The Relationship between Antigen Concentration, Antigen Internalization, and Antigenic Complexes: Modeling Insights into Antigen Processing and Presentation

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Abstract. Native antigen is processed and subsequently presented on the surface of antigen-presenting cells, an important step in the elicitation of an immune response. The early events of antigen processing and presentation include: ingestion of a native antigen, intracellular degradation to expose an antigenic peptide fragment, binding of this fragment with an MHC class II molecule, and display of this newly formed complex on the cell surface. Through the development of a mathematical model, a set of mathematical equations which describes the time-dependent appearance, disappearance, and movement of individual molecules, quantitative insight can be gained into the pathways and rate-limiting steps of antigen presentation.

The credibility of the model has been verified by comparison to literature data. For example, it has been shown experimentally that macrophages require 60 min for effective antigen presentation, whereas B cells require 6–8 h. The mathematical model predicts these presentation times and identifies the difference in the cell's respective pinocytic rates and sizes as important parameters. B cells capture antigen in their environment through nonspecific fluid-phase pinocytosis as well as by binding antigen to their surface immunoglobulin, allowing receptor-mediated uptake. Uptake of antigen via receptor-mediated endocytosis has been reported to require 1,000-fold less antigen than uptake via nonspecific pinocytosis. The mathematical model clearly predicts this decrease in concentration. The model also makes quantitative predictions for the number of MHC class II–antigen complexes needed to produce T cell stimulation.

T HE recognition of complex antigens by T helper cells requires the participation of accessory cells. These accessory cells, termed antigen-presenting cells (APC),[1] prepare the antigen for recognition by the T cell. The APC, commonly a B cell or macrophage, ingests the extracellular antigen, processes it so as to expose an immunogenic peptide sequence, and expresses this fragment on its membrane surface in a complex with the proper genetic restriction molecule, an MHC class II molecule (Ia). It is believed that the antigen, in the context of this complex, can then be recognized by the T cell receptor (Watts and McConnell, 1987; Allen et al., 1987). This series of events is commonly referred to as antigen processing and presentation (Moller, 1978; Unanue, 1984; Chesnut and Grey, 1985).

Recent experiments have provided data on the ingestion of a native antigen, the intracellular processing of that antigen in an acidic compartment, and the surface expression of the MHC class II–antigen (Ia–Ag) complex. Yet many unanswered questions remain regarding the initial interaction of antigen with APC. For example, literature data relating anti-gen concentration with subsequent T cell response, typically assayed by measuring IL 2 secretion or tritiated thymidine uptake, give no information on the number of Ia–Ag complexes required to produce such a response. In addition, differences in the rate of antigen processing between types of APC have been observed but not explained (Chesnut et al., 1982; Grey et al., 1982). Macrophages have been shown to effectively stimulate a population of T cells after an antigen processing time of 60 min (Ziegler and Unanue, 1981), while B cells require a processing time of 8 h to stimulate a population of T cells (Lakey et al., 1988; Gosselin et al., 1988; Eisenlohr et al., 1988); the reason for this dramatic difference is not known. Further, it has been reported that a difference in the method of antigen internalization can dramatically affect the amount of T cell stimulation. When using their surface immunoglobulins (Ig) and receptor-mediated endocytosis to internalize antigen, B cells can effectively stimulate a population of T cells with one-thousandth the antigen concentration needed for the same stimulation when only fluid-phase uptake is used (Casten and Pierce, 1988; Snider and Segal, 1987). Although receptor-mediated endocytosis is known to affect a concentration enhancement (Linderman and Lauffenburger, 1989; Steinman et al., 1983; Swanson, 1985), no method is currently available to quan-

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[1] *Abbreviations used in this paper:* APC, antigen-presenting cell; Ag, antigenic peptide fragment; FPP, fluid-phase pinocytosis; RME, receptor-mediated endocytosis.

titatively predict the dependence of T cell stimulation on Ag concentration and method of Ag ingestion.

The goal of this paper is to present a mathematical model to accurately describe the initial events of antigen presentation: the internalization and processing of antigen by an APC and the display of Ia–Ag complexes on the surface of the APC. Specifically, the development of such a model allows us to address the relationship between antigen concentration and the number of Ia–Ag complexes displayed on the APC, the differences in processing time between macrophages and B cells, and the difference between two methods of antigen ingestion, nonspecific fluid phase uptake and receptor-mediated endocytosis. We identify the key rate-limiting processes in antigen presentation and compare our model predictions with reported data. By focusing on these early events in the APC/T cell interaction, we provide a framework for quantitatively relating antigen dose with aspects of the ensuing immune response.

Background

To formulate a mathematical model sufficiently general to describe a variety of APC/T-cell systems, we draw on experimental data that are briefly summarized here. Glutaraldehyde fixation of live APC before pulsing with native antigen prevents stimulation of T helper cells (Thomas, 1978), while pulsing with antigenic peptide fragments allows stimulation (Lakey et al., 1988), evidence that native antigen must first be internalized before the T cell can be stimulated. Internalization of native antigen can occur via at least two methods: nonspecific fluid-phase pinocytosis (FPP) and receptor-mediated endocytosis (RME) (Steinman et al., 1983). Both macrophages and B cells internalize antigen using fluid-phase pinocytosis (Werdelin and Buus, 1988). B cells can also bind some antigens via their surface immunoglobulin receptors (Gosselin et al., 1988; Lanzavecchia, 1985), trap these receptor-ligand complexes in coated pits, and internalize the complexes via RME. In model systems, antigen can also be targeted to other APC surface molecules, including MHC class I and II molecules, by using antibody heteroconjugates (Snider and Segal, 1989; Casten and Pierce, 1988).

MHC class II molecules are also present on the APC surface. When the cell's membrane pinches off to form intracellular vesicles, these molecules are then internalized (Pletscher and Pernis, 1983). Recent evidence has shown that the MHC Class II molecules are not concentrated in coated pits before internalization (Tse et al., 1986).

When native antigen is administered, the APC must process or partially degrade the antigen molecule and select an antigenic fragment for presentation to the T cell. Processing of the native antigen typically takes place intracellularly; however, proteolytic enzymes on the surface of the cell may also play a role in degrading some antigens (Buus and Werdelin, 1986; Werdelin and Buus, 1988). It is believed that molecules internalized via FPP or RME are delivered to endosomes (Cresswell, 1985; Germain, 1986; Long; 1989), vesicular compartments with a pH in the range of 5.0 to 6.0 (Tycko and Maxfield, 1982; Murphy and Roederer, 1986; Yamashiro and Maxfield, 1984; Anderson and Orci, 1988). The acidic endosome provides an optimal environment for proteases. Cathepsin-D has been shown to be present in the endosome and capable of protein degradation in this environment (Diment and Stahl, 1985; Ferguson et al., 1973).

Use of the catabolic agents ammonia and chloroquine leads to a decrease in both antigen presentation and T cell stimulation (Ziegler and Unanue, 1982). In general, these agents are known to increase the pH of intracellular organelles and would therefore be expected to interfere with the limited proteolysis needed for processing. Chloroquine has also been shown to affect the maturation of Ia by preventing the dissociation of the invariant portion of the molecule (Nowell and Quaranta, 1985). In addition, McCoy and co-workers have shown that APC deficient in endosomal but not lysosomal acidification are impaired in their ability to present antigen (McCoy et al., 1989). Together, the experimental results support the premise that the antigen is first internalized and then denatured in an endosome.

The next step in the process is the selection of the proper proteolytic fragment for presentation by the APC. Recent evidence suggests that the MHC class II molecule and the proper immunogenic fragment have an affinity for one another; the K_D found for the interaction between purified Ia and peptide fragments is 10^{-5} and 10^{-6} M at a pH of 5.6 and 7.2, respectively

(Babbitt et al., 1985; Buus et al., 1987). As the role of the intramembraneous structures on the activity of Ia is unknown, these data must be taken as a first approximation. It is also thought that autologous peptides may be bound to these purified Ia molecules. The use of inhibitory peptides restricted by the same Ia in in vitro whole cell competition assays further suggests an interaction between the MHC Class II molecule and the immunogenic fragment (Guillet et al., 1987).

Surface expression of the complexed antigenic fragment and the MHC class II molecule on the APC is a critical event in the process of antigen presentation. As discussed above, it is believed that the antigenic fragment and the MHC class II molecule interact (Luescher et al., 1988; Phillips et al., 1986); the site of this interaction is not known. MHC class II molecules from the surface of APC have been shown to be present in vesicles containing internalized surface immunoglobulin, suggesting that native antigen and MHC class II molecules may be internalized together during endocytosis (Pletscher and Pernis, 1983). In addition, Cresswell has shown that recently synthesized Ia are present in the same vesicles as transferrin–neuraminidase, which is internalized via RME (Cresswell, 1985). Phillips and co-workers (Phillips et al., 1988) have evidence for the presence of Ia-insulin peptide complexes in endosomal compartments. These data suggest that Ia and antigenic peptides have the opportunity to bind in the endosome, the same compartment in which the native antigen is likely degraded (Werdelin and Buus, 1988). In some cases, it is possible for the binding event to take place on the APC membrane, particularly when an antigenic peptide is provided and no internalization is needed (Watts et al., 1985). A variation of this method occurs for viral antigens that are exocytosed after internal degradation and then diffuse within the APC membrane, to associate with the Ia molecule (Mills, 1986). These two latter cases are not the focus of this work.

For systems in which the antigenic complex is formed intracellularly, a means of transporting the complex to the cell surface to allow for interaction with a T cell must be available. One theory is that complexes as well as free Ia molecules are sorted in the endosome from materials to be routed to the lysosome (Germain, 1986; Werdelin and Buus, 1988) and then transported to the cell surface via the recycling pathway. Although antigen might also be degraded in lysosomes, no route to shuttle the immunogenic peptides from lysosomes back to the cell surface has been elucidated, supporting the theory that limited proteolysis and binding occur in the endosome.

Ia–Ag complexes at the cell surface are believed to be recognized by T cell receptors (Dembic et al., 1986). The Ia serves not only as the genetic restriction molecule but is also believed to hold the antigen in the proper conformation for T cell recognition and protect it from further proteolysis at the cell surface (Guillet et al., 1987; Allen et al., 1984; Donermeyer and Allen, 1989).

Mathematical Model

As summarized above, experimental evidence suggests that the steps leading to the surface expression of Ia–Ag complexes include ingestion of the native antigen, intracellular degradation of the antigen, binding of the antigenic peptide fragment and the Ia molecule, and the eventual presentation of this complex on the APC surface. Our model for these initial events in antigen processing and presentation is presented in Fig. 1.

It is assumed that the free Ia molecules are uniformly distributed on the surface of the cell and are transported into the cell with rate constant k_v as the membrane pinches off to form endocytic vesicles. At the same time, native antigen is also taken up into endocytic vesicles. The rate at which native antigen is ingested via FPP is a function of how quickly the cell membrane pinches off to form endocytic vesicles and the extracellular antigen concentration. The rate constant for antigen internalization, k_u, is proportional to k_v. This proportionality, based upon consideration of cell surface area SA_c, endosomal surface area SA_v, and endosomal volume V_v, is:

$$k_u = \frac{k_v V_v (SA_c)}{(SA_v)}$$ (1)

In deriving this relationship, we have assumed that all endosomes have the same size and that the total surface area and volume of endosomes formed is equal to the total membrane surface area and volume of fluid internalized, respectively.

If an appropriate antigen receptor is present on the cell surface, receptor-ligand complexes form and dissociate with overall rate constants k_f^* and k_r^* and can cluster in coated pits. These complexes are internalized with a rate constant k_e^*. It is assumed that free receptors are not internalized (Drake et al., 1989; Wiley and Cunningham, 1981).
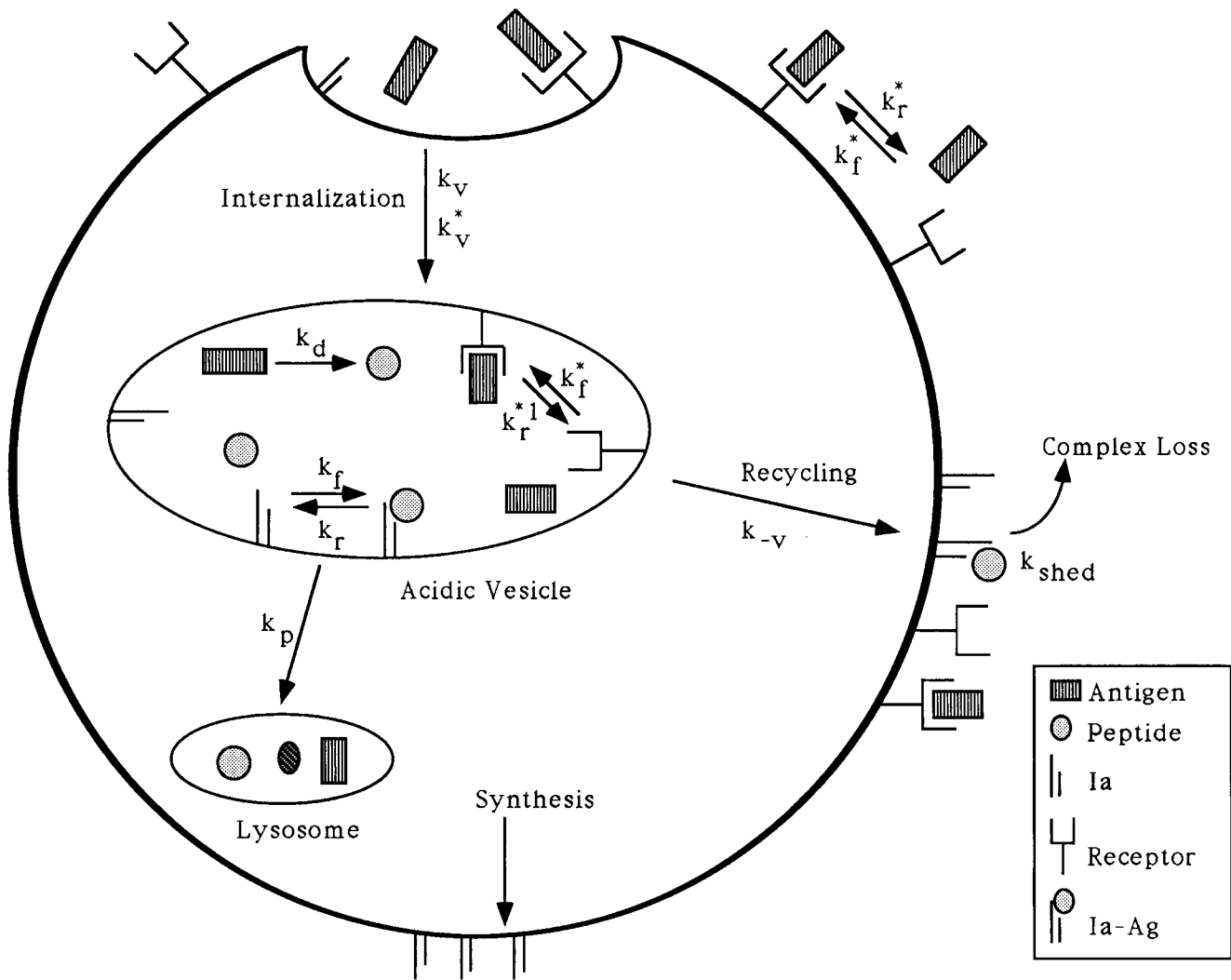
Figure 1. Schematic of antigen processing and presentation. Shown are the steps included in the formulation of the model: ingestion of the antigen, degradation of the antigen, formation of the Ia–Ag complexes, and transportation of Ia-Ag complexes to the surface for presentation.

We assume that the contents of endocytic vesicles, the result of FPP and/or RME, are rapidly delivered to mature endosomes. Once inside endosomes, receptor–ligand complexes dissociate with a rate constant k_r^{*1}. As it is usually believed that dissociation rather than association kinetics are most affected by this change in pH (Mellman and Unkeless, 1980; Mellman et al., 1984), this dissociation rate constant is increased above k_r^*, thereby enhancing the rate of dissociation. Rebinding of native antigen to receptors can occur; lacking evidence to the contrary, we set the rate constant for rebinding to be identical to that for binding at the cell surface.

Native antigen undergoes limited proteolysis with a rate constant k_d in the endosome. As in vitro ligand concentrations are often small relative to the Michaelis–Menton constant K_m, degradation can be approximated by first-order kinetics with an apparent rate constant k_d equal to the maximum rate of reaction V_{max} divided by K_m. The kinetic parameters for many of these enzymes have been tabulated (Laidler, 1958). After proteolysis, antigenic fragments are able to associate with and dissociate from Ia molecules inside the vesicle with overall rate constants k_f and k_r, respectively. It is assumed that the intracellular complexes are uniformly distributed throughout the vesicles.

Ia–Ag complexes, receptor–ligand complexes, Ia, antigen, and peptide fragments do not remain in the endosome indefinitely. All undegraded antigen and unbound peptide fragments are assumed to be shuttled to the lysosome with a rate constant k_p to undergo further degradation. Ia–Ag complexes, free Ia, free receptors, and any undissociated receptor-ligand

complexes are assumed to be recycled to the cell surface with a rate constant k_{-v}. The assumption of perfect recycling of Ia and Ia–Ag complexes is appropriate as a first approximation if these molecules behave similarly to MHC Class I molecules, which have a long $t_{1/2}$ of 14 h (Tse et al., 1986). Note that the concentration of each molecular species in intracellular vesicles is the result of the competing effects of internalization, degradation, binding, routing to the lysosome, and recycling to the cell surface and is strongly dependent upon the magnitude of the rate constants for each step.

It is assumed that once an Ia–Ag complex is returned to the cell surface it cannot be taken up again. This assumption is reasonable if the complexes bind to the T cell receptor shortly after their appearance on the APC surface or if APC can selectively exclude these complexes from the internalization pathway, as has been shown for some surface markers on other cells (Tse et al., 1986). Ia–Ag dissociation is extremely slow and is ignored in our model, for the half-life of antigenic complexes has been measured to be 27 h (Buus et al., 1987). The cell is assumed to lose complexes from the APC surface with a rate constant k_{shed}, accounting for complex loss via degradation or shedding. In addition, we allow for newly synthesized free Ia to be added on the surface of the cell. Finally, we note that the assumptions made here are meant to be valid only for the time period of antigen processing and presentation.

The mathematical formulation of this model, describing the rates of change of the numbers or concentrations of the relevant molecular species, can now be written. The most general formulation of the model consists of

Table I. Model Parameters, Their Definitions, and Estimated Physiological Range of Values

Parameter	Definition	Estimated physiological range	References
k_v	Rate constant for internalization	0.0075–0.05 (*1/min*)	Tse et al., 1986 Harding and Unanue, 1989 Chesnut et al., 1982 Swanson, 1985 Watts and Davidson, 1988
k_v^*	Rate constant for internalization of receptor–ligand complexes	0.030–0.1 (*1/min*)	Cuchens and Buttke, 1984
k_{-v}	Rate constant for vesicle recycle	0.012–0.03 (*1/min*)	Tse et al., 1986 Watts and Davidson, 1988
SA_c	Surface area of the cell	400–2,850 (*μm^2*)	Sherman and Sherman, 1983 Steinman and Cohn, 1972 Swanson, 1985
SA_v	Surface area of an endosome	1.5 (*μm^2*)	Marsh et al., 1986
V_v	Volume of an endosome	0.04 (*μm^3*)	Marsh et al., 1986 Tse et al., 1986
N	Number of endosomes containing Ia molecules per cell	8–20	Tse and Pernis, 1984
A_0	Initial number of Ia molecules	1.0×10^5–4.0×10^5	Casten and Pierce, 1988 Harding and Unanue, 1989
R_0	Initial number of receptors	10^4–10^6	Delisi, 1980 Casten and Pierce, 1988 Lanzavecchia, 1985
L_0	Extracellular concentration of native antigen	Varies	
k_f	Association rate constant for the binding of Ia and peptide fragment	19.1–247.5 (*$1/M \cdot min$*)	Buus and Werdelin, 1986 Phillips et al., 1988
k_r	Dissociation rate constant for Ia-peptide complex	4.2×10^{-4} (*1/min*)	Buus and Werdelin, 1986
k_f^*	Association rate constant for the binding of receptor and ligand	4×10^5–6×10^6 (*$1/M \cdot min$*)	Buus et al., 1987 Watts and Davidson, 1988 Casten and Pierce, 1988 Lanzavecchia, 1985
k_r^*	Dissociation rate constant for receptor–ligand complexes at neutral pH	0.36–0.0036 (*1/min*)	Buus et al., 1987 Watts and Davidson, 1988 Casten and Pierce, 1988 Lanzavecchia, 1985
k_r^{*1}	Dissociation rate constant for receptor–ligand complex at acidic pH	0.1–0.2 (*1/min*)	Watts and Davidson, 1988 Mellman and Unkeless, 1980 Mellman et al., 1984
k_d	Rate constant for degradation of native antigen	0.012–0.105 (*1/min*)	Diment and Stahl, 1985 Ferguson et al., 1973 Linderman and Lauffenburger, 1989 Zigmond et al., 1982
k_p	Rate constant for routing of molecules to lysosome	0.01–0.015 (*1/min*)	Lauffenburger et al., 1987
k_{shed}	Rate constant for loss of complexes from APC surface	8.2×10^{-4} (*1/min*)	Tse et al., 1986

10 independent equations. This set of equations can be reduced to the set of eight independent equations given in the Appendix when the total number of Ia and receptors are held constant according to:

$$A_0 = A + B + C + C^*, \qquad (2)$$

$$R_0 = R + (RL) + R^* + (RL)^* \qquad (3)$$

The symbols used in the equations are: A = number of Ia molecules on the cell surface, number/cell; B = number of Ia molecules in endosomes, number/cell; C = Ia–Ag complexes in endosomes, number/cell; C^* = number of Ia–Ag complexes on the cell surface, number/cell; R = number of receptor molecules on the cell surface, number/cell; (RL) = number of receptor-ligand complexes on the cell surface, number/cell; R^* = number of receptor molecules in endosomes, number/cell; $(RL)^*$ = number of receptor-ligand complexes in endosomes, number/cell. A_0 and R_0 are defined as the total number of Ia and receptors, respectively, expressed by the APC at time zero.

Incorporation of these physical constraints allows us to set the rate constant for loss of complexes from the cell surface, k_{shed}, whereas the rate of synthesis of Ia molecules varies implicitly to satisfy Eq. 2. For the results presented in this paper, we choose the initial conditions of no antigen inside or bound to the surface of the APC and all Ia molecules and receptors unbound and on the surface of the APC.

The model equations describing the antigen processing and presentation events were solved numerically to give the concentration of each of the individual species as a function of time. The predictions of the model can be examined for a variety of parameter values.

Parameter Estimation

Our model suggests that the initial events of antigen presentation can be characterized in terms of the parameters listed in Table I together with physiological ranges or estimates for these parameters. Rate constants for recycling, routing to the lysosome, and the surface area and volume of an endosome are parameters obtained from studies of the endocytic pathway that do not explicitly involve antigen presentation, as we assume that the process of antigen presentation uses the endocytic pathway as a means of trafficking Ia, antigen, antigenic complexes, receptors, and receptor-ligand complexes. The rate constants for receptor-ligand binding and the internalization of these complexes are obtained from studies of immunoglobulin interactions on the surface of B cells. Rate constants for vesicle formation, association and dissociation of the Ia–Ag complex, degradation, and complex shedding are experimental results from macrophage or B cell presentation assays.

The range of parameter values can be attributed to a number of different factors. For example, the value of k_v varies over an order of magnitude to account for a wide range of studied systems that exhibit internalization behavior. This natural range may thus allow the model to distinguish between efficient and inefficient APC. On the other hand, the rate constant k_f, the association constant for the binding of Ia and the peptide fragment, exhibits a wide range of estimated values due in part to the uncertainty involved in the methods of Ia purification and the role of membraneous structures in binding. Finally, the parameters SA_c and SA_v have been experimentally determined only for one system (Marsh et al., 1986), and therefore a physiological range of values is not known. Overall, it is apparent that a range in parameter values can be due to uncertainties in the parameter itself or to naturally occurring differences in the behavior of systems that have been studied.

In some cases, we have further analyzed the literature data cited to extract the parameter values needed for our model. This analysis often involves making additional assumptions about the system under investigation and can increase the amount of uncertainty involved in the estimation of a parameter. For example, data on the degradation of molecules in a macrophage endosome is reported as amount of TCA-soluble material as a function of time (Diment and Stahl, 1985). To determine a degradative rate constant, a reaction order must be assumed. As Diment and Stahl show that the amount of TCA-soluble material is linear with time, a first-order reaction was assumed. The value of k_d we determined from this analysis agrees well with related data in the literature (Ferguson et al., 1973). Similar analyses were performed to obtain the rate constants for internalization, association, and dissociation of the receptor-antigen complex. Finally, the data from Buus and co-workers on the rate of association and dissociation of the Ia–Ag complex was obtained using purified Ia molecules and peptide in solution. As we assume that complex formation during antigen processing occurs between Ia molecules diffusing in a fluid bilayer membrane and antigen fragments free in solution, we modify the reported rate constants to consider mass transfer as well as reaction kinetics according to the theory of DeLisi

and co-workers (k_r is unaffected; k_f is decreased by a factor of 3) (Delisi, 1980). Additionally, we modify the reported rate constants, k_f and k_r to account for the fact that ∿10% of the purified Ia were actually participating in these binding assays (Buus et al., 1988). These modified values for k_f and k_r are listed in Table I.

Results

General Behavior of the Model

We first examine the general behavior of our model for a macrophage APC, the case of antigen internalization via fluid phase pinocytosis alone. By reducing the system of equations given in the Appendix to include only fluid-phase internalization, our general model can be reduced to a set of five equations. For the experimental conditions under consideration, the variation in the extracellular ligand concentration with time is insignificant. We therefore assume that the extracellular antigen concentration, L_0, remains constant.

For a particular set of parameter values, the model predicts the macrophage relative species numbers or concentrations as a function of time, as shown in Fig. 2, a and b. The parameter values used to determine the species profiles as a function of time correspond to physiological values found in the literature for macrophage behavior and are listed in the figure legend. As internalization begins, there is a quick rise in the intracellular pool of free Ia, B/A_0, and a corresponding decrease in the free surface Ia population, A/A_0. The macrophage is able to quickly concentrate the native antigen, L^*/L_0, in intracellular endosomes, generating a significant concentration of antigenic fragments, Ag/L_0, for complex formation.

MHC class II molecules must bind to antigenic fragments for the APC to begin accumulating intracellular Ia–Ag complexes, C/A_0. Initially, the rate of transport of antigenic peptide fragments to the lysosome competes with the binding of MHC class II molecule and fragment; this binding is further impeded by the low affinity between the interacting species. As antigenic peptide concentration increases, the rate of complex formation increases. Surface Ia–Ag complexes, C^*/A_0, begin appearing on the APC plasma membrane after a short delay and continue to accumulate during the time of antigen processing and presentation. This time delay or lag time has been reported as a requirement that APC must be cultured with antigen for a characteristic processing time in order to effectively stimulate a T cell population (Roosnek et al., 1988; Jensen, 1988; Gosselin et al., 1988; Lanzavecchia, 1986). This processing time is thus directly related to the time necessary to accumulate significant numbers of Ia–Ag complexes on the surface of the APC.

Antigen-presenting Cell Type

Although both macrophages and B cells can function as APC using only FPP as an antigen uptake mechanism, they exhibit significant differences in the antigen processing time required to effectively stimulate a population of T cells. Macrophages are large scavenger cells that actively remove debris from areas such as the peritoneal cavity, while B cells are concentrated in immunologically active organs such as the spleen and lymph nodes and have a primary role as antibody producing cells (Nester, 1978), suggesting that their different roles may in some way lead to a difference in the
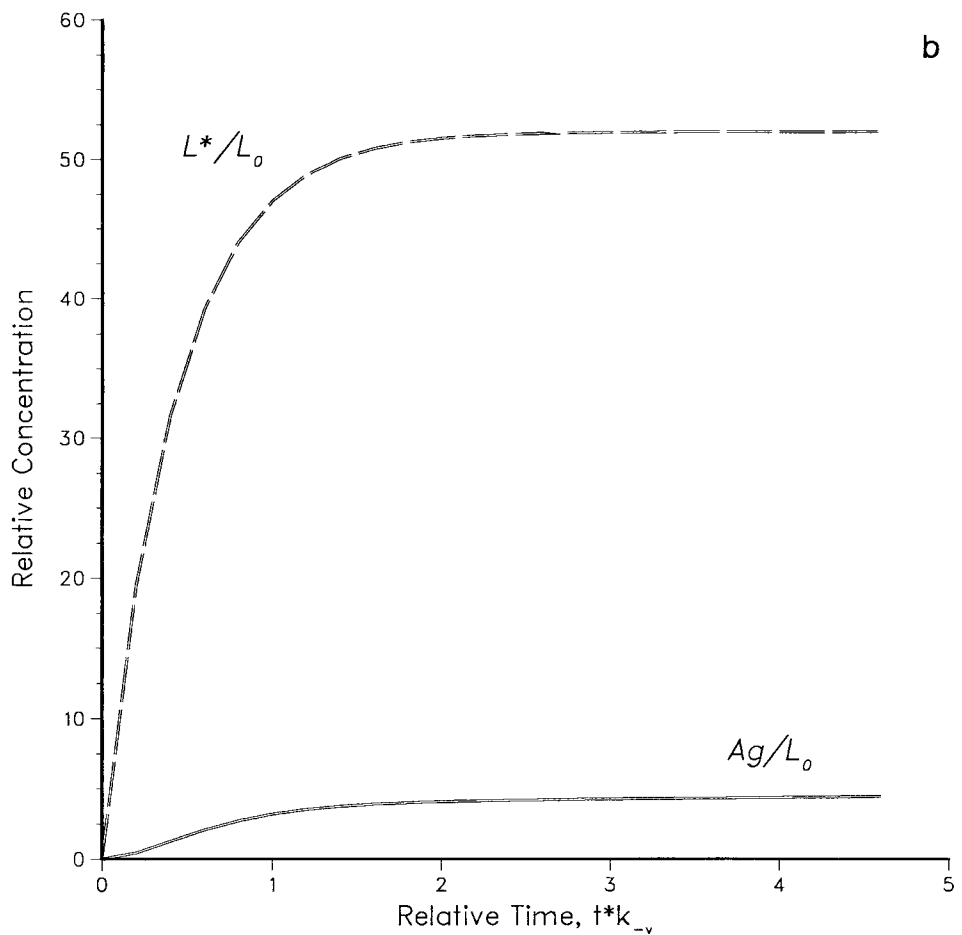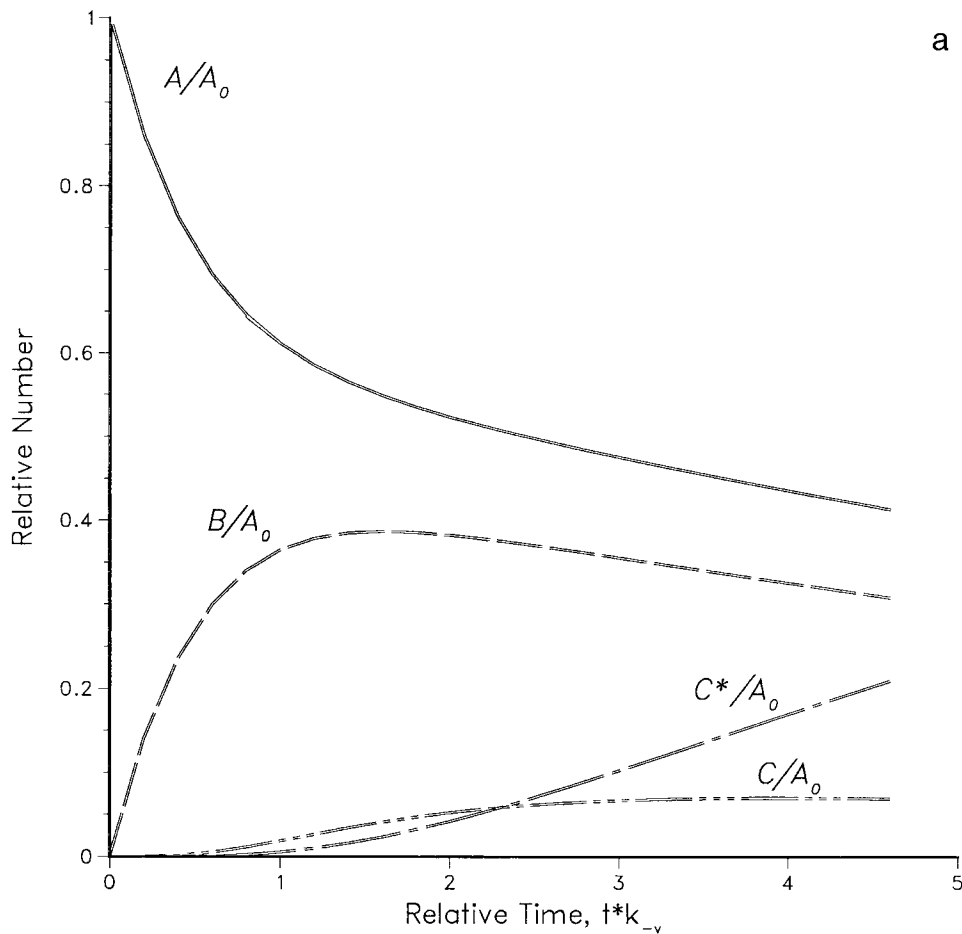
Figure 2. The general behavior of the model. Relative species number or concentration as a function of relative time. The plots are representative of macrophage presenting cells for typical assay conditions. For reference, a relative time of three is equal to 60 min. Parameter values: $A_0 = 1.1 \times 10^5$ Ia/cell; $L_0 = 10\ \mu M$; $k_v = 0.042\ min^{-1}$; $k_{-v} = 0.05\ min^{-1}$; $N = 10$; $k_r = 4.2 \times 10^{-4} \cdot min^{-1}$; $k_f = 250\ M^{-1} \cdot min^{-1}$; $k_d = 0.012\ min^{-1}$; $k_{shed} = 8.2 \times 10^{-4} \cdot min^{-1}$; $SA_c = 2,170\ \mu m^2$; $SA_v = 1.5\ \mu m^2$. (*a*) Relative number of free Ia on the cell surface, free Ia divided by total Ia per cell, A/A_0. Relative number of free Ia inside the cell, B/A_0. Relative number of intracellular Ia–Ag complexes, C/A_0. Relative number of cell surface Ia–Ag complexes, C^*/A_0. (*b*) Relative concentration of intracellular native antigen, intracellular concentration divided by extracellular concentration, L^*/L_0. Relative concentration of intracellular peptide fragments, Ag/L_0.

necessary processing time. Macrophages have been shown to require an antigen processing time of 60 min before optimal T cell stimulation can occur (Jensen, 1988; Ziegler and Unanue, 1981; Allen et al., 1984), whereas B cells have been shown to require a processing time of 6–8 h (Lakey et al., 1988; Eisenlohr et al., 1988; Gosselin et al., 1988). This processing period provides the necessary time for APC to process and present antigen in the context of MHC class II molecules. After this pulse time, the APC has accumulated sufficient Ia–Ag complexes on the surface of the APC to bind T cell receptors and thus stimulate a T cell.

If our model is to apply well to both cell types, the dramatic difference in processing times between the two cell types, noted above, must be due only to a difference in one or more of the characteristic parameters listed in Table I. There is evidence to suggest that macrophages and B cells have inherently different rate constants for FPP (Chesnut et al., 1982; Swanson, 1985; Harding and Unanue, 1989). This difference is shown by the range of values for k_v presented in Table I. The two cells also differ in size, macrophages having two to three times more surface area and volume than B cells (Sherman and Sherman, 1983; Swanson, 1985). We have found no literature data to suggest that any other model parameters are significantly different between macrophages and B cells.

To test whether these differences in the internalization rate constant and cell size could account for the dramatic differ-

ence in presentation times, two simulations using our model equations were performed. The parameter values chosen for the macrophage correspond to those used in Fig. 2, *a* and *b*, whereas the parameter values chosen for the B cell are identical except for a smaller value for the cell size, SA_c, and a smaller rate constant for internalization, k_v. For illustrative purposes we have chosen 0.042 and 0.0075 min⁻¹ for the rates of internalization for macrophages and B cells, respectively (Harding and Unanue, 1989), which approximately correspond to the median values reported in the literature. In Fig. 3, the results are plotted as Ia–Ag complex density on the surface of each APC type as a function of time. These model predictions show an important result. The B-cell surface density of Ia–Ag complexes expressed after 6–8 h of antigen processing, ∼5 complexes/μm², is equal to the macrophage surface density of Ia–Ag complexes expressed after 50–65 min of antigen processing. These processing periods agree well with the experimental observations discussed above. Based on the simple postulate that equal densities of Ia–Ag complexes on the surface of an APC will result in equal amounts of T cell stimulation, our model predicts that an Ia–Ag complex density of ∼5 complexes/μm² will produce maximal T cell stimulation. This complex density is reached after a different antigen processing time for each APC type.

Complex density on the APC surface, rather than absolute number of complexes, is shown in Fig. 3 for three reasons.
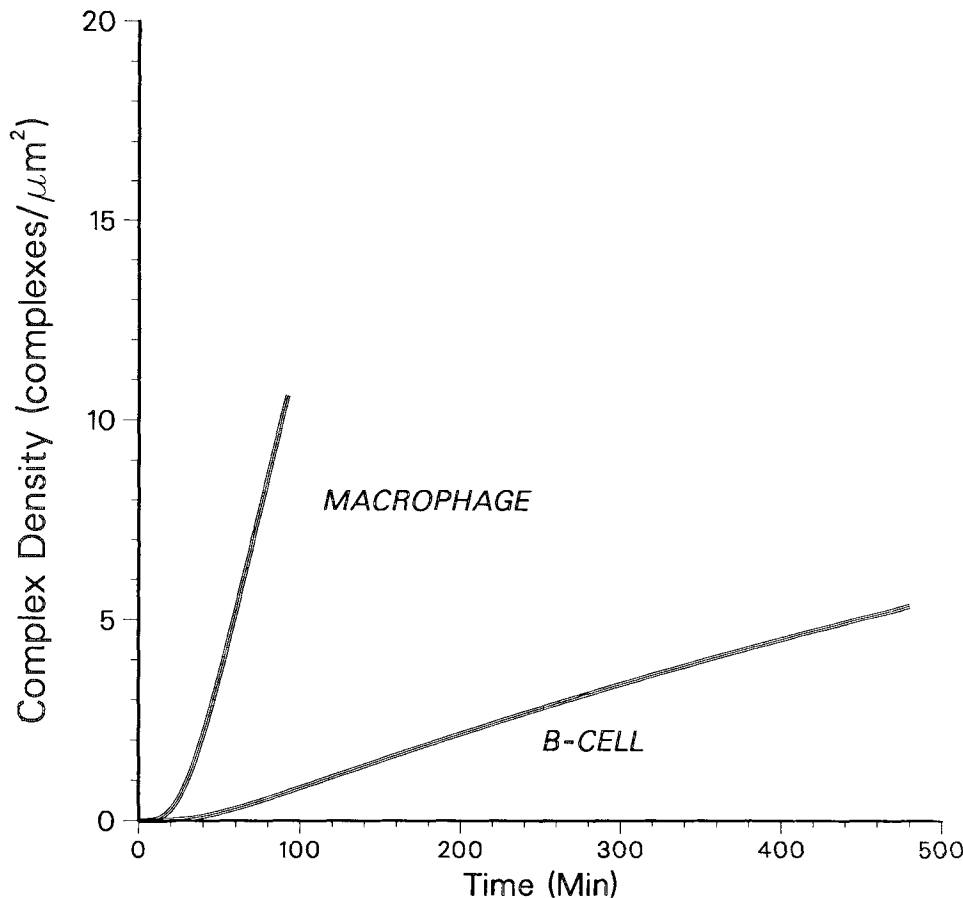

Figure 3. Ia–Ag complex density as a function of time for macrophage and B cell APC and fluid phase ingestion of antigen. These parameters were identical for both cell types: A_0 = 1.1 × 10⁵ Ia/cell; L_0 = 10 μM; k_{-v} = 0.05 min⁻¹; N = 10; k_r = 4.2 × 10⁻⁴ min⁻¹; k_f = 250 M⁻¹ · min⁻¹; k_d = 0.012 min⁻¹; k_{shed} = 8.2 × 10⁻⁴ · min⁻¹; SA_v = 1.5 μm². Rate constants for internalization and surface areas of cells: macrophage, k_v = 0.042 min⁻¹, SA_c = 2,170 μm²; B cell, k_v = 0.0075 min⁻¹, SA_c = 900 μm².

First, complex density allows us to directly relate each cell type without considering the inherent differences in cell size. Second, we suggest that T cells see only a small and relatively constant area of the APC surface. Thus the number of Ia–Ag complexes available for binding in a contact area between the APC and the T cell is roughly equal to the total number of cell surface complexes times the fraction of APC cell surface area in the contact area. If we assume that the complexes are uniformly distributed over the APC surface, then the number of Ia–Ag complexes available for binding is also equal to the complex density times an unknown contact area. If the contact area is constant, then the number of complexes available for binding is dependent only on the complex surface density, the model prediction we plot. Third, if absolute numbers of complexes on macrophage and B cell surfaces are compared using the same parameter values as in Fig. 3, at 60 min the macrophage expresses 11,000 complexes on its surface, while at 8 h the B-cell expresses only 4,500 complexes. Clearly this prediction for total complex number does not easily explain the observed difference in processing times between the two cell types.

Our model predicts that the processing time differences between macrophages and B cells discussed above result from differences in size, SA_c, and the internalization rate constant k_v. We now focus our attention on the internalization rate constant and its effect on the antigen processing time. In Fig. 4, the rate constant for internalization is varied over a range from 0.004 to 0.045 min^{-1}. To produce a surface density of 5 complexes/μm^2, which results in maximal

T cell stimulation, the processing period required varies from >8 h to only 55 min as the rate constant for internalization is increased. For the case of antigen internalization via FPP alone, the process of antigen presentation is clearly rate-limited by internalization. This step of the process involves not only uptake of native antigen, but also the movement of Ia molecules from the cell surface to intracellular vesicles, where binding to antigenic fragments can occur. B cells, with their smaller internalization rate, are unable to concentrate antigen and intracellular Ia to the same extent as macrophages, thereby providing fewer antigenic fragments and fewer Ia molecules for complex formation. As a result, B-cells accumulate surface Ia–Ag complexes more slowly than macrophages. Given additional time for processing, however, B cells can present antigen as effectively as macrophages.

Receptor-mediated Concentration Enhancement

We now consider the case of antigen internalization via RME. B cells, for example, are able to bind some antigens to their surface immunoglobulin and thus enhance the rate of antigen internalization over that of FPP. In particular, we aim to explain a recent observation by Casten and Pierce (Casten and Pierce, 1988). These researchers found that B cells that ingest antigen via RME can effectively stimulate a population of T cells with approximately one-thousandth the concentration of extracellular ligand needed for comparable stimulation when antigen is internalized via nonspecific
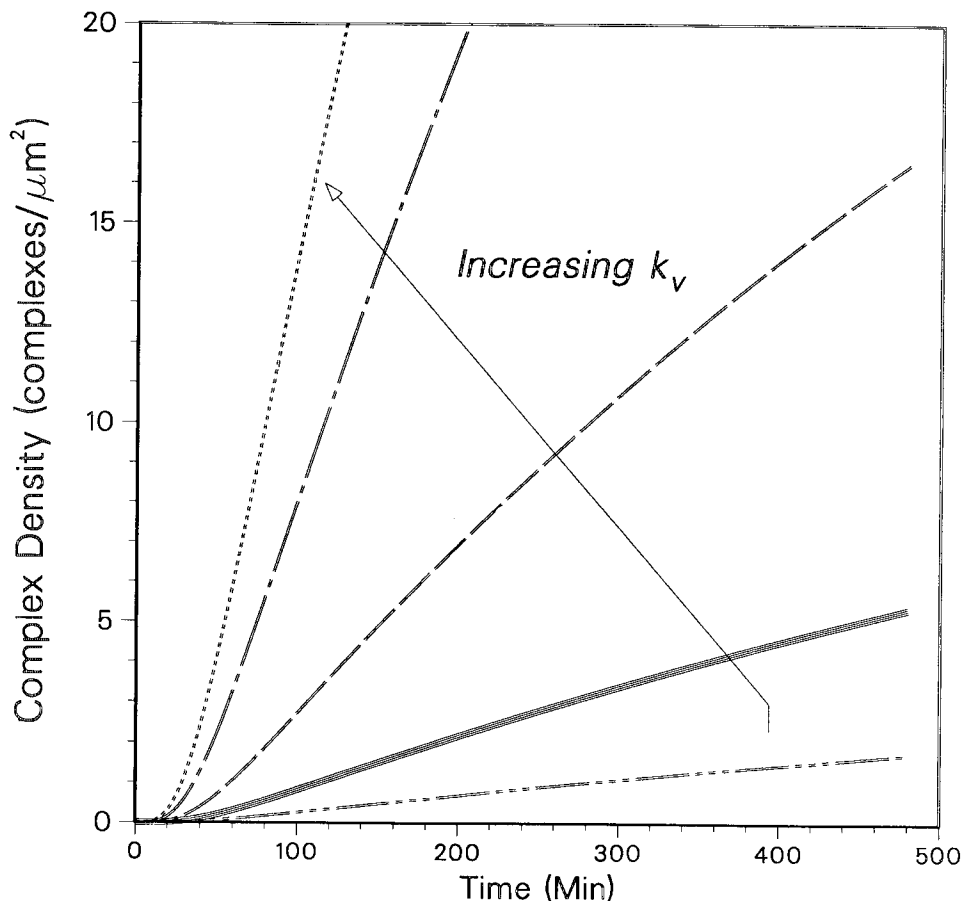

Figure 4. Ia–Ag complex density as a function of time and the rate constant for internalization. The parameters used were identical to the B cell parameters for Fig. 3 except for k_v. Values for k_v: 0.004 min^{-1}; 0.0075 min^{-1}; 0.015 min^{-1}; 0.03 min^{-1}; 0.045 min^{-1}.

uptake. For their studies of antigen uptake via RME, the antigen pigeon cytochrome c is conjugated to anti-Ig. We have replotted their results in Fig. 5 to show this dramatic concentration effect. The figure presents the percent of maximal stimulation as measured by the IL 2-dependent growth of CTLL cells as a function of extracellular antigen concentration. For example, to activate their T cell population to 50% of maximal, the fluid-phase uptake mechanism requires an extracellular antigen concentration of ~ 1 μM, whereas the receptor-mediated uptake mechanism requires ~ 0.003 μM of antigen.

To compare the experimental observations of RME and FPP uptake mechanisms shown in Fig. 5 with our model, we must first consider the behavior of the receptor–ligand complex involved in RME. The rate of internalization of these receptor–ligand complexes, which cluster in coated pits, is greater than the rate of internalization of MHC class II molecules, which are not trapped in coated pits. We also assume, for simplicity, that bivalent antibody binding can be approximated with the overall association and dissociation rate constants, k_f^r and k_r^r. After delivery of receptor–antigen complexes to endosomes, we assume in this model that the antigen must first dissociate from the receptor in order for degradation of antigen to occur. Our model also assumes that receptor–antigen complexes and free receptors are shuttled to the APC surface along with the Ia–Ag complexes and the free Ia.

Model equations were solved using identical parameter values for the fluid phase and the receptor-mediated cases.

Additional parameters needed for the case of receptor-mediated uptake of antigen include the initial number of surface receptors, the forward and reverse binding constants for the receptor–antigen complex on the cell surface and inside the cell, and the rate of internalization of receptor–antigen complexes. K_d was taken directly from Casten and Pierce; k_r^r was estimated from Watts and Davidson (Watts and Davidson, 1988) to calculate k_f^r. Our results are plotted in Fig. 6 as the number of surface complexes expressed as a function of the extracellular antigen concentration. As cells of the same size are compared, we chose to plot complex number rather than complex density on the cell surface. All points correspond to a processing time of 8 h, during which the antigen is continuously present. Although Casten and Pierce incubate antigen, APC, and T cells together for a 24-h period in their assay, Lakey and co-workers (1988) have shown that these B cells require an 8-h processing time prior to culture with T cells to produce maximal T cell stimulation for both RME and FPP uptake mechanisms.

The data of Fig. 5 show that 50 and 100% T cell stimulation occur at antigen concentrations of ~ 0.003 and 0.1 μM for RME and ~ 1.0 and 4.0 μM for FPP uptake mechanisms. Using our predictions for complex number shown in Fig. 6, we can determine that the numbers of complexes expressed for RME uptake at 0.003 μM antigen concentration and for FPP uptake at 1.0 μM are approximately equal. If we were instead to assume that the receptor-antigen complexes are shuttled to the lysosome for further degradation and these
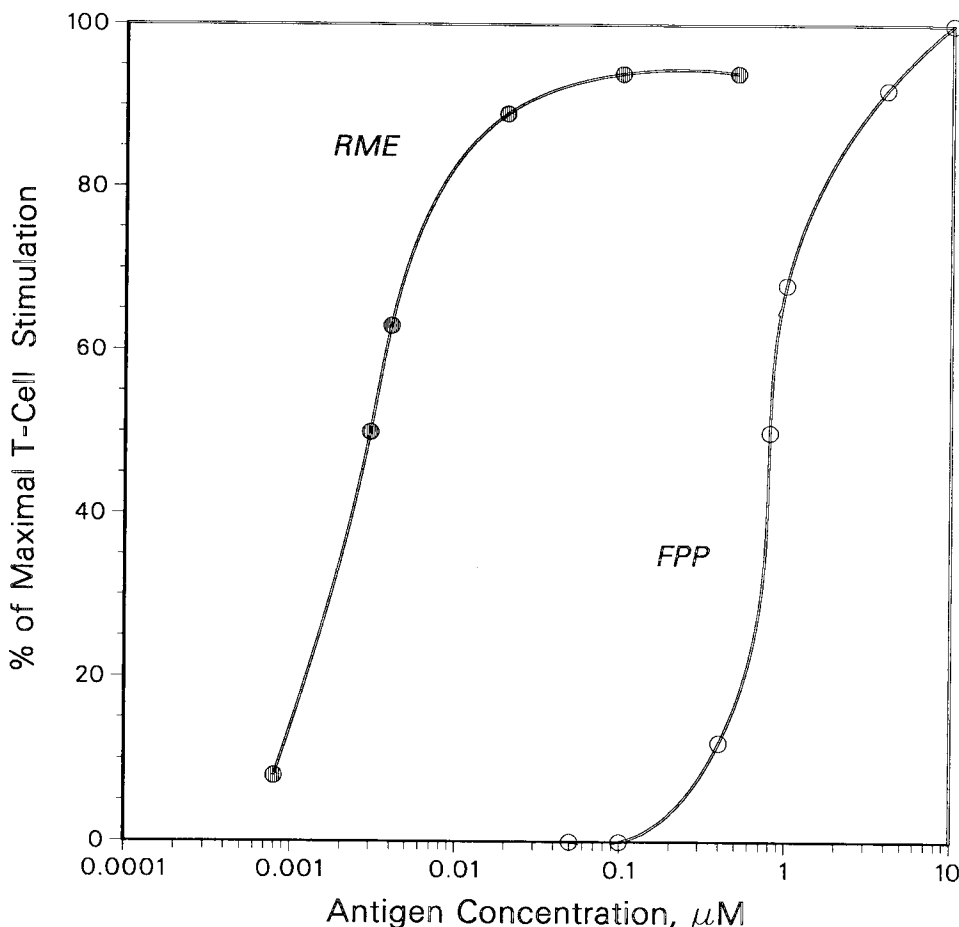

Figure 5. T cell stimulation as a function of antigen concentration when antigen is internalized via receptor-mediated endocytosis or fluid phase pinocytosis. Data are taken entirely from Casten and Pierce (1988). TPc9.1 T cells were cultured with purified murine splenic B cells as APCs and pigeon cytochrome c for fluid-phase ingestion or pigeon cytochrome c coupled to Ig antibody for receptor-mediated ingestion. The results are expressed as the percentage of the maximal T cell response obtained for the fluid-phase case.
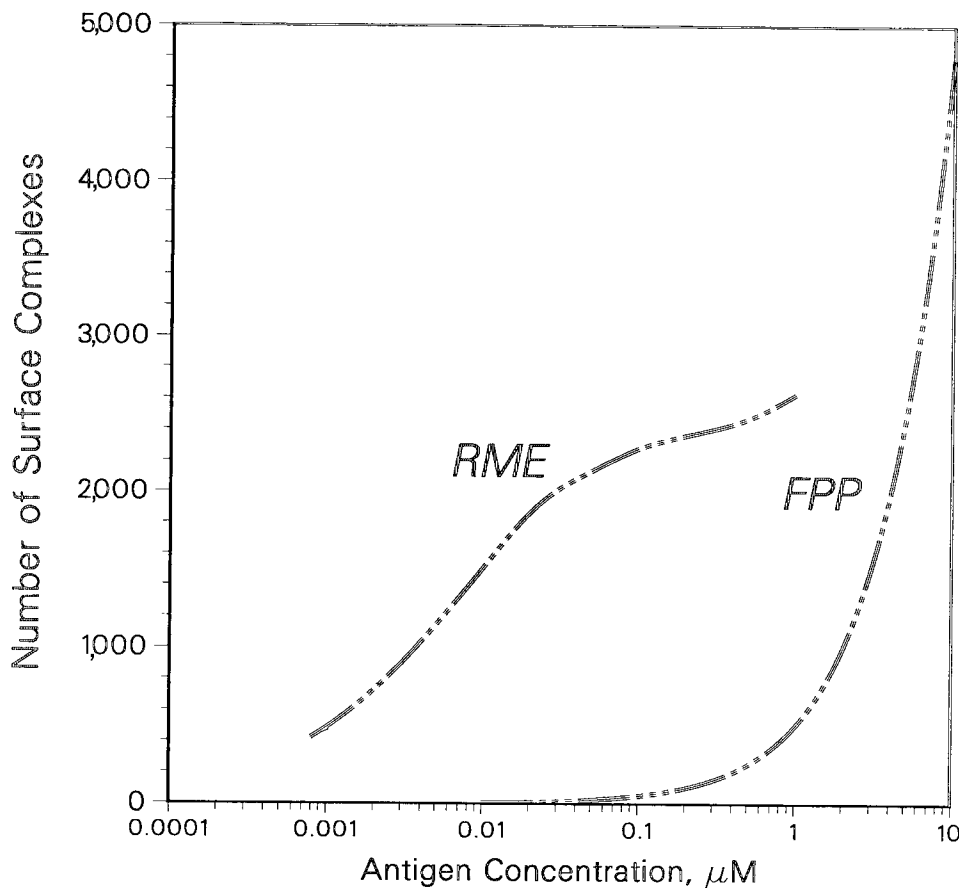
Figure 6. Number of Ia–Ag complexes as a function of antigen concentration for receptor-mediated endocytosis and fluid-phase pinocytosis as predicted by the mathematical model. The complex number shown is for a pulse time of 8 h. The parameters used are for B cell APC. FPP parameters: as stated for Fig. 3 except for antigen concentration, L_0 which varies from 0.01 to 10.0 μM. RME parameters: $R_0 = 6.5 \times 10^5$ rec./ cell; $k_r^* = 0.0416$ min^{-1}; $k_r^* = 2.72 \times 10^{-3}$ min$^{-1} \cdot$ M^{-1}; $k_r^{x1} = 0.272$ min^{-1}; $k_f^* = 4.08 \times 10^5$ min$^{-1} \cdot$ M^{-1}; L_0 varies from 0.0008 to 1.5 μM.

receptors are replaced by newly synthesized receptors (Drake et al., 1989; Linderman and Lauffenburger, 1988), there is little effect on this model prediction. Assuming again that equal numbers of complexes produce equal amounts of stimulation, this equality not only suggests the validity of our model but also provides the estimate that 500 complexes on the cell surface are necessary to produce 50% T cell stimulation. Similarly, we can determine that the number of surface complexes needed to produce 100% T cell stimulation for either RME or FPP uptake mechanism is ∼2,300. We note that these estimates of surface complex number are specific to the system of Casten and Pierce.

Our estimates for the number of complexes on the B cell surface that produce 50 and 100% T cell stimulation, 500 and 2,300 complexes, correspond to complex surface densities of 0.55 and 2.55 complexes/μm², respectively. When we compared macrophages and B cells using FPP uptake earlier, we estimated that 5.0 complexes/μm² would give maximal T cell stimulation. This apparent inconsistency can be easily explained. The data of Fig. 5 show that T cell stimulation is saturable. The extracellular ligand concentrations that correspond to a saturated response in the system of Casten and Pierce can be determined from the data in Fig. 5: for RME uptake T cell stimulation saturates at ∼0.1 μM, while fluid phase uptake saturates at ∼4 μM. Extracellular ligand concentrations above these saturation values, such as the extracellular antigen concentration of 10 μM used for Fig. 3, may provide a greater number of Ia–Ag complexes on the surface of the APC, but these complexes do not appear

to contribute any further to T cell stimulation. Therefore, the density of complexes determined from Fig. 3, 5 complexes/ μm², is an overestimate of the complex density necessary for 100% stimulation of a T cell population, at least for this particular system. The number of complexes determined from a comparison of the data in Fig. 5 with our model predictions of Fig. 6 provides a better estimate on the number of complexes needed to produce maximal or 100% stimulation.

Our model predicts that the association constant for the formation of the receptor–ligand complex, k_f^*, is an important parameter in the determination of the Ia–Ag complex profile on the surface of the cell. Model predictions for the effect of k_f^* on complex expression are shown in Fig. 7. We first consider our results for low antigen concentration (∼0.0005 μM). As k_f^* and therefore the affinity K_a is increased, the APC is able to bind antigen at the cell surface more effectively, leading to an increase in intracellular antigen and therefore enhanced Ia–Ag complex expression. At intermediate antigen concentrations (∼0.002–0.5 μM), an increase in k_f^* does not necessarily increase complex expression: large k_f^* and therefore K_a values do not allow the receptor–antigen complex to remain dissociated inside the cell, preventing the production of peptide fragments and thus complex formation. In fact, our model predicts that the lowest of the three affinities shown, $K_a = 1.5 \times 10^7$ M^{-1}, will allow the greatest number of complexes to be expressed over much of this concentration range. This affinity is low enough to allow the RME mechanism to effectively shuttle
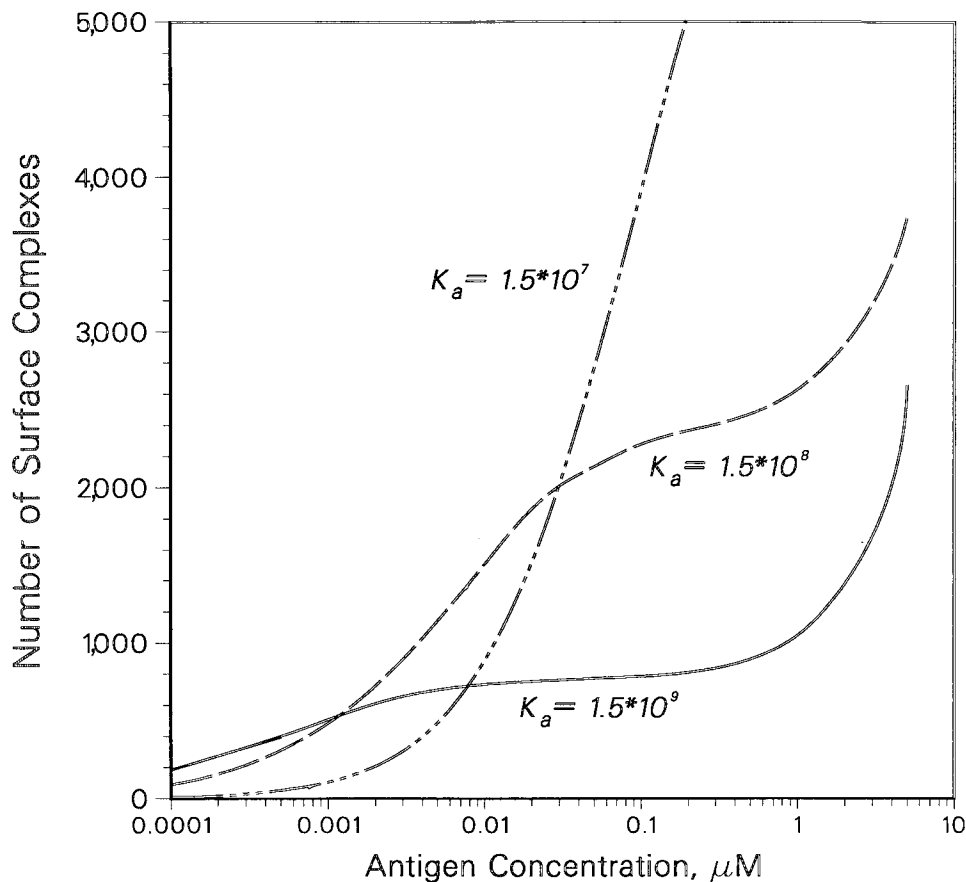
Figure 7. Number of Ia-Ag complexes as a function of time and the affinity of antigen for receptor. Our model was used to predict complex number for a pulse time of 8 h. The parameter values used are identical to those stated in Fig. 6 except for the forward rate constant, k_f^*, for receptor-antigen binding on the APC surface. k_f^* values of 4.08×10^6 $min^{-1} \cdot M^{-1}$, 4.08×10^5 $min^{-1} \cdot M^{-1}$, and 4.08×10^4 $min^{-1} \cdot M^{-1}$ were used with the k_r^* value of 0.00272 min^{-1} to give K_a values of 1.5×10^7, 1.5×10^8, and 1.5×10^9 M^{-1}, respectively and L_0, which varies from 0.0001 to 5.0 μM.

antigen into the cell. The more effective the shuttle, the more antigen deposited in intracellular vesicles, the more complexes formed, and the more similar the shape of the curve is to that for FPP. Finally, all three curves show a steep increase at the high extracellular ligand concentration of 3 μM. This can be attributed to the contribution from fluid phase internalization, which results in a sharp increase in the number of surface complexes produced at an extracellular ligand concentration of 3 μM (see Fig. 6).

Finally, we can use our model to compare surface complex expression on APC for two common types of experiments. In the pulse method, APC are incubated with antigen at 4°C until binding has reached equilibrium, antigen is then washed away, and the cells are subsequently warmed to 37°C for the duration of the processing period. In the continuous method, the focus of our earlier discussion, APC are incubated at 37°C in the continuous presence of antigen. As shown in Fig. 8, the pulse method shows a slight advantage in complex expression over most of the processing time for the low antigen concentrations of 0.01 μM, whereas at the higher antigen concentration of 1.0 μM the continuous method allows accumulation of a slightly greater number of surface complexes. Our results suggest that there is little difference in the final complex profile between these two methods of cell preparation.

Discussion

In this paper, we have formulated a mathematical model, shown schematically in Fig. 1, to describe the initial events

of antigen processing and presentation and to quantitatively predict the appearance of Ia-Ag complexes on the surface of an APC. Our analysis of these events involved a delineation of the reaction and transport kinetics occurring within and on the surface of an APC together with several assumptions. We have assumed, for example, that antigen processing occurs along the endocytic pathway and that free Ia molecules are internalized and delivered to endosomes together with antigen. In the endosome, degradation of antigen and binding of antigenic peptide fragments to Ia molecules occurs. Ia-Ag complexes are then recycled to the APC surface to be presented to a T cell. Use of the model required the estimation of a number of physiologic parameters from a variety of reported data, as shown in Table 1.

We tested the validity of our model predictions in two ways. First we showed that our model predicts that macrophages and B cells express equal Ia-Ag complex densities at ~60 min and 8 h, respectively. These predicted processing periods are in good agreement with the experimentally observed lag times for T cell stimulation (Ziegler and Unanue, 1981; Lakey et al., 1988). As a second test of our model, we compared presentation for antigens internalized via nonspecific fluid-phase uptake or receptor-mediated uptake. Our results show that receptor-mediated antigen ingestion produces an equivalent T cell response to that of nonspecific antigen uptake for much lower extracellular antigen concentrations, in quantitative agreement with the data of Casten and Pierce (Casten and Pierce, 1988), and qualitative agreement with the data of Snider and Segal (Snider and Segal, 1987).
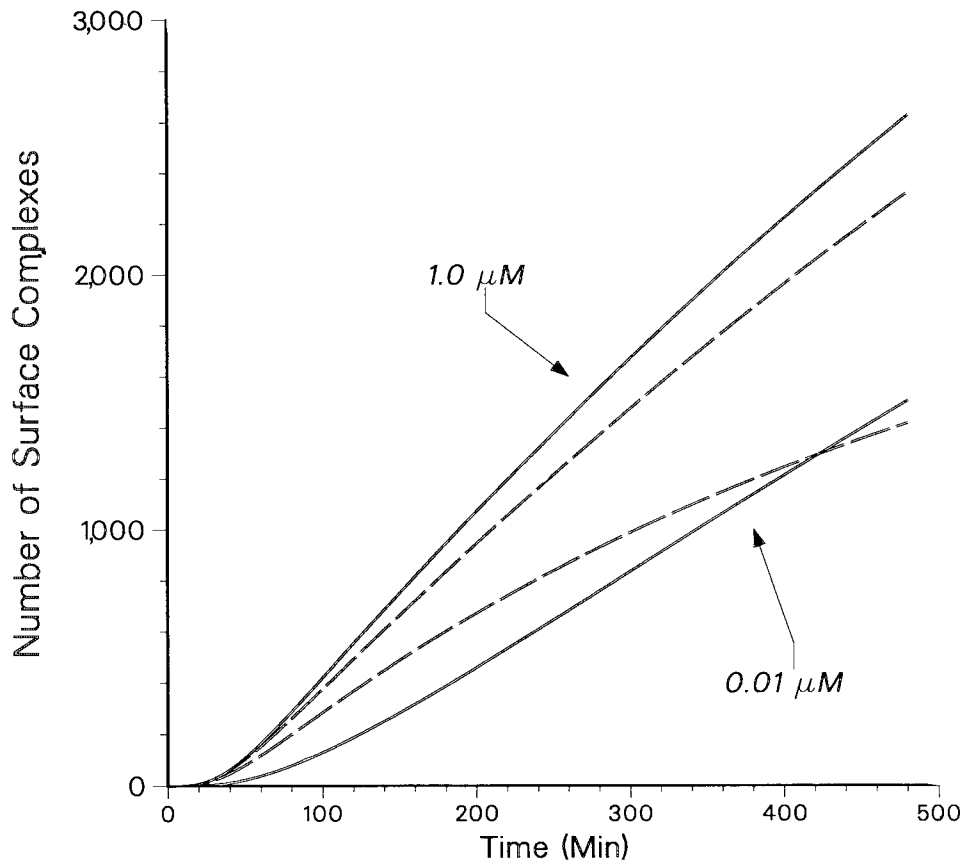
Figure 8. Ia–Ag complex profile as a function of the experimental assay: pulse or continuous method. The parameter values used are identical to those stated in Fig. 6. Top two curves correspond to an antigen concentration of L_0 = 1.0 μM and lower two curves correspond to an antigen concentration of L_0 = 0.01 μM. Solid lines represent a pulse experiment (defined in text) and dashed lines represent a continuous experiment.

Our mathematical model for antigen processing has allowed us to make quantitative predictions for the number of Ia–Ag complexes expressed on the surface of the APC. For some representative parameter ranges, these results have been plotted (Figs. 3 and 6). Significantly, we estimate that in the system of Casten and Pierce, B cell complex surface densities of 0.55 and 2.55 complexes/μm^2, corresponding to ~500 and 2,300 complexes/cell, result in 50 and 100% T cell stimulation. Although these particular results are specific to their system, the model can easily be applied to other systems as well. Our prediction for the number of complexes needed for T cell stimulation compares well with the approximations of other researchers (Lanzavecchia et al., 1988; Watts and McConnell, 1986).

Our model does not currently allow for the binding of endogenous or self peptides to Ia. Several researchers have suggested that foreign peptide may compete with self peptides for Ia binding (Cuchens and Buttke, 1984; Lorenz and Allen, 1988). This aspect of antigen processing can be added to the current model once more information is available about the affinities and concentrations of these peptides.

We assume in our model that antigen internalized bound to cell surface molecules or receptors such as Ig must first dissociate from these receptors before proteolytic degradation in the endosome can occur. Clearly, a portion of the antigen which falls outside the binding site of the receptor may be degraded while the receptor-antigen complex is still essentially intact (Watts and Davidson, 1988); however, this does not insure that the immunogenic peptide is included in

the peptide fragments produced. If the immunogenic peptide fragment is produced before receptor-antigen complex dissociation, our model would need to be modified to account for this fact. In addition, we assume here that each antigen molecule internalized can produce one antigenic peptide fragment. For large or polymeric antigens which contain more than one copy of an immunogenic peptide sequence, our model could be trivially modified.

The mathematical model we have presented here is testable. Variables such as the number of surface receptors, receptor-antigen affinity, concentration of extracellular antigen, and antigen processing time can be experimentally manipulated and the results of such experiments can be compared to model predictions. The techniques of Snider and Segal, who target antigen to a variety of cell surface molecules using antibody heteroconjugates, may be useful for such experiments (Snider and Segal, 1989). Surface expression of Ia–Ag complexes, one of the key quantities our model predicts, cannot currently be directly measured; however, indirect comparisons of our model predictions are still possible. For example, in this paper we have made the simple assumption that equal densities of Ia–Ag complexes on the surface of APC result in equal amounts of T cell stimulation. Although we realize that T cell stimulation is the result of a complex series of signals, involving not only the T cell receptor/Ia/peptide complex but a variety of adhesion molecules on the T cell and APC, recent evidence suggests that our approach is valid at least as a first approximation. T cell responses to antigenic peptides incubated with membranes

containing purified Ia have been measured and it has been found that a constant product of the concentration of Ia and the concentration of antigenic peptide fragments elicits a constant amount of IL 2 production, the T cell response measured (Krieger et al., 1988; Fox et al., 1987). If simple binding occurs between Ia and peptide, then the number of Ia–Ag complexes is a function only of this product of Ia and peptide concentrations and our assumption is supported. Certainly as more information about the production of T cell responses becomes available, this information can be used to give a more precise comparison of model predictions with experimental measures of T cell response. For example, Kupfer and Singer have recently shown a rapid redistribution of the TCR/CD3 complex into the contact area upon APC:T cell interaction. Future models will need to consider the translational diffusion of the Ia–Ag complex and the movement of the complex into the contact area.

Appendix

The mathematical formulation of our model of antigen processing and presentation, allowing antigen internalization via RME and/or FPP, is given by the following equations which describe the individual species concentrations or numbers as a function of time. The equations have been formulated to include the physical constraints given by Eqs. 2 and 3.

$$\frac{dR}{dt} = -k_f^* R L_0 + k_r^*(RL) + k_{-v}R^* \tag{A1}$$

$$\frac{d(RL)}{dt} = k_f^* R_{L0} - k_r^*(RL) - k_v^*(RL) + k_{-v}(R_0 - R - (RL) - R^*) \tag{A2}$$

$$\frac{dR^*}{dt} = -k_{-v}R^* + k_r^{*1}(R_0 - R - (RL) - R^*) - k_f^* R^* L^* \tag{A3}$$

$$\frac{dL^*}{dt} NV_vN_A = \frac{k_vV_v}{\sigma} L_0N_A + k_r^{*1}(R_0 - R - (RL) - R^*) - k_f^* R^* L^* - L^* V_v NN_A(k_d + k_p) \tag{A4}$$

$$\frac{dB}{dt} = k_v(A_0 - B - C - C^*) + k_rC - k_fBAg - k_{-v}B \tag{A5}$$

$$\frac{dC}{dt} = k_fBAg - k_rC - k_{-v}C \tag{A6}$$

$$\frac{dC^*}{dt} = k_{-v}C - k_{shed}C^* \tag{A7}$$

$$\frac{dAg}{dt} NV_vN_A = k_dL^*NV_vN_A + k_rC - k_fBA_g - k_pAgNV_vN_A \tag{A8}$$

The symbols used in the equations are as defined in the body of the test along with the following additions: σ = ratio of SA_v to SA_c; V_v = volume of an endosome, μm^3; N_A = Avogadro's number, molecules/mole; N = number of endosomes.

For the case of antigen internalization via FPP, the set of equations can be simplified by eliminating Eqs. A1, A2, and A3, the equations describing the receptor populations.

The set of coupled differential equations can be solved on most personal computers using numerical routines such as Gear or Runge-Kutta methods (Gear, 1971; Carnahan et. al., 1969).

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