD4\textsuperscript{pos} cells, CD8\textsuperscript{bright} cells, B cells and NK cells always added to approximately 100% (data not shown). After the first mAb administration, T cell numbers dropped sharply, and they gradually reappeared during the treatment with a steady state level after 5 to 8 days of about 80% of the starting values. On subsequent mAb administrations, T cell numbers dropped again, although not as sharply as on the first day, and recovered.

Fig. 1. IgA-CD3 serum levels during the treatment. A, trough levels during the treatment until 24 h after the last administration. Geometric mean and standard error of the mean for the 15 patients who received a full treatment course. B, sequential levels on the first (closed triangles), second (open triangles), fourth (closed circles), and tenth (open circles) day. Geometric mean for the 15 patients who received a full treatment course.

Fig. 2. IgA-CD3 serum trough levels (geometric means) during the treatment for three groups of patients, showing three pharmacokinetic profiles. Closed circles, group A (increase-decrease); open circles, group B (steady state); closed squares, group C (continuous increase).
within 24 h to original levels. Therefore, massive cell death induced by the mAb seems not very likely.

**TCR/CD3 Complex Expression.** For the 15 patients who received a complete course, the mean amount of total and free CD3 antigen on CD4^{pos} T cells on a per cell basis in time is depicted in Fig. 4B. After the first mAb administration, CD3 antigen expression fell sharply, increased a little before the second mAb administration, fell again and remained low throughout the treatment. By the end of the treatment, a slight increase was observed, albeit only in the samples taken before the daily mAb administration. After cessation of the treatment, CD3 antigen rapidly reappeared. No difference in CD3 antigen disappearance and reappearance was found between CD4^{pos} lymphocytes and CD8^{bright} lymphocytes. We could detect IgA-CD3 bound in vivo (expressed as a percentage of IgA-CD3 bound in vitro on a pretreatment sample), but it was always very low except at 30 min after the first injection. The percentages of free CD3 antigen and IgA-
CD3 bound in vivo always added to approximately the percentage of total CD3 antigen, underlining the reliability of our method (data not shown). Disappearance and reappearance of free TCRβ antigen followed a curve very similar to that of free CD3 antigen (data not shown), showing that TCR/CD3 complex expression could reliably be estimated by measuring CD3 antigen expression only. Individual patients displayed different reductions of their pretreatment level of TCR/CD3 complexes. On a per cell basis and after 4 days of treatment TCR/CD3 complex expression ranged from 0.9 to 21.2% (free) and from 3.9 to 23.7% (total).

**Absolute Amount of CD3 Antigen in Peripheral Blood.** We used the product of the absolute T lymphocyte number and the percentage of CD3 antigen expression as an estimate of the absolute amount of CD3 antigen in peripheral blood. The mean of the absolute amount of CD3 antigen in peripheral blood was reduced to less than 5% of baseline within 30 min after the first administration and remained low until the end of the treatment (Fig. 4C). By the end of treatment, a slight increase was observed, albeit only in the samples taken before the daily mAb administration. After cessation of the treatment, CD3 antigen rapidly reappeared. As indicated above, this observation could explain the difference between the plasma disappearance curves on day 1 compared with days 2 and after. Individual patients displayed different reductions of their pretreatment amount of CD3 antigen. After 4 days of treatment, CD3 antigen ranged from 0.5 to 8.7% of the amount pretreatment.

To support our hypothesis that changes in the amount of target antigen might have important consequences for the pharmacokinetics, we simulated a multidosed curve based on the following assumptions: 1) IgA-CD3 is eliminated in two ways: spontaneously through a first order process and through complex formation with the target antigen. The latter process is faster than the former. 2) CD3 antigen has zero-order production, and two modes of elimination: spontaneously through a first order process and through complex formation with IgA-CD3. 3) Complex formation is a first order process depending on both the amount of IgA-CD3 and CD3 antigen, and elimination of complex is also a first order process.

It is evident that a steady state is reached after approximately 4 days (Fig. 5). On the first day elimination of IgA-CD3 is biphasic and on later days elimination of IgA-CD3 is monophasic. The simulated curves resemble our observations (Fig. 1, A and B; Fig. 2, group B; Fig. 4C).

**Pharmacodynamics.** The importance of TCR/CD3 complex blockade and down modulation is supported by the experiments in vitro (Fig. 6). Complete inhibition of proliferation in mixed lymphocyte cultures only arrived when about 90% of the TCR/CD3 complex was down-modulated by IgA-CD3, with only 1% of the CD3 molecules freely available. We therefore focused our pharmacodynamic analysis in vivo on TCR interactions and particularly on the presence of free CD3 antigen. Figure 7A shows expression levels of free CD3 antigen in vivo in 18 patients, as a function of the corresponding IgA-CD3 serum levels. The differences between individuals and the differences in time with respect to CD3 antigen expression appeared to be a direct result of different IgA-CD3 serum levels. In a bilogarithmic graph, a linear relationship became apparent, that could be described by the following equation: \( \log M = s \cdot \log C + i \) or \( M = 10^s \cdot C^i \) in which \( M \) (modulation) = free CD3 antigen expression and \( C \) (concentration) = IgA-CD3 serum level. For the given data, \( s \) (slope) = \(-0.5907 \) and \( i \) (intercept) = 1.965. Analogous analysis in individual patients revealed only insignificant differences (data not shown). Therefore, variation in the amount of free and total CD3 antigen was determined by the pharmacokinetic differences between individuals, and not by individual susceptibility on a pharmacodynamic level.

Based on the above equation, free CD3 antigen expression <10% required a serum level >43 ng/ml and expression <5% required a serum level >139 ng/ml. These results agree very well with our in vitro data (Fig. 6).

The pharmacodynamic relationship between IgA-CD3 serum levels and T cell numbers was more complex. Clearly, T cells were most efficiently removed from the circulation when they expressed normal levels of CD3 antigen, as at the moment of the first mAb administration. On later administrations, only a limited proportion of T cells was removed from the circulation, depending on the CD3 antigen level at the moment of administration (Fig. 7B).
Human Anti-Mouse Antibodies

Human anti-mouse antibodies were detectable in 13 of the 15 patients who received a complete course and in one of three with an incomplete course 2 weeks after treatment. Usually, these IgG antibodies were present already in the first post-treatment serum sample in which mAb was not detectable anymore. No difference in the serum half-lives of mAb between patients with high ($n = 7$) or low ($n = 7$) or without ($n = 4$) measurable IgG anti-mouse antibodies was demonstrated. The relationship between serum level and CD3-expression remained the same by the end of the therapy (Fig. 7A, highlighted data), showing that the “bioactivity” of the mAb did not change.

Discussion

In this paper we analyzed the interactions between IgA-CD3 and its target, human T lymphocytes. We showed that the disappearance of almost all CD3 antigen from the peripheral blood during the first day of treatment could explain the different plasma disappearance curve on days 2 through 10, as compared with the curve on day 1. When a low amount of antigen was present in the peripheral blood, as on days 2 through 10, a one compartment model could describe the plasma disappearance curve. When a normal amount of antigen was present in the peripheral blood, as on day 1, a two-compartment model could describe the plasma disappearance curve. It follows that the pharmacodynamic properties of this drug are a major factor in its pharmacokinetics.

However, although we could explain a delay in reaching a steady state with our simulation based on target antigen depletion, the observed data still differed from the model. The mean trough levels increased during the first week of therapy and decreased thereafter, so that no steady state was reached at all. Interestingly, we observed three patterns of trough levels and half-lives in individual patients. In some patients trough levels and half-lives continued to rise throughout the treatment. In others, a steady state was reached. In a third group of patients trough levels and half-lives resembled the curve of the mean trough levels and half-lives.

Starting from the model, one could assume that production and/or reappearance of CD3 antigen is not a constant but that it changes in time, depending on the individual. In all three groups, half-life on day 4 was longer than on day 2. In some of the patients this process of increasing half-lives continued throughout the treatment. These findings suggest that production and/or reappearance of CD3 antigen decreases in all patients initially and continues to do so in some. The decreasing half-lives from day 4 to day 10 in others suggest that here the production and/or reappearance of CD3

Fig. 6. In vitro experiment showing the relationship between inhibition of alloantigen specific proliferation and TCR/CD3 expression. PBMC of nine healthy subjects were used for one-way mixed lymphocyte reactions, each with two different stimulators. Of the 18 mixed lymphocyte reactions, 17 showed significant proliferation. A, proliferation on day 6 for these 17 cultures (cpm, mean and standard error of the mean). The open square represents proliferation in the absence of IgA-CD3. Closed squares represent proliferation in the presence of IgA-CD3. B, CD3 antigen expression after 4 h of incubation for these same nine subjects (mean and standard deviation). Open circles, total CD3 antigen; closed circles, free CD3 antigen.

A

B

Fig. 7. A, expression levels of free CD3 antigen on a per cell basis as a function of IgA-CD3 serum levels. Data from all 18 patients are included. Plus signs represent observations made on the tenth day of treatment. B, T cell depletion as a function of pre-administration CD3 expression. Observations made on the tenth day of treatment from the 15 patients who received a full treatment course are shown.

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Antigen increases finally. One could speculate on the causes of these supposed decreases and increases. However, our data do not allow any conclusion regarding production and/or reappearance of target antigen. We only know the net result of all processes involved.

Antibody formation, which might affect $K_{out\text{, mAb}}$, provides a second, and maybe additional explanation for the differences between the observed data and the model. The timing of decreasing half-lives and of decreasing trough levels is in agreement with our knowledge of the timing of antibody formation. Development of human anti-mouse antibodies can have profound influence on therapy with murine monoclonal antibodies. In the first place, HAMAs, when present in high titer and/or high avidity, may completely remove murine antibodies from the circulation, immediately after administration. This may happen when a second course of murine antibodies is given to a sensitized patient (Prins et al., 1999). In the second place, HAMAs may more subtly reduce the half-life of the murine antibody by increasing its clearance through immune complex formation. In the third place, it is conceivable that anti-idiotypic HAMAs reduce the ‘bioactivity’ of the mAb, by binding to its antigen binding site. Some authors hold that this may happen without an effect on plasma clearance (Chatenoud et al., 1986).

After termination of the mAb treatment, we observed human IgG anti-mouse antibodies in the majority of patients. As we observed only modest decreases in serum half-life, we must assume that the anti-mouse antibody response is suppressed during the therapy. Our HAMA measurements neither prove nor falsify the above explanation for the decreasing half-lives in some of the patients. On the one hand, one patient had a decreasing half-life without IgG HAMAs. On the other hand, some patients with IgG HAMAs had continuously increasing half-lives. What would be needed, are assays that identify HAMAs during mAb treatment. However, such assays are at least difficult and maybe impossible because of antigen (i.e., mAb) excess during the treatment.

The literature on the murine IgG2a CD3 mAb OKT3 is inconclusive about the question whether a steady state is reached during treatment with this mAb. Some authors observed a steady state (Goldmann et al., 1986 and Goldmann et al., 1988) whereas others observed continuously rising trough levels (Hesse et al., 1991 and Alloway et al., 1994). These different observations might be due to the different settings (kidney versus heart or liver, rejection treatment versus induction, each with its specific immunosuppressive protocol) in which the studies took place (Alloway et al., 1994). Interestingly, we observed the different patterns in one and the same setting. Probably, the different patterns reflect individual differences.

Using a quantitative measurement of CD3 antigen expression on a per cell basis, we were able to describe TCR/CD3 complex expression as a function of IgA-CD3 serum levels, as well as T cell depletion as a function of preadministration TCR/CD3 complex expression. In addition, the in vitro experiments stressed the importance of TCR/CD3 complex expression for alloantigenic responsiveness. However, T cell depletion might also be important for clinical effectiveness, although it was only a short-lasting phenomenon. Therefore, the combined parameter of CD3 antigen depletion should also be considered.

We were not able to relate any of the effect parameters to clinical efficacy. A number of factors could be responsible. In the first place, because the CD3 antigen was always strongly reduced, the margins may have been too small to detect a significant correlation. In the second place, rejection processes may differ in severity; moreover, when T cell-independent processes play an additional role, CD3 antibodies are less likely to be successful. In the third place, our measurement of CD3 antigen expression was performed on peripheral blood, which might not have been representative for the situation throughout the body, for instance in the rejecting kidney. Finally, our readout is not inhibition of the rejection process, but functional recovery of the affected kidney, which may depend on a number of other factors, e.g., extent of damage caused by the rejection process, pre-existing damage in the donor organ, or damage done as a result of the transplantation procedure.

What should be the target level to pursue for CD3 antigen expression? Recent studies suggest that a T cell needs triggering of at least 8000 TCR/CD3 complexes for proliferation and cytokine production (Viola and Lanzavecchia, 1996). When sufficient costimulation is provided, 1500 TCR/CD3 complexes would suffice. The number of TCR/CD3 complexes on a peripheral blood T cell is thought to be around 50,000. A reduction to 5%, as is achieved on average in our patients, would therefore certainly impair T cell function.

The experiment in vitro shown in Fig. 6 demonstrates that IgA-CD3 concentrations of >100 ng/ml both reduce CD3 antigen expression and inhibit mixed lymphocyte reactivity for more than 95%. However, it is not known whether such a concentration should be reached throughout the body or only in the peripheral blood and whether it should be exceeded throughout the day or only part of the day. A practical approach to this problem would be to try to keep serum levels throughout the day >100 ng/ml, so that recirculating lymphocytes at any time of the day would encounter inhibitory concentrations of the mAb. The dosing scheme that we applied in our patients was copied from the scheme for OKT3. In view of the sometimes very short (around 4 h) serum half-life that we observed in our patients, it would be prudent to give the IgA-CD3 antibody in divided daily doses in the future.

In conclusion, we found that the pharmacokinetic profile of IgA-CD3 is determined to a large extent by the pharmacodynamic effects of the mAb itself. Although we could not predict the clinical response to the therapy, we were able to describe accurately the relation between mAb serum levels and the mechanistically important effect of TCR/CD3 down modulation. This stresses the importance of describing antigenic modulation of target antigens in a quantitative way.

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References


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