Pharmacokinetic Model for a Primary Antibody Response

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Abstract □ Antigen distribution and clearance studied *in vivo* may be viewed as a pharmacokinetic problem complicated by the intervention of the immune response. A model characterizing the clearance of the simple antigen ϕX174 from the bloodstream of various experimental animals, as well as the subsequent serum antibody response, was developed. The present model is the simplest of the possible models and no doubt will have to be modified when considering more complex antigens, additional distribution modes, and differences in antibody reactivity. For the primary (IgM) immune response, however, early events in the immunological response can be adequately accounted for in a consistent and quantitative manner.

Keyphrases □ Antibody response, primary—pharmacokinetic model developed □ Antigens—distribution and clearance, pharmacokinetic model described for primary antibody response □ Pharmacokinetic models—primary antibody response

The time course during which drugs are absorbed, distributed, metabolized, and excreted is commonly studied by using pharmacokinetic models (1, 2). A pharmacokinetic model takes into account the various ways by which a drug may be distributed between different body compartments and attempts to account quantitatively for the disposition of drug in any one compartment at any time. Particular compartments, such as blood or urine, may be explicitly identified because there are the biophases assayed for drug content.

However, other compartments, such as adipose tissue, have their presence implied from the nature of the drug concentration–time profile. A model is constructed depicting the transfer of drug from one compartment to another, the transfer being characterized by one or more rate constants, and subsequently a mathematical expression is derived to replicate the observed drug concentration–time profile. As a number of models may serve to fit the same data, it is usual to prefer the scheme that involves the fewest number of possible compartments and is compatible with experimental results.

In certain immunological studies, it is the practice to inject an antigen into a test animal and to assay blood samples, withdrawn at timed intervals, for remaining antigen and for the presence of antibody. Antigen distribution and clearance, when studied in this manner, may be viewed as a pharmacokinetic problem complicated by the intervention of the immune response. With this perspective, a pharmacokinetic model was developed which accounts for the major events taking place in the initiation of the primary (IgM) immune response. This model deals only with early events in the primary antibody response; that is, IgM is considered to be the sole antibody involved in the removal of antigen from the circulation.

In constructing the model, use is made of generally accepted concepts regarding the nature of the primary antibody response. It is shown that this model characterizes the primary immune response toward the bacteriophage ϕX174 in different mammalian species. With antigens more complex than ϕX174, a more involved pharmacokinetic model may be necessary.

MODEL

Construction—Injection of small amounts of an antigen, such as ϕX174, into the circulatory system of guinea pigs (3, 4), mice (5, 6), or fetal lambs (7) provides a means of following the primary antibody response in a quantitative manner. The elimination of antigen from the circulation occurs in two phases (Fig. 1). The first and slower elimination is due to nonimmune catabolism, while the second and rapid elimination is interpreted as being caused by the production and release into the circulation of specific antibody (3, 4). Designating A as the amount of antigen and B as the amount of antibody present at a particular time t, antigen elimination may be represented by either of three generalized competing pathways:

$$A \xrightarrow{k_p} \text{nonimmune elimination}$$

$$A + B \xrightarrow{k_c} \text{immune elimination}$$

Scheme I

$$A \xrightarrow{k_p} \text{nonimmune elimination}$$

$$A + B \xrightarrow{k_c} AB \xrightarrow{k_a} \text{immune elimination}$$

Scheme II

$$A \xrightarrow{k_p} \text{nonimmune elimination}$$

$$A + B \underset{k_r}{\overset{k_f}{\rightleftharpoons}} AB \xrightarrow{k_a} \text{immune elimination}$$

Scheme III

where k_p, k_c, k_a, k_f, and k_r are rate constants and AB represents the amount of antigen–antibody complex. Both the antigen and the antigen–antibody complex are thought to be removed through the reticuloendothelial system (8), which suggests that the pathways (Schemes I–III) should have a common elimination compartment. However, since blood levels of antigen are monitored and not contents of the elimination compartment, it is permissible to view the nonimmune and immune clearances as separate competing elimination modes.

Scheme I shows the more general clearance model; it simply designates that antigen may be removed from the circulation either by nonimmune catabolism or by reaction with antibody. The rate law appropriate to elimination of antigen by this pathway is given by:

$$-\frac{dA}{dt} = k_pA + k_cA(B) \qquad \text{(Eq. 1)}$$

Scheme II designates that, in the immune elimination, the antigen–antibody complex is formed in an irreversible manner and that the complex is eliminated. So long as only antigen blood levels are monitored, the rate law for this pathway is indistinguishable from Eq. 1. Scheme III is similar to Scheme II insofar as the antigen–antibody complex is the species eliminated due to the immune response. However, it differs from Scheme II by considering the formation of the antigen–antibody complex to be reversible. In this instance, the rate law appropriate to antigen elimination, under steady-state conditions, is given by:

$$-\frac{dA}{dt} = k_pA + \left(\frac{k_fk_a}{k_r + k_a}\right)A(B) \qquad \text{(Eq. 2)}$$

Equation 2 differs from Eq. 1 by having a more complex form to the second-order rate constant, but Eqs. 1 and 2 are kinetically indistinguishable in practice. For practical purposes, then, antigen elimination may be represented by the rate law:

$$-\frac{dA}{dt} = k_p A + k_o A(B) \qquad \text{(Eq. 3)}$$

where k_o is the experimental second-order rate constant.

Equation 3 can serve as a basis for interpreting ϕX174 clearance curves if an expression for B can be arrived at that allows integration. This problem may be approached by noting that antigen stimulates lymphocytes toward replication and differentiation into mature plasma cells. Mature plasma cells are nonreplicating, and the number of these cells ultimately produced seems to depend on the amount of initially administered antigen (3, 4, 9). The population P of these nonproliferating antibody-producing cells at any time t may then be denoted by the expression:

$$P = P_A(1 - e^{-k_d t}) \qquad \text{(Eq. 4)}$$

in which P_A is the optimum population of these cells achieved for a particular initial dose of antigen, and k_d is a parameter characterizing the rate of differentiation.

For extended periods, much longer than the time period of present concern, the tendency for mature plasma cells to "die off" after having attained their optimum number must be considered. This can be done by representing the population of antibody-producing cells P as subject to the consecutive process:

$$U \xrightarrow{k_d} P \xrightarrow{k_i} \text{nonantibody producing cells}$$

Scheme IV

where U is the population of replicating cells from which differentiation is occurring, and k_i is a constant characterizing the inactivation of mature plasma cells. By presuming that both differentiation and inactivation are apparent first-order processes, the population of antibody-producing cells is represented by:

$$P = \frac{k_d U_A}{k_i - k_d}(e^{-k_d t} - e^{-k_i t}) \qquad \text{(Eq. 5)}$$

where, by this representation, U_A is viewed as the total number of lymphocytes stimulated to divide by a given dose of antigen. Earlier models for Scheme IV (10, 11) were not as explicit as Eq. 5.

Within the timespan during which Eq. 4 is useful in characterizing the population of antibody-producing cells, it may be presumed that the amount of antibody liberated from the antibody-producing cells is reflective of their population (11). In the simplest instance, the amount of antibody produced is directly proportional to the number of antibody-producing cells:

$$B = k_b P \qquad \text{(Eq. 6)}$$

where k_b is a constant characterizing the average amount of antibody produced per cell. This relationship seems reasonable, since it has been demonstrated that the amount of antibody present has no effect on the production of antibody or on the proliferation of antibody-producing cells (12).

Substitution of Eq. 4 into Eq. 6 provides an expression for the average amount of antibody present at any time:

$$B = B_A(1 - e^{-k_d t}) \qquad \text{(Eq. 7)}$$

where $B_A = k_b P_A$. Upon introducing Eq. 7 into Eq. 3 and solving, the resulting expression is:

$$\ln\left(\frac{A}{A_0}\right) = -(k_p + k_A)t + \frac{k_A}{k_d}(1 - e^{-k_d t}) \qquad \text{(Eq. 8)}$$

Here $k_A = k_o B_A$, and A_0 is the initial amount of antigen in the blood. Equation 8 thus represents the amount of antigen in the circulation at any time.

Application—A typical clearance curve for the elimination of the bacteriophage ϕX174 from the blood of a mouse (Swiss male) is shown in Fig. 1. Equation 8 allows this curve to be readily interpreted. There is minimal production of antibody, implying $k_A = 0$,

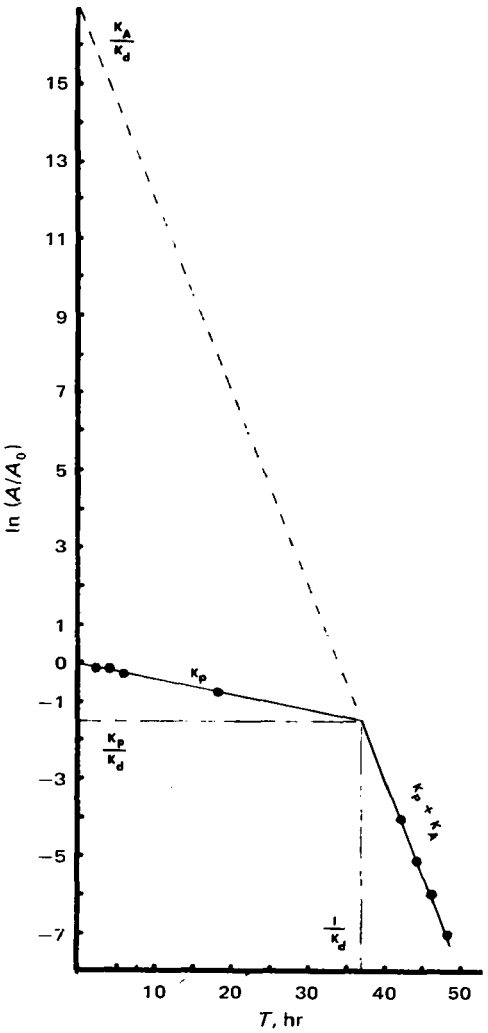

Figure 1—*Clearance curve for the elimination of the bacteriophage ϕX174 from the blood of a mouse (Swiss male).*

in the time period immediately following injection of antigen. During this stage, the elimination of antigen can be characterized by:

$$\ln\left(\frac{A}{A_0}\right) = -k_p t \qquad \text{(Eq. 9)}$$

As antibody production increases, the competition between nonimmune and immune antigen elimination modes becomes evident by a curvature in the clearance curve (not shown in Fig. 1). This competitive elimination is characterized by Eq. 8. When antigen-stimulated antibody production is at its optimum, the exponential term in Eq. 8 becomes negligible and Eq. 8 reduces to:

$$\ln\left(\frac{A}{A_0}\right) = -(k_p + k_A)t + \frac{k_A}{k_d} \qquad \text{(Eq. 10)}$$

Computer simulations showed that, with k_d in the 0.02–0.40 range, Eq. 8 reduces to Eq. 10 well within the time of a clearance experiment.

The linear equations (Eqs. 9 and 10) provide a basis for obtaining the individual rate constants from the clearance curves. A least-squares fit of the experimental points for the nonimmune and immune clearances of antigen is made to gain estimates for the slopes and intercepts represented in the respective relations. Nonimmune clearance provides the slope k_p, while immune clearance gives a slope of $k_p + k_A$. The difference between the two slopes provides an estimate for k_A. The reciprocal of the intercept for the immune clearance least-squares equation is identified as k_d/k_A. Hence, multiplication by k_A provides an estimate of k_d.

An alternative, and more precise, means of gaining an estimate

for k_d involves equating the two least-squares equations for nonimmune and immune clearances to determine the point of intercept. Equating the doses leads to an estimate of the inflection time:

$$t_i = \frac{1}{k_d} \qquad \text{(Eq. 11)}$$

while equating the times leads to an estimate for the inflection dose:

$$\ln\left(\frac{A_i}{A_0}\right) = -\frac{k_p}{k_d} \qquad \text{(Eq. 12)}$$

These estimates are shown schematically in Fig. 1. The reciprocal of the inflection time provides k_d.

RESULTS AND DISCUSSION

Table I summarizes values for the rate constants k_p, k_A, and k_d, which have been estimated from Fig. 1 and from similar studies (3–7). When literature values were used for the estimates, only those clearance curves having at least three experimental values describing nonimmune and immune clearances were analyzed by least squares. A comparison of the values suggests the following:

1. The rate of nonimmune clearance, as measured by k_p, is essentially independent of the administered dose of antigen and seems to differ from one mammalian species to another. This is consistent with the well-known species variation in the phagocytic index (13). The agreement between the values of k_p for the mouse should be noted, because the clearance curves were obtained in three independent laboratories. Comparison between the results gained in separate laboratories is possible since the magnitude of experimental slopes, and not points, is compared. Therefore, while the level of response of an animal toward clearing antigen may vary, the time course of clearance as measured by k_p should be essentially the same, animal to animal, if a similar clearance mechanism operates in each instance.

2. The rate of differentiation to antibody-producing cells, as reflected by k_d, is essentially the same for all mammalian species considered. The average value is 0.027 hr^{-1}. There seems to be a limited trend between the value of k_d and the dose of antigen administered, which can be represented by least-squares equations:

$$k_d(\text{mouse}) = -8.12\,(\pm 0.22) \times 10^{-3} \ln A + 6.80\,(\pm 0.11) \times 10^{-2} \qquad \text{(Eq. 13)}$$

n	r	s
3	0.999	0.00025

$$k_d(\text{guinea pig}) = -5.64\,(\pm 0.92) \times 10^{-3} \ln A + 6.74\,(\pm 0.92) \times 10^{-2} \qquad \text{(Eq. 14)}$$

n	r	s
3	0.986	0.00267

where n is the number of data points, r is the correlation coefficient, s is the standard error of the estimate, and the values in parentheses following each coefficient are the standard errors of the estimate of the slope and intercept, respectively. However, in view of the low values for the slopes of these relationships, one may, to a good approximation, consider k_d to be essentially independent of the dose of antigen administered.

3. The rate of immune clearance, as measured by k_A, seems to differ from one mammalian species to another. This finding is not unexpected, considering that the reticuloendothelial system is involved in both immune and nonimmune clearances (8) and that rates of nonimmune clearance differ among species. No apparent relationship between k_A and the initial dose of antigen is readily discerned. More precise data, involving a larger number of data points for the immune clearance, may make discernible a relationship between k_A and the initial dose of antigen. With the data at hand, however, one may presume that k_A is essentially constant for each species. Under this presumption, the ratio k_A/k_p provides a measure of the magnitude of the immune response for each species. This ratio is about 10.7, 5.9, and 1.8 for the mouse, fetal lamb, and guinea pig, respectively. Of the species considered, the mouse is the most and the guinea pig is the least immunologically sensitive animal toward ϕX174.

Table I—Pharmacokinetic Parameters from ϕX174 Clearance Curves

Species	Antigen Dose, PFU/ml[a]	k_p, hr^{-1}	k_A, hr^{-1}	k_d[b], hr^{-1}	Reference
Mouse	1.5×10^4	0.037	0.371	0.034	5
Mouse	9×10^4	0.033	0.329	0.028	6
Mouse	6×10^5	0.039	0.460	0.021	—
Guinea pig	5×10^4	0.115	0.200	0.042	3
Guinea pig	6×10^6	0.104	0.251	0.027	3
Guinea pig	6×10^8	0.163	0.251	0.019	3
Fetal lamb (116 days)	5×10^6	0.060	0.368	0.023	7
Fetal lamb (103 days)	1×10^{10}	0.064	0.368	0.021	7

[a] PFU = number of plaque-forming units. [b] Determined using point of intercept method.

As is well known (3, 4), there is no detectable specific antibody to ϕX174 (IgM) in the blood during the immune clearance of the antigen; but shortly after antigen clearance, an exponential build-up of antibody is observed and it reaches an optimum value approximately 96 hr after immunization. This build-up of antibody can be expressed by a rearranged form of Eq. 7:

$$\ln\left(\frac{B_A}{B_A - B}\right) = k_d' t \qquad \text{(Eq. 15)}$$

Early (72–96 hr after injection) serum antibody responses toward ϕX174, interpolated from published (3, 5, 7) figures, are essentially linear when plotted in accord with Eq. 15 (Fig. 2). This linear representation is limited in the respect that, when serum antibody levels B are considerably less than the optimum B_A, information content is lost since the ratio $B_A/(B_A - B)$ is near 1 in these instances.

An ambiguity is associated with the determination of k_d' values from serum antibody responses as opposed to k_d values from antigen clearance curves, and it seems related to the immunological sensitivity of the test animal. If the model applies to both antigen clearance and serum antibody response, one expects the k_d values determined from each experiment to agree within the limits of ex-

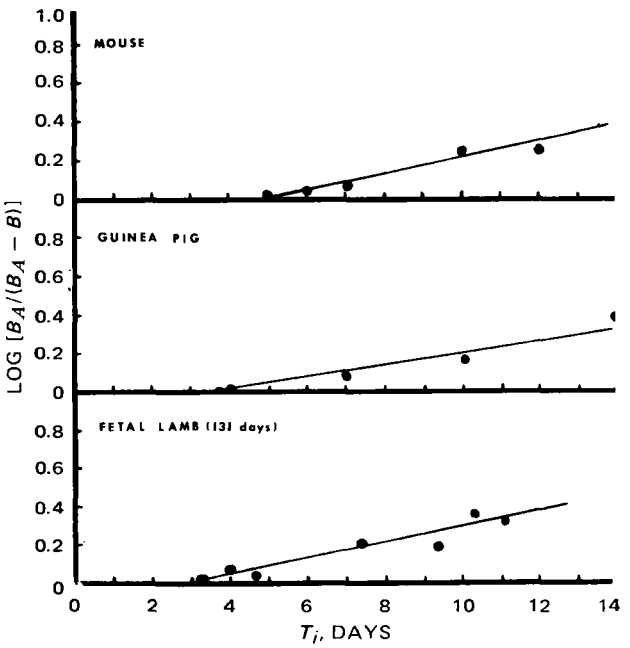

Figure 2—*Linear representation of serum antibody response.*

Table II—Comparison of k_d Values

Species	Antigen Dose, PFU/ml	Optimum Antibody Produced (Estimated)	k_d, hr^{-1} Antigen Clearance	k_d, hr^{-1} Serum Antibody Response	Reference
Guinea pig	2×10^9	50[a]	0.023	0.019	3
Fetal lamb (131 days)	6×10^8	10[a]	0.022	0.0038	7
Mouse	3×10^9	80×10^2[b]	0.021[c]	0.0056	6

[a] Assay in terms of K values. [b] Assay in terms of SD_{50} values. [c] Initial dose of antigen, 6×10^5 PFU/ml.

perimental uncertainty. This appears to be so with the guinea pig as the test animal (Table II). However, for the two other species, mouse and fetal lamb (131 days), the k_d' value determined from the serum antibody response is about 20 times less than the k_d value determined from antigen clearance curves.

Evidently, immunological sensitivity implies that antigen stimulates not only the production of antibody-producing cells but also the production of antibody from these cells. On this basis, animals such as the guinea pig, which are relatively insensitive immunologically toward ϕX174, respond to antigen primarily through the production of antibody-producing cells. Values of k_d determined from clearance curves and from serum antibody responses are then expected to be in agreement. However, animals that are immunologically sensitive toward ϕX174 have their antibody-producing cells stimulated by antigen so as to produce more antibody. Serum antibody responses thus lead to k_d' values characterizing the rate of production of antibody in the absence of antigen, while antigen clearance curves lead to estimates of k_d characterizing the rate of production of antibody in the presence of antigen.

CONCLUSIONS

The developed pharmacokinetic model enables an interpretation of cellular events from *in vivo* experiments on the time course of the primary antibody response toward ϕX174. A modified model may be necessary for more complex antigens, especially in relation to their distribution between blood and body tissues. However, the present model generally describes the major events occurring in the initiation of the primary antibody response. It does so in a quantitative manner, without straining or forcing an interpretation. It provides a basis for comparing results from differing sources, a framework into which kinetic information from the study of isolated events in the immunological initiation process may be fitted, and offers a means of gaining a preliminary mechanistic interpretation.

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ACKNOWLEDGMENTS AND ADDRESSES

Received October 23, 1974, from the *School of Pharmacy, Temple University, Philadelphia, PA 19140*

Accepted for publication January 6, 1975.

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