Pharmacokinetics of Monoclonal Immunoglobulin G1, F(ab′)2, and Fab′ in Mice

David G. Covell, Jacques Barbet, Oscar D. Holton, Christopher D. V. Black, R. J. Parker, and John N. Weinstein

Laboratory of Mathematical Biology [D. G. C., J. B., O. D. H., C. D. V. B., J. N. W.] and Office of the Chief, Division of Cancer Etiology [R. J. P.], National Cancer Institute, NIH, Bethesda, Maryland 20892

ABSTRACT

The pharmacokinetics of an immunoglobulin G1 (IgG1) and its F(ab′)2, and Fab′ fragments following i.v. administration in mice has been studied by constructing a physiologically based, organ-specific model to describe antibody biodistribution, catabolism, and excretion. The antibody selected for study (MOPC-21) has no known binding sites in the body and therefore is useful for defining antibody metabolism by nontumor tissues. Whole IgG (a) remains in the body for 8.3 days, the majority of time in the carcass (53.0% of the total residence time); (b) has a distribution volume exceeding that of plasma plus interstitial fluid; (c) distributes into these volumes rapidly for most enteral organs (equilibration time <2.6 min for liver, spleen, kidney, and lung), slower for the gut (<20 min), and slowest for carcass (<260 min); (d) produces interstitial:plasma concentration ratios of >85 for enteral organs and 0.18 for carcass; (e) has the greatest percentage of its catabolism due to the gut (72.8%), followed by the liver (20.5%), then the spleen (3.6%); (f) has the highest extraction on a single pass by the gut (0.14%) and (g) cycles through the interstitial spaces of the body at least 2.8 times/g of organ weight before being metabolized or excreted. When compared with whole IgG, the Fab′ fragment (a) is cleared from the body 35 times faster; (b) has a larger total distribution volume; (c) distributes more rapidly into this volume; (d) produces higher interstitial:plasma concentration ratios; (e) is catabolized principally by the kidney (73.4% of total catabolism), followed by the gut (22.9%), then the spleen (3.1%); (f) is extracted from the circulation to the extent of 3.4% on each pass through the kidney, and less by gut (1.0%) and spleen (0.14%) and (g) cycles through non-kidney interstitial spaces at least 0.4 cycles/g of tissue weight before metabolism or excretion. The F(ab′)2 fragment has pharmacokinetic characteristics that fall between those of whole IgG and Fab′. These results (a) provide pharmacokinetic criteria for selecting whole IgG, F(ab′)2, or Fab′ for various in vivo applications; (b) provide a framework for predicting cumulative tissue exposure to antibody labeled with different isotopes; and (c) provide a reference metabolic state for the analysis of more complex systems that do include antibody binding.

INTRODUCTION

Recent advances in immunology and nuclear medicine have led to improvements in tumor localization with radiolabeled monoclonal antibodies (1–9). Although many achievements in the in vivo application of radiolabeled antibodies can be cited [see reviews by Gallagher (10), Burchiel (11), and Sfakinakis (12)], the development and clinical availability of monoclonal antibodies specific for most tumor-associated antigens rank (12), the development and clinical availability of monoclonal antibodies (1–9). Although many achievements in the in vivo application of radiolabeled antibodies can be cited [see reviews by Gallagher (10), Burchiel (11), and Sfakinakis (12)], the development and clinical availability of monoclonal antibodies specific for most tumor-associated antigens rank (12), the development and clinical availability of monoclonal antibodies specific for most tumor-associated antigens rank. Our laboratory has been conducting experiments on the fate of i.v. administered iodine-labeled IgG, F(ab′)2, and Fab′ fragments of an anti-LYB8.2 monoclonal antibody (clone CY34) (37) and of the MOPC-21 myeloma protein in mice. The anti-LYB8.2 antibody and its F(ab′)2 and Fab′ fragments bind to an allelic determinant on mouse B-lymphocytes, whereas the MOPC-21 antibody has no known target antigen in the mouse. Data from these studies have been used to develop a compartmental model of the pharmacokinetics of MOPC-21 and its fragments. Complete experimental details can be found in Holton et al. and will be discussed only briefly here. One-tenth μg of either whole 131I-IgG or its 131I-labeled fragments, the latter prepared according to the method described by Parham and coworkers (38), tested by sodium dodecyl sulfate:polyacrylamide gel electrophoresis (39) and purified by conducted by those expert in the technique (27).

Recent research suggests that the antibody fragments F(ab′)2, and Fab′ may improve the tumor uptake ratio (27–30). Investigations to date, which have been limited but encouraging, suggest that most of the improvements in tumor uptake ratios are due to a more rapid plasma clearance of antibody fragments, producing lower background levels (11, 27).

The levels of background radioactivity, which when elevated tend to lower the tumor uptake ratio, are determined by the kinetic factors of antibody biodistribution, catabolism, and excretion (22). Therefore it would be useful to know how these pharmacokinetic factors compare among whole IgG and its fragments. Most previous analyses of the metabolism of antibodies have lumped various body pools into a single (e.g., intravascular or extravascular) compartment (31–33) and thus have not permitted close examination of regions of the body that are known to have different antibody concentrations. Studies in which individual tissues have been examined for antibody have pointed to the need for a better understanding of antibody metabolism by the various organs (34–36).

An alternative approach for investigating how kinetic factors influence levels of background radioactivity is to develop a physiologically based, organ-specific model that describes the pharmacokinetics of radiolabeled antibody. A relatively simple model, which we will report in detail here, defines the pharmacokinetics of an i.v. injected murine, homologous whole IgG1 (MOPC-21) and its F(ab′)2 and Fab′ fragments in a mouse system that has no known antigen binding sites. This system avoids the additional complexities related to specific binding of antibody to tumor antigen. The resulting model will be used in the following three ways: (a) to define quantitatively the pharmacokinetic differences between whole IgG and its fragments; (b) to examine how these pharmacokinetic differences may be used for improved in vivo use; and (c) to provide a reference metabolic state for the analysis of more complex systems that do include antibody binding.

MATERIALS AND METHODS

Experimental Background

Our laboratory has been conducting experiments on the fate of i.v. administered iodine-labeled IgG, F(ab′)2, and Fab′ fragments of an anti-LYB8.2 monoclonal antibody (clone CY34) (37) and of the MOPC-21 myeloma protein in mice. The anti-LYB8.2 antibody and its F(ab′)2 and Fab′ fragments bind to an allelic determinant on mouse B-lymphocytes, whereas the MOPC-21 antibody has no known target antigen in the mouse. Data from these studies have been used to develop a compartmental model of the pharmacokinetics of MOPC-21 and its fragments. Complete experimental details can be found in Holton et al. and will be discussed only briefly here. One-tenth μg of either whole 131I-IgG or its 131I-labeled fragments, the latter prepared according to the method described by Parham and coworkers (38), tested by sodium dodecyl sulfate:polyacrylamide gel electrophoresis (39) and purified by

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1 B. Rhodes, S. Burchell, K. Breslow, and D. Laven, unpublished observations (see Ref. 11).


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high-performance liquid chromatography, was injected via the tail vein into C57BL/6 mice. Groups of 4 mice were sacrificed serially over the next 24 h, and measurements were made of antibody in plasma, gut, liver, kidney, spleen, lung, and carcass. The total body recovery and plasma TCA	extsuperscript{3}-precipitable fraction of label were also determined. Averaged (n = 4) data at each time point were used for model development. Data from longer term studies on plasma clearance were also used. The present paper will focus only on the data for MOPC-21 IgG and its fragments.

Model Development. The construction of a useful model of nonspecific antibody metabolism must be firmly based on the major physiologic/pharmacological features of the system (40). Since we can never expect to have enough details to model the entire system, a simpler model is proposed that contains those units which appear to be important globally in antibody pharmacology. For this analysis our model includes the processes related to (a) circulation of plasma, (b) exchange of antibody across the capillary wall, (c) return of antibody from the interstitial space to the bloodstream via lymph, (d) interaction of antibody with "cell-associated" material, and (e) formation of metabolic products. The "cell-associated" regions of the model represent antibody interaction with non-plasma, non-interstitial spaces, which may include direct contact with cells or with matrix within the tissue. Our data do not permit a distinction between the two; hence, we will refer to such interactions as "cell associated." Cell-associated antibody can be irreversibly converted to non-antibody metabolites. The five processes listed above define the rate of antibody transfer between capillary plasma, interstitial fluid, and the cell-associated space for each organ in the model. This analysis is comparable to that of fitting a three-compartment linear model to the data for each organ. All of the organs are linked together in a single model, hence permitting analysis of kinetic interactions among the various organs. Consequently, most of the complexity of the proposed model is due to having data from a relatively large number of organs (n = 6) rather than from specification of a complex model for each organ. A detailed description of the model can be found in "Appendix B." Definitions of model parameters are contained in "Appendix A."

Three model parameters for each organ were treated as unknowns whose values were determined by fitting the model to the data. These were (a) the PS (ml/min), which describes the movement of antibody between capillary plasma and interstitial fluid, (b) the V, (ml), which describes the apparent distribution volume of antibody in the non-plasma and non-interstitial fluid volumes in each organ, and (c) the Kav (ml/min), which defines the conversion of antibody to non-antibody metabolites for each organ. The remaining model parameter values were obtained from published literature (see "Appendix B" and Ref. 41).

The mass balance equations for the proposed antibody model were simulated and analyzed using the CONSAM computer program (42, 43). Estimates of unknown model parameters were obtained by adjusting their values such that the variance-weighted squared error between model simulations and experimental measurements was minimized. The variance of each parameter estimate was obtained from the covariance matrix using standard weighted least-squares methods (44).

Calculations. MRT of antibody in each organ defines the average time that antibody molecules entering the system via i.v. injection remain in that organ (45, 46). MRT is equivalent to the "area under the curve" for a plot of fraction of dose in each organ against time (47). The sum of organ mean residence times equals the average time that antibody remains in the animal. Consequently, MRT measures the cumulative exposure to antibody that a tissue site receives following injection. In terms of interpreting tumor uptake ratios, nontumor regions with high exposure to antibody will have greater background levels and thus require greater localization of tumor-specific antigen for positive immunodetection. Regions with low exposure will produce low background levels. However, low exposure may also indicate limited access of specific or nonspecific antibody to that region. MRT will be used to quantify these effects. Details of MRT calculations can be found in Ref. 47.

A measure of tissue catabolism is the extraction of antibody from capillary plasma on a single pass through an organ. Extraction (E_organ) is defined as

$$E_{\text{organ}} = 1.0 - \frac{\text{rate}_{\text{out}}}{\text{rate}_{\text{in}}}$$

and will be calculated for each organ by model simulation.

One goal of tumor localization is to maximize uptake by tumor-specific antigen and to minimize nonspecific background. A factor that affects the uptake of tumor-specific antibody is whether the antibody has sufficient opportunity to bind to the target antigen. Clearly, getting to the antigenic site is a prerequisite to binding. From our model it is possible to calculate the average number of times that an antibody molecule can be expected to pass through each compartment. This process is referred to as cycling, and the net number of cycles of antibody through each region in the model is calculated as the product of the compartmental mean residence time (min) and the compartmental turnover time (min	extsuperscript{-1}) (47). Regions where net cycling is high will have the greatest opportunity for specific antibody to locate and presumably bind to antigen.

Determination of Unknown Model Parameters. Having constructed a mathematical model to describe antibody metabolism it was necessary to determine whether the unknown parameters of the model could be estimated from the available data (48). This issue, commonly referred to as "the inverse problem," has been discussed in detail by Jacquez (49). Its solution involves adjusting the values of the unknown parameters until the model can accurately simulate the experimental data. If the data are sufficiently rich in information content (50) and the parameters are appropriately chosen, the unknown parameter values can be uniquely determined from the experimental data. Theoretical parameter identifiability for linear systems has been thoroughly documented (51). Unfortunately, the proposed theory for large linear systems is not easily applied.

As an alternative a parameter sensitivity analysis can be performed (45). This analysis involves determining whether changes of unknown parameters produce appreciable changes in the model's response to a standard input. If the parameter change produces a large change in the simulated response, that parameter is considered to be a sensitive parameter. Sensitive parameters are more likely to be estimated accurately from the data than are less sensitive ones. In this study it was necessary to determine whether changes in the parameters PS, V, and Kav produce large changes in the model simulations.

Fig. 1 shows the effects of model simulations of changing values for the three unknown model parameters for a typical organ. From this analysis it is apparent that an increase in PS results in a corresponding increase in the peak value of organ radioactivity. An increase in V, which results in a decrease in the rate constant (R/V,) for antibody transfer from the cell-associated compartment, results in a slower fall of the curve at longer time points. An increase in the catabolic clearance constant, Kav, results in a much more rapid fall off of radioactivity after the peak value. These simulations suggest that the unknown model parameters produce increases or decreases in the simulation curves at

![Fig. 1. Sensitivity plots for IgG in a representative organ over time (A) and the corresponding fraction of radioisotopically labeled IgG in the plasma that was TCA precipitable (B). Simulations are for a reference set of parameters (---), and a 5-fold increase in V, (---), or Kav, (---) for that organ. Parameter values for kidney IgG pharmacokinetics are used as reference values.](image-url)
IgG KINETICS IN MICE

very different temporal locations. Accordingly, the proposed model appears capable of distinguishing whether changes in PS, \( K_{\text{elim}} \), or \( V_c \) occur with different antibody fragments. Although this analysis does not guarantee parameter identifiability, it does demonstrate that, in the proposed model, the unknown parameters are sensitive.

RESULTS

The proposed compartmental model was capable of simulating all of the experimental data (see Fig. 2). The model parameters used for each simulation are listed in Tables 1 and 2. The model-derived values for the unknown parameters PS, \( K_{\text{elim}} \), and \( V_c \) could be estimated from the data with reasonable precision. The coefficient of variation for estimated parameters ranged from a low of 10% to a high of 120%. However, most of the parameters had a coefficient of variation around 45%. The values of \( K_{\text{elim}} \) were the most difficult to obtain from fitting our data. This is presumably the result of not having direct measurements of non-antibody metabolites in each organ.

The PS determines the transcapillary movement of antibody. Larger PS values produce greater flux. On a per gram organ weight basis, PS was greatest in the lung (0.53 ml·min\(^{-1}\)·g\(^{-1}\)), liver (0.35 ml·min\(^{-1}\)·g\(^{-1}\)), and spleen (0.20 ml·min\(^{-1}\)·g\(^{-1}\)). The surprisingly high value for lung may be due to its extensive capillary network. The large values of PS per gram organ weight for liver and spleen are consistent with the existence of fenestrations in the walls of sinusoids in these organs (52). Values of PS per gram weight for the other organs decreased in the following order: kidney (0.09 ml·min\(^{-1}\)·g\(^{-1}\)), gut (0.006 ml·min\(^{-1}\)·g\(^{-1}\)), and carcass (0.0003 ml·min\(^{-1}\)·g\(^{-1}\)). In a few instances our parameter estimates for PS can be compared with published values. Garlick and Renkin (53) studied the transport of large dextran molecules and albumin from plasma to interstitial fluid and lymph in the hindlimb of dogs. A very satisfactory agreement is observed between our PS values for carcass and their estimates for molecules with equivalent Stokes' radii [e.g., \( PS_{\text{dextran10}} = 0.0023 \text{ ml/min} \) versus \( PS_{\text{IgG}} = 0.0036 \text{ ml/min} \); \( PS_{\text{dextran20}} = 0.0061 \text{ ml/min} \) versus \( PS_{\text{(ab')2}} = 0.0041 \text{ ml/min} \); \( PS_{\text{dextran10}} = 0.029 \text{ ml/min} \) versus \( PS_{\text{Fab'}} = 0.050 \text{ ml/min} \). The general trend of increasing PS with decreasing fragment size is a consistent feature of our results. The liver was an exception, with PS being greatest for whole IgG and decreasing

Fig. 2. Experimental data and model simulations of IgG, F(ab')\(_2\), and Fab' for plasma (A), liver (B), gut (C), lung (D), spleen (E), kidney (F), carcass (G), TCA-precipitable fraction in plasma (H), and total body recovery (I). The standard deviation for the early time points has been omitted for clarity. Points, mean; bars, SD.
by 40% for both fragments. This reduction in $PS$ indicates that the transcapillary flux of antibody in the liver must depend on factors other than molecular size. One possible interpretation of this decrease in $PS$ may be related to an enhanced diffusibility of the smaller fragments into the interstitial matrix or to a greater association of antibody with cellular material (55). On an organ weight basis, $V_c$ values for IgG were ordered as follows: liver (0.033 ml/g) < lung (0.062 ml/g) < spleen (0.083 ml/g) < kidney (0.088 ml/g) < gut (0.094 ml/g) < carcass (0.31 ml/g). $V_c$ values for liver, spleen, carcass, and lung were comparable between whole IgG, F(ab')2, and Fab'. Substantial increases in $V_c$ were observed in the kidney and gut for Fab' when compared with those for whole IgG and F(ab')2. This suggested a preferential "trapping" of Fab' fragments in the cell-associated compartment of kidney and gut.

An important consideration in immunodetection is the steady-state ratio of antibody concentration in the interstitial space to that of circulating plasma. This ratio is related to the concentration of antibody in the lymph (56). Calculated ratios ($I/P$) for all organs of the model and the $T_a$ are listed in Table 3. In all cases the $I/P$ ratio was less than 1 and indicated that the steady-state interstitial concentration was less than the plasma concentration. This observation has also been made for γ-globulins (57–59) and for dextrans (60). In addition, the regional differences in the interstitial:plasma antibody concentration ratios predicted by the model have been observed for dextrans, with organs such as liver and gut producing the highest and carcass the lowest concentrations (61). The general small decrease in PS may be related to loss of Fc receptor-mediated uptake of whole antibody by Kupffer cells in the liver (54). Additional investigations of this possibility are required.

The values of catabolic clearance ($K_{elim}$) reflect each organ's ability to degrade antibody that reaches the cell-associated compartment. The clearance values for all organs were non-zero except for lung. That is, only in the case of lung did the parameter iteration scheme move $K_{elim}$ to zero. The gut had the largest value of clearance for whole IgG. Order of magnitude smaller clearance values for whole IgG were observed for the remaining organs (liver > carcass > spleen > kidney).

The values of catabolic clearance for F(ab')2 and Fab' fragments changed very little when compared with their values for whole IgG in liver spleen and carcass. A 2-fold increase in $K_{elim}$ for F(ab')2 and a 7-fold increase in $K_{elim}$ for Fab', when compared with whole IgG, was observed for gut. Fits to the gut data for Fab' were poor, suggesting that a different approach to the modeling of catabolism by the gut may be necessary. A greater than 5000-fold increase over the whole IgG clearance value for kidney was observed for both of the fragments. The latter result is due to our inability to fit the data for F(ab')2 and Fab' without introducing an irreversible loss from the cell-associated compartment in the kidney. This loss is apparently related to catabolism of fragments. However, the non-antibody metabolites produced via this pathway do not return to the plasma but instead are excreted directly from the body. Consequently these non-antibody metabolites cannot contribute to lowering the TCA-precipitable fraction in plasma. Regardless of how the comparison is made, kidney clearance values for the F(ab')2 and Fab' fragments were much greater than those for any other organ.

The parameter $V_c$ determines the size of the cell-associated compartment in each organ. This term represents an apparent volume of distribution of antibody in the regions that do not include the plasma or interstitial volumes. Larger values of $V_c$ result in less material returning to the interstitial compartment per unit time. Increases in $V_c$ may be related to an enhanced association of antibody with cellular material (53). On an organ weight basis, $V_c$ values for IgG were ordered as follows: liver (0.033 ml/g) < lung (0.062 ml/g) < spleen (0.083 ml/g) < kidney (0.088 ml/g) < gut (0.094 ml/g) < carcass (0.31 ml/g). $V_c$ values for liver, spleen, carcass, and lung were comparable between whole IgG, F(ab')2, and Fab'. Substantial increases in $V_c$ were observed in the kidney and gut for Fab' when compared with those for whole IgG and F(ab')2. This suggested a preferential "trapping" of Fab' fragments in the cell-associated compartment of kidney and gut.
trend for smaller antibody fragments to produce concentrations closer to that of plasma is consistent with expectations based on molecular size. The interstitial concentration of Fab’ is no lower than 80% of the plasma concentration for all organs, and for liver, kidney, and spleen, it is almost at concentration equilibrium with plasma.

The time to reach steady state for whole IgG and F(ab’)2 is shortest for liver, spleen, and lung (<1.1 min), intermediate for kidney and gut (<3.4 and <22.0 min, respectively), and longest for carcass (<270.0 min). Organs reaching steady state in the shortest times show only slightly smaller values of $T_m$ with the Fab’ fragment. The remaining organs have at least a 60% reduction in $T_m$ for the Fab’ fragment. In the case of carcass, the $T_m$ of 251 min for whole IgG was reduced to 17.7 min for Fab’.

**DISCUSSION**

Our analysis has demonstrated that a simple model of antibody metabolism, while being neither unique nor comprehensive, is useful for examination of the pharmacokinetics of a nonspecific murine IgG1 and its F(ab’)2 and Fab’ fragments in mice. This model can be used to establish a set of pharmacokinetic measures for evaluating the in vivo use of radiolabeled antibody. The results of this analysis apply most directly to the situation of murine monoclonal used in healthy mice that have very little tumor burden. Consequently, conclusions about background levels, cumulative tissue exposure, and antibody access to tumor antigen, as these quantities relate to in vivo immunodetection and therapy, apply to this case. Extrapolation of these results to heterologous systems is not straightforward and will require additional experimentation. We believe that in the future human monoclonal antibodies will be used for immunodiagnosis and immunotherapy. It remains to be determined, however, whether the mouse-mouse system is an appropriate model for the human-human system.

Numerous factors will be important in choosing which antibody to use for a desired application. The final decision will be affected by biochemical factors such as antibody binding to tumor cells, fragmentation, cross-reactivity, labeling isotope; physiological factors such as blood and lymph circulation to normal and tumor tissue, and the immune response to challenge by foreign antibody; and pharmacological factors such as biodistribution, catabolism, and excretion. No attempt will be made in this discussion to address issues beyond those of antibody pharmacology. Furthermore, application of these results to antibodies labeled with metal-chelates will require modification of the model to account for cellular trapping of the chelate.

Two measures will be used to quantify antibody pharmacokinetics for comparisons in vivo. (a) Mean residence time per gram organ weight expresses antibody exposure for various regions of the body. High exposure to tumor and low exposure to normal tissues are desirable for both therapy and immunodetection. (b) Access of antibody to regions of the system where tumor antigen may exist is a prerequisite of antigen-antibody binding. Whether or not antibody actually binds to antigen when given the opportunity will depend on the rate-limiting step in the kinetics of the binding process. However, organs with the highest access can be expected to have the most opportunity to bind antigen located within that organ. The number of cycles of antibody through the various regions of each organ will be used as a measure of access. These two measures will be used to examine how the pharmacokinetics of whole IgG, its F(ab’)2 fragment, and its Fab’ fragment relate to their use for immunodetection and therapy. Although we have no direct evidence that these measures will be different for different doses of antibody administered i.v., investigations on the fate of s.c. administered MOPC-214 and s.c. administered anti-H2K* (62) demonstrate no dose-dependent pharmacokinetics.

The model-calculated mean residence times in the body for whole IgG, F(ab’)2, and Fab’ of 8.5, 0.5, and 0.2 days, respectively (see Table 4), are in satisfactory agreement with published literature (11, 31–33). Plasma, lung, and the enteral organs liver, gut, and spleen have the highest levels of exposure to whole IgG on a per gram weight basis. The kidney and carcass have the lowest levels of exposure to whole IgG, despite the fact that they account for over 57% of the total body mean residence time (53.0% from carcass and 4.2% from gut).

The total body residence time for F(ab’)2 was 1/17th the value for whole IgG. This is reflected by nearly equivalent reductions for all organs in mean residence time per gram weight. That is, the 17-fold decrease in organ exposure to F(ab’)2 when compared with whole IgG, occurs in all organs, and as a result the distribution of the total body residence time across all organs does not change. Taken together these results suggest that F(ab’)2 produces an order of magnitude reduction in organ exposure when compared with whole IgG.

The total body residence time of Fab’ was 2-fold shorter than that of F(ab’)2. This factor of 2 reduction in total body antibody exposure was found in all organs except the gut, where only a 12% decrease in exposure was observed, and the kidney, where a 2-fold increase in exposure was observed. This result is consistent with the shift in the distribution of total body residence time for Fab’, such that 34% of the total residence time could now be accounted for by gut and kidney (17.2% for gut and 17.1% for kidney).

The distribution of residence times between the capillary, interstitial, and cell-associated compartments of the model is summarized in Table 5. For all organs except carcass, the capillary plasma compartment had the greatest fraction of the total organ’s residence time for whole IgG and F(ab’)2. This suggests that whole nonspecific IgG and F(ab’)2 reside for the most part in the plasma compartment of the body. For Fab’ most of the residence time is due to the cell-associated space (except for the liver and lung, where most of the residence time is due to capillary plasma). The general trend for distribution of antibody within the capillary plasma, interstitial, and cell-associated spaces is consistent with the premise that the smaller
antibody fragments are more capable of leaving the capillary space to enter interstitial and cell-associated regions. Antibody fragments are more capable of leaving the capillary space to enter interstitial and cell-associated regions. Antibody fragments are more capable of leaving the capillary space to enter interstitial and cell-associated regions. Antibody fragments are more capable of leaving the capillary space to enter interstitial and cell-associated regions. Antibody fragments are more capable of leaving the capillary space to enter interstitial and cell-associated regions. Antibody fragments are more capable of leaving the capillary space to enter interstitial and cell-associated regions. Antibody fragments are more capable of leaving the capillary space to enter interstitial and cell-associated regions. Antibody fragments are more capable of leaving the capillary space to enter interstitial and cell-associated regions. Antibody fragments are more capable of leaving the capillary space to enter interstitial and cell-associated regions. Antibody fragments are more capable of leaving the capillary space to enter interstitial and cell-associated regions. Antibody fragments are more capable of leaving the capillary space to enter interstitial and cell-associated regions. Antibody fragments are more capable of leaving the capillary space to enter interstitial and cell-associated regions. Antibody fragments are more capable of leaving the capillary space to enter interstitial and cell-associated regions. Antibody fragments are more capable of leaving the capillary space to enter interstitial and cell-associated regions. Antibody fragments are more capable of leaving the capillary space to enter interstitial and cell-associated regions. Antibody fragments are more capable of leaving the capillary space to enter interstitial and cell-associated regions. Antibody fragments are more capable of leaving the capillary space to enter interstitial and cell-associated regions. Antibody fragments are more capable of leaving the capillary space to enter interstitial and cell-associated regions. Antibody fragments are more capable of leaving the capillary space to enter interstitial and cell-associated regions. Antibody fragments are more capable of leaving the capillary space to enter interstitial and cell-associated regions.

Table 5 Mean residence time distribution within organ expressed as percentage of total mean residence time in each organ

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Whole (%)</th>
<th>F(ab')</th>
<th>Fab' (%)</th>
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<tr>
<td>Gut Capillary</td>
<td>42.4</td>
<td>46.5</td>
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</tr>
<tr>
<td>Intestinal</td>
<td>23.9</td>
<td>25.7</td>
<td>9.5</td>
</tr>
<tr>
<td>Cell associated</td>
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<td>27.8</td>
<td>77.7</td>
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<td>Liver Capillary</td>
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<td>43.5</td>
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<td>42.5</td>
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<tr>
<td>Cell associated</td>
<td>11.0</td>
<td>5.5</td>
<td>11.6</td>
</tr>
<tr>
<td>Kidney Capillary</td>
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<td>49.6</td>
<td>6.0</td>
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<td>5.2</td>
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<td>Cell associated</td>
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<td>88.7</td>
</tr>
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<td>Spleen Capillary</td>
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<td>33.3</td>
</tr>
<tr>
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<td>25.0</td>
</tr>
<tr>
<td>Cell associated</td>
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<td>41.7</td>
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<td>Carcass Capillary</td>
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<td>19.3</td>
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<tr>
<td>Cell associated</td>
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<td>Intestinal</td>
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<td>32.8</td>
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</tr>
<tr>
<td>Cell associated</td>
<td>15.5</td>
<td>9.0</td>
<td>27.1</td>
</tr>
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</table>

| Table 6 Percentage of extraction per wet weight by catabolizing organs

<table>
<thead>
<tr>
<th>Organ</th>
<th>Whole</th>
<th>F(ab')</th>
<th>Fab'</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gut</td>
<td>0.140 (72.8)</td>
<td>0.227 (43.3)</td>
<td>1.02 (22.9)</td>
</tr>
<tr>
<td>Spleen</td>
<td>0.013 (3.6)</td>
<td>0.028 (3.3)</td>
<td>0.14 (3.1)</td>
</tr>
<tr>
<td>Liver</td>
<td>0.008 (20.5)</td>
<td>0.004 (3.1)</td>
<td>0.013 (0.3)</td>
</tr>
<tr>
<td>Kidney</td>
<td>0.0007 (1.7)</td>
<td>0.059 (50.3)</td>
<td>3.39 (73.4)</td>
</tr>
<tr>
<td>Carcass</td>
<td>0.0006 (1.4)</td>
<td>0.0006 (0.1)</td>
<td>0.0098 (0.2)</td>
</tr>
</tbody>
</table>

* Numbers in parentheses, percentage of total extraction by all organs.

The net number of cycles of antibody per gram tissue through the interstitial and cell-associated regions of each organ in the model is listed in Table 7. There is a general tendency for the number of cycles for each organ to be greatest for whole IgG, intermediate for F(ab')2, and lowest for Fab'. The best case, in terms of frequent visits by antibody, is for whole IgG in the liver where 2291 cycles through the interstitial space are predicted. The worst case is for whole IgG in the carcass, where only 43 visits are predicted. When this result is expressed on a per gram basis, the difference is even more dramatic (2490 cycles/g in the liver versus 2.8 cycles/g in the carcass). Whether or not the relatively low access of antibody in the interstitial space of the carcass explains the difficulties of localization within tumor when using whole IgG will depend, among other factors, on how closely tumor vasculature compares with that of muscle.

The tendency for the number of cycles to decrease for F(ab')2 and Fab' is consistent with the progressively shorter total body retention for each antibody fragment. F(ab')2 cycles 1/3rd as often, and Fab' cycles 1/5th as often as whole IgG for most organs. There are two notable exceptions to these observations. (a) The Fab' fragment cycles through the interstitial space of the carcass 2 times more often than F(ab')2. This observation may be explained on the basis of the greater transcapillary flux of the smaller antibody fragment. Consequently, if the Fab' and F(ab')2 fragments have equal affinity for tumor antigen, the Fab' fragment would have a 2-fold greater chance of visiting the site of tumor (and binding to it). Whole IgG appears to have the greatest chance of visiting the interstitial space in the carcass, with cycling occurring 8-fold more often than in the case of Fab'. (b) The number of cycles of Fab' through the interstitial space of the kidney was nearly 20-fold greater than that for F(ab')2. This observation is consistent with the greater role of kidney in Fab' catabolism.

When comparisons of number of cycles are made between organs, for either whole IgG or its fragments, those organs receiving the highest plasma flow and having the largest values of permeability-surface area product also have the largest values...
for cycling through the interstitial space. The order of ranking for cycling per organ weight of whole IgG and F(ab')2 through the interstitial space of each organ is lung > liver > spleen > kidney > gut > carcass. For Fab' the order of ranking has shifted so that the kidney has the largest value for cycling, followed by lung, spleen, liver, gut, and carcass.

The pharmacokinetic results presented above can be used to make comparisons between whole IgG and its fragments for their use in immunodetection or therapy. Although explicit criteria for antibody selection have not yet been specified, the differential pharmacokinetics of these molecules will clearly be important.

This analysis has shown that i.v. administered antibody rapidly distributes into the nonplasma spaces of organs with high capillary permeability (e.g., liver, spleen, and kidney) and more slowly into organs with lower capillary permeability (e.g., gut and carcass). The extreme case is that of whole IgG in the carcass, for which the model-predicted distribution time was nearly 5 h. Under no circumstances was the distribution volume for antibody within an organ restricted to capillary and interstitial spaces. The nonplasma, noninterstitial space was smaller than the sum of plasma and interstitial spaces for each organ except the carcass (for all fragments) and the gut and kidney (for Fab' only).

Whole IgG is retained in the body for a considerably longer time than either fragment. Consequently, tissue exposure persists longer for IgG than for either fragment. On a per gram basis, the liver, spleen, gut, and lung have at least 2-fold more exposure when compared with kidney or carcass. Long total body retention of whole IgG may be an advantage in terms of therapy. In the worst case, each antibody molecule visits the interstitial space of the carcass (where tumor could be located) at least 43.6 times or 2.8 times on a per gram tissue weight basis.

The F(ab')2 fragment is pharmacokinetically different from whole IgG mostly on the basis of its 17-fold shorter total body retention time. Other characteristics, such as distribution volumes and speed of distribution into these volumes, are comparable with those of whole IgG. The shorter length of stay reduces exposure, when compared with whole IgG. The reduction in exposure appears to be distributed equally across all compartments (except the kidney, where slightly more uptake is observed). As a result of the shorter total body retention, F(ab')2 cycles only 2.7 times through the interstitial space of the carcass, that is, 0.2 times per gram weight. This may be insufficient for good localization of tumor served by capillaries with continuous endothelia.

The Fab' fragment is pharmacokinetically quite different from whole IgG or F(ab')2, apparently due to its smaller size. When compared with whole IgG or F(ab')2, it (a) distributes more rapidly into a larger total distribution volume, (b) produces higher interstitial/plasma concentration ratios, (c) is more readily extracted from the circulation and catabolized by kidney, spleen, and gut, (d) is cleared from the body 35 times faster, and (e) cycles through non-kidney interstitial spaces less often. The in vivo differences between F(ab')2 and whole IgG, due to shorter total body retention, also apply for Fab'. And, as in the case of F(ab')2, it remains to be determined whether the decreased number of cycles through the interstitium by Fab' is sufficient for acceptable localization.

In conclusion, the modeling approach presented here has yielded some results that are obvious and others that are not. The more obvious results, which have also been found by others who have not used formal modeling, relate to the lower cumulative tissue exposure when using fragments instead of whole IgG. The less obvious results, those more dependent on formal modeling, relate to pharmacokinetic interpretation terms of volume of distribution, permeability-surface area product, organ clearance, organ extraction, mean residence time, and number of cycles through various body regions. These results provide a basis for quantitative comparisons between antibodies (and their fragments) for their in vivo use. A physiologically rational model of antibody kinetics also provides a general framework for the presentation of kinetic data and may thus serve as a basis for a wider range of applications. As an example, this analysis has shown that there may be significant trade-offs when using rapidly cleared antibody fragments. The smallest fragment, with the fastest clearance, also cycles the fewest times through regions in the body where tumor may exist. This characteristic may prove to offset any advantages in localization due to lower background exposure. The physiological model presented here is currently being extended to include binding to tumor and circulating antigen. It is also being used, in conjunction with clinical data, in the generation of models for antibody administration in humans.

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APPENDIX A: GLOSSARY OF TERMS

- $C_{p,j}$ labeled antibody concentration in plasma (pm)
- $C_{a,j}$ labeled antibody concentration in capillary plasma of the $j$th tissue (pm)
- $C_{j,j}$ cell-associated labeled antibody concentration in interstitium of the $j$th tissue (pm)
- $C_{c,j}$ cell-associated labeled antibody concentration for the $j$th tissue (pm)
- $Q_{j}$ plasma flow to $j$th tissue (ml/min)
- $V_{p,j}$ capillary plasma volume of the $j$th tissue (ml)
- $V_{i,j}$ interstitial volume of the $j$th tissue (ml)
- $V_{c,j}$ cell-associated volume of the $j$th tissue (ml)
- $R/V_c$ transfer rate of antibody from cell-associated to interstitial compartment (min⁻¹)
- $R/V_i$ transfer rate of antibody from interstitial to cell-associated compartment (min⁻¹)
- $K_{cin}$ catabolic clearance of antibody from cell-associated compartment (ml/min)
- $L$ lymph flow rate (ml/min)
- $N$ Staverman reflection coefficient
- $j$ organ (gut, liver, spleen, kidney, carcass, lung)

APPENDIX B

In this section the mathematical model for antibody pharmacokinetics used to fit the data is developed. A physiological flow model, similar to that proposed by Bischoff and Dedrick for the anticancer agent methotrexate (40), is proposed to describe the circulation of plasma through the body. The convective and diffusive components of transcapillary exchange are modeled as proposed by Landis and Pappenheimer (71). Catabolism of antibody is assumed to occur only when antibody interacts with cell-associated material of the organs.

The circulation portion of the model consists of plasma flowing through highly perfused organs (e.g., liver, lung, kidney, spleen, and gut) and more slowly perfused organs (e.g., muscle, skin, etc.). A
schematic of the model is shown in Fig. 3. Plasma antibody is assumed to distribute to each organ at a rate determined by the plasma flow to that organ. The amount of antibody delivered to each organ per unit time (pmol/min) is determined as the product of the plasma flow to the organ \( Q_{\text{organ}} \) (ml/min) and the plasma antibody concentration \( C_{\text{pl}} \). The amount of antibody returning to plasma from each organ per minute is the product of the plasma flow to the organ \( Q_{\text{organ}} \) and the antibody concentration in the capillary plasma of the organ \( C_{\text{cap}} \).

The transcapillary exchange component of the model describes the movement of antibody between plasma and interstitial fluid. A schematic of this system is shown in Fig. 4. This exchange is assumed to occur via diffusive and convective pathways through the capillary endothelium and basement membrane. The transport equation describing the net flux of antibody \( J, \) pmol/min) between plasma and interstitial fluid is

\[
J = 0.5J_e(1 - \sigma)(C_p + C_i) + PS(C_p - C_i)
\]

where \( C_p \) and \( C_i \) are the capillary plasma and interstitial concentrations of antibody, \( J \) is the net rate of volume flow through the capillary membrane (ml/min), \( \sigma \) is the reflection coefficient for restricted movement of molecules by convective flux, and \( PS \) has been defined as \( DA/d \) (ml/min), where \( D \) is the free diffusion coefficient (cm²/min), \( A \) is the total cross-sectional area of the aggregate of transcapillary pores (cm²), and \( d \) is the average distance across the capillary wall. This formulation assumes that an "ideal" membrane containing a uniform distribution of pores of equal dimensions separates capillary plasma and interstitial fluid. The value of \( J \) for each organ will be taken as equivalent to the net rate of lymph flow, \( L \) (73).

Antibody in interstitial fluid of each organ may exchange with the capillary plasma (as described above) or enter the lymphatic system and return directly to circulating plasma. The rate at which antibody leaves the interstitium in the lymph and returns directly to plasma is \( C.L \). Alternatively, antibody may associate nonspecifically with either cells or matrix within the tissue. Our data do not permit a distinction between the two; hence, we will refer to such interactions as cell associated. In the model, cell-associated antibody can be irreversibly converted to non-antibody metabolites, which enter the plasma and are subsequently excreted from the body. In the absence of catabolism the concentration of antibody in the interstitium would eventually equilibrate with the concentration in the cell-associated compartment.

In summary, a linear model is proposed to describe the kinetics of nonspecific antibody pharmacokinetics in normal mice. The salient features of the model include plasma flow to each organ, plasma antibody distribution to the interstitial fluid of each organ, the formation and flow of lymph, and the uptake and catabolism of antibody by cell-associated material of each organ. Mass balance equations for the model are as follows. For plasma:

\[
V_p dC_{pl}/dt = -(Q_{\text{Liver}} + Q_{\text{Kidney}} + Q_{\text{Carcass}})C_{pl} + Q_{\text{lung}}C_{plung}
\]

and for the \( j \)th organ:

\[
V_{\text{organ}} dC_{\text{organ}}/dt = Q_{\text{organ}} C_{\text{organ}} - (Q_{\text{organ}} + L_j)C_{\text{organ}} - PS(C_{\text{organ}} - C_{\text{interstitial}}) - L_j(1 - \sigma)C_{\text{organ}}
\]

A set of equations analogous to those proposed for the lung describes the liver, with the modification that liver receives venous effluent from the gut and spleen. A definition for the terms in this model can be found in "Appendix A."

To permit interpretation of data on plasma TCA-precipitable fraction, a model for non-antibody metabolites has been included in the analysis (see Fig. 5). Irreversible losses of antibody from liver, kidney, gut, spleen, and carcass due to catabolism constitute inputs directly into the non-antibody metabolite model. Non-antibody metabolites are assumed to distribute between plasma and the rest of the total body water and be excreted from the body (74).

Not all the parameters of the proposed model can be determined from information contained in our experiments. However, values for certain parameters can be predicted from data in the literature, and

Fig. 3. Compartmental model for IgG pharmacokinetics. Antibody is distributed to each organ according to the arterial plasma flow to that organ \( Q \). Total organ measurements of antibody were made for liver, spleen, gut, kidney, carcass, lung, and plasma.

Fig. 4. Organ model for exchange of antibody between capillary plasma, interstitial space, and cell-associated compartment. \( Q \) is plasma flow (ml/min), \( L \) is lymph flow, and \( C \) and \( V \) are the antibody concentrations and distribution volumes for capillary plasma \( C_p, V_p \), interstitial \( C_i, V_i \), and cell-associated \( C_c, V_c \) compartments.
Fig. 5. Non-antibody metabolite model. Catabolic losses from the antibody model (shown in Fig. 4) constitute inputs into the plasma compartment of the non-antibody metabolite model. Non-antibody metabolites distribute throughout body water and are excreted.

realistic model constraints can be imposed to reduce further the number of unknown parameters. In this analysis, literature values for plasma flows to each organ, $Q_j$, and the plasma and interstitial distribution volumes, $V_p$ and $V_i$, for each organ were used (40, 75). These values are listed in Table 1 and remained constant throughout the modeling analysis. The flux of lymph for visceral and nonvisceral organs was assumed to be 2 and 4% of the plasma flow to each organ, respectively. The reflection coefficient, $\alpha$, for each organ was fixed at 0.95 for the whole antibody, 0.90 for the Fab(\'b\prime)_2 fragment, and 0.5 for the Fab fragment. These values are consistent with estimates of reflection coefficients for proteins of equivalent Stokes' radii (56, 76, 77). The rate constant, $R$, which determines the time for antibody in the interstitial and cell-associated compartments to reach concentration equilibriums (under conditions of no catabolism) was assumed to be 0.02/min. This value is sufficiently large that it never becomes rate limiting. The remaining parameters in the model were treated as unknown values to be determined from the data. The unknown model parameters are $PS$, $K_{elim}$, and $V_F$.
Pharmacokinetics of Monoclonal Immunoglobulin G₁, F(ab′)₂, and Fab′ in Mice


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