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Antigen Processing and Presentation: How Can a Foreign Antigen be Recognized in a Sea of Self Proteins?

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A mathematical model describing the time-dependent events of antigen processing and presentation is utilized to quantitatively analyze the importance of newly synthesized Ia molecules as well as Ia molecules internalized from the cell surface in the formation of Ia–antigen complexes, the T cell receptor ligand. It has recently been shown that antigen presenting cells are not selective for the proteins they process and present. Therefore, we also investigate the ability of macrophages and B cells to process and present antigen in the presence of competing proteins often present in the extracellular environment. A set of criteria is formulated based upon experimental data to determine the validity of two model variations. We draw two major conclusions from our simulations. First, we determine that macrophages and B cells can present between 1–3 Ia–antigen complexes μm^{-2} for antigen concentrations in the range of 4–7 μM while in the presence of approximately 0–10 μM competing proteins or peptides. Second, we find it likely that antigen presenting cells, both B cells and macrophages, need to internalize Ia molecules from the cell surface in order for a sufficient number of Ia–antigen complexes to be presented. Binding of antigen to newly synthesized Ia alone does not, given experimentally reported values for Ia synthesis, allow sufficient Ia–antigen complex formation.

Introduction

The body's ability to mount a successful immune response to a variety of invading antigens is dependent on the ability of specialized cells, termed antigen presenting cells (APC), to process and subsequently present antigen prior to recognition by the responding immune cells. The responding immune cells, termed T helper lymphocytes (T cells), only respond to foreign antigen when it is presented on the surface of APC in the context of a genetic restriction molecule, MHC Class II (Ia) (Rosenthal & Shevach, 1973; Unanue & Cerottini, 1989). Antigen processing and presentation involves internalization of native antigen followed by the subsequent unfolding and/or degradation of this antigen. Once antigenic fragments of native antigen become available, binding can occur between peptide antigen and Ia. These complexes, denoted Ia–Ag, are then shuttled to the cell surface for presentation to T cells. (Allen, 1987; Chesnut & Grey, 1985; Allen *et al.*, 1987).

Although the cellular immune response is mediated by T cells, APC play a critical role in determining the extent to which antigen is presented for eventual stimulation. Experimental data have shown how various forms of foreign protein antigens are handled by two types of APC, macrophages and B cells (Ziegler & Unanue, 1981; Lakey *et al.*, 1988; Casten & Pierce, 1988). Yet in all these systems, the cellular

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assays are conducted in the presence of additional proteins. APC are unable to distinguish foreign protein or antigen from other proteins present in the extracellular environment as fluid phase internalization is non-specific (Schmidtke & Unanue, 1971) and MHC restriction is not strictly discriminatory (Rosloniec *et al.*, 1990). Whether the proteins are naturally occurring in the system, for example, proteins present in mouse serum used in the cultures of mouse cells (Lorenz *et al.*, 1990) or non-stimulatory analogs of foreign antigen (Adorini *et al.*, 1989), both represent competition to the effective presentation of stimulatory Ia–Ag complexes. Throughout this paper we will use the terms competing protein and self protein interchangeably to denote the non-antigenic protein and the terms foreign and antigenic protein to denote the stimulatory protein. The quantitative relationship between increasing doses of competing protein and the resultant decrease in stimulatory Ia–Ag complexes and therefore in T cell stimulation is not known.

In a related issue, for some time immunologists have disagreed over the role of newly synthesized Ia in the antigen processing pathway. Newly synthesized Ia have recently been shown to be present in endocytic vesicles (Guagliardi *et al.*, 1990; Cresswell, 1985). The presence of newly synthesized Ia in endocytic vesicles has led many to hypothesize that in order to minimize the competing effects of self peptides it is only the newly synthesized Ia which have the opportunity to bind to antigenic peptides (Guagliardi *et al.*, 1990). This argument assumes that once a peptide fragment is bound, be it self or foreign, the binding rate constants are such that the peptide in the Ia binding pocket is not likely to dissociate (Buus *et al.*, 1987; Babbitt *et al.*, 1985). Alternatively, experimental evidence has shown that peptide fragments can effectively compete with preformed Ia–Ag complexes in living cells when the competing peptide is administered hours after the initial dose of antigen (Adorini *et al.*, 1989). This has led some to suggest that Ia–Ag complexes are formed primarily from internalized surface Ia (Cresswell, 1990; Germain, 1986). This surface pool of Ia represents a large reservoir in comparison to newly synthesized Ia.

Our aim is to assess the effects of competing proteins as well as the role of Ia synthesis in antigen processing and presentation. Previously, we have formulated a mathematical model describing the events of antigen processing and presentation (Singer & Linderman, 1990). In this paper, we modify and extend our analysis to establish a quantitative relationship between antigen and competing protein doses and the number of Ia–Ag complexes expressed by APC. We will address the APC sensitivity to a range of competing protein doses and discuss the implications for suppression of an undesired immune response. In addition, we will assess the importance of newly synthesized Ia molecules in the pathway of Ia–Ag complex presentation.

Mathematical Model

Our model schematic for the events of antigen processing and presentation, which allows us to incorporate competing proteins and to examine the role of newly synthesized Ia molecules, is presented in Fig. 1; note that each of the steps has an

associated rate constant. Antigen, including foreign as well as competing proteins and peptides, is internalized via non-specific fluid phase uptake and is quickly delivered to an acidic vesicle, an endosome. The intraendosomal pH lies between 5·0 and 6·0 (Tycko & Maxfield, 1982; Murphy & Roederer, 1986), providing an optimal acidic environment for proteases such as Cathepsin D (Diment & Stahl, 1985; Ferguson *et al.*, 1973) to degrade a variety of protein antigens present in the vesicle. We assume that all proteins are degraded with the same first-order rate constant. Data from Ferguson show only minor differences in Cathepsin D activity on a variety of substrates (Ferguson *et al.*, 1973); this data compares well with the *in vivo* data of Diment & Stahl (1985). As peptide fragments of these protein antigens become available, binding can occur to form Ia–Ag complexes. Peptide antigens can also be taken up; these require no further processing before binding to Ia can occur. Any undegraded antigen or unbound peptides are assumed to be shuttled to the lysosome for further degradation, while all species of Ia molecules, including Ia–Ag complexes, are assumed to be cycled to the cell surface.

In the most general scheme, we allow Ia molecules to reach endosomes from two pathways: newly synthesized Ia are delivered to endosomes in order to intersect the exocytic pathway (Cresswell, 1985; Guagliardi *et al.*, 1990), and Ia are constitutively internalized from the cell surface (Salamero *et al.*, 1990; Harding & Unanue, 1989; Snider & Segal, 1989; Pernis, 1985). We are interested in quantitatively assessing the role of each pathway on the presentation of Ia–Ag complexes in order to determine the relative contribution of newly synthesized Ia molecules.

Following the schematic of Fig. 1, equations were written to describe the time-dependent accumulation of the species present in the system. We have constructed a model formalism which generalizes the system to allow uptake and processing of n protein species and m peptide species. In our model equations, we use the subscript i to refer to exogenous protein-derived species ($i = 1, 2, \ldots, n$) and subscript j to refer to exogenous peptide-derived species ($j = 1, 2, \ldots, m$). The dimensionless equations are shown below. In general, we write a differential equation for each species, including internalization, binding, synthesis, export, and degradation events as appropriate†. Definitions of the variables and dimensionless parameters are provided in Appendix A. For the Ia-containing species, including free Ia on the cell surface (a), Ia complexes on the cell surface derived from protein antigens (x_i), and Ia complexes on the cell surface derived from peptide antigens (x_j), intracellular free Ia (b), intracellular Ia complexes derived from protein antigens (c_i), and intracellular Ia complexes derived from peptide antigens (c_j), we write the dimensionless equations:

$$\frac{\mathrm{d}a}{\mathrm{d}\tau} = -K_v a + b + \rho\left[\sum_{i=1}^{n} x_i + \sum_{j=1}^{m} x_j\right] - \alpha a - \sum_{j=1}^{m} \varphi_j a \tag{1}$$

$$\frac{\mathrm{d}x_i}{\mathrm{d}\tau} = c_i - x_i(\alpha + \rho) \quad \text{for} \quad i = 1, 2, \ldots, n \tag{2}$$

† We have previously presented these equations in dimensional form for a one antigen scenario (Singer & Linderman, 1990).

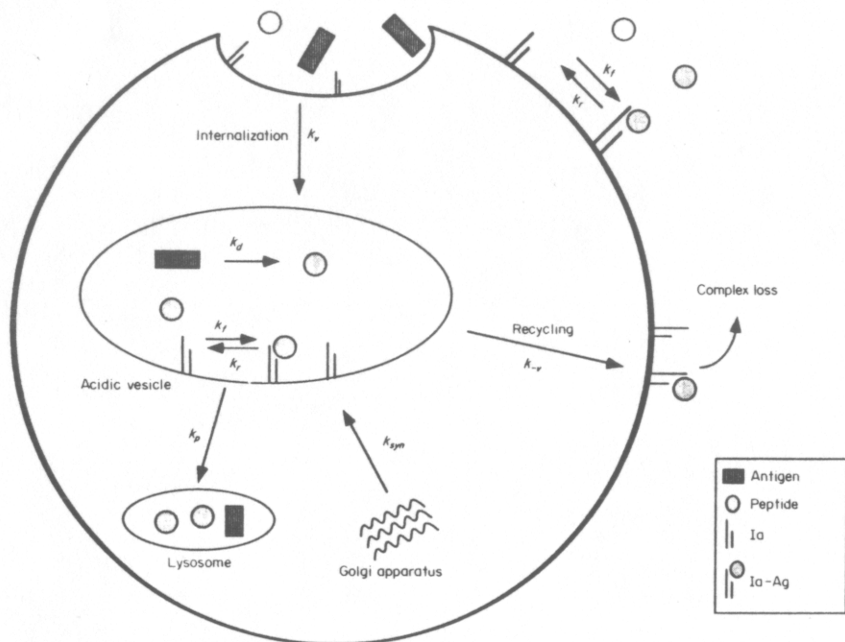

FIG. 1. Model schematic of antigen processing and presentation.

$$\frac{\mathrm{d}x_j}{\mathrm{d}\tau} = c_j - x_j(\alpha + \rho) + \varphi_j a \quad \text{for} \quad j = 1, 2, \ldots, m \tag{3}$$

$$\frac{\mathrm{d}b}{\mathrm{d}\tau} = K_v a - b + \rho\left[\sum_{i=1}^{n} c_i + \sum_{j=1}^{m} c_j\right] + \alpha\left[a + \sum_{i=1}^{n} x_i + \sum_{j=1}^{m} x_j\right] - b\left[\sum_{i=1}^{n} \varphi_i (ag)_i + \sum_{j=1}^{m} \varphi_j (ag)_j\right] \tag{4}$$

$$\frac{\mathrm{d}c_i}{\mathrm{d}\tau} = b \sum_{i=1}^{n} \varphi_i (ag)_i - c_i(\rho + 1) \quad \text{for} \quad i = 1, 2, \ldots, n \tag{5}$$

$$\frac{\mathrm{d}c_j}{\mathrm{d}\tau} = b \sum_{j=1}^{m} \varphi_j (ag)_j - c_j(\rho + 1) \quad \text{for} \quad j = 1, 2, \ldots, m. \tag{6}$$

The accumulation of peptide fragments derived from protein antigens $(ag)_i$ and peptide fragments derived from peptide antigens $(ag)_j$ are described, respectively, by:

$$\frac{\mathrm{d}(ag)_i}{\mathrm{d}\tau} = \delta l_i - \gamma_i \varphi_i b(ag)_i + \rho \gamma_i c_i - \Phi(ag)_i \quad \text{for} \quad i = 1, 2, \ldots, n \tag{7}$$

$$\frac{\mathrm{d}(ag)_j}{\mathrm{d}\tau} = \frac{K_v}{\sigma N} - \gamma_j \varphi_j b(ag)_j + \rho \gamma_j c_j - \Phi(ag)_j \quad \text{for} \quad j = 1, 2, \ldots, m. \tag{8}$$

Finally, the time-dependent intracellular accumulation of native proteins (l_i) in endosomes can be formulated as follows:

$$l_i = \frac{K_v}{\sigma N(\delta+\Phi)}[1-\exp(-(\delta+\Phi)\tau)] \quad \text{for} \quad i=1,2,\ldots,n. \tag{9}$$

Equation (9) represents an exact solution for l_i as a function of the dimensionless time, τ, and with the assumption that no protein is initially present inside endosomes. Note that the major quantity of interest, the number of Ia–Ag complexes present on the APC surface, is given by the solution of these equations for x_i, where x_i = number of cell surface Ia–Ag complexes derived from protein i/total number of Ia molecules.

In its present form, our model does not allow changes in the total number of Ia molecules in the system. Therefore, the synthesis rate corresponds exactly to the number of Ia that are eliminated from the cell surface via shedding and/or routing to degradation pathways. The sorting process inside the endosome allows all species of Ia to be recycled and all unbound antigen forms to be shuttled to the lysosome. This assumption provides a lower estimate for the number of Ia complexes than would be predicted if antigen is allowed to cycle to the exterior along with the Ia species, during which time additional binding could occur.

This set of nine equations describes the coupled interactions between Ia and any number of protein and peptide species taken up by the APC. Later in this paper, we will consider two cases of this general model framework: case 1 will simulate the interactions of an antigenic protein species and a competing protein species ($n=2$, $m=0$) within the APC; case 2 will simulate the interactions of an antigenic protein species and a competing peptide species within the APC in the presence of a serum protein ($n=2$, $m=1$). These simulations represent the most common experimental designs.

Values for model parameters have been estimated from literature data and presented previously (Singer & Linderman, 1990) with the following exceptions. In an early study, using Ia and peptide antigen free in solution and the method of equilibrium dialysis, Buus *et al.* (1987) obtained a value of 2 $(\text{M min})^{-1}$ for the forward association rate constant between Ia and peptide antigen. To obtain more physiologically relevant values, more recent assays have been performed in the presence of lipids to obtain a value of 330 $(\text{M min})^{-1}$ (Roof *et al.*, 1990) and using photoaffinity-labeled antigen binding to the surface of APC to obtain a value of 120 $(\text{M min})^{-1}$ (Phillips *et al.*, 1986). We use an intermediate value of 250 $(\text{M min})^{-1}$ for the forward association rate constant and have found that our conclusions are insensitive to the value of this rate constant over the reported range of values, 120–330 $(\text{M min})^{-1}$. Studies evaluating the rates of peptide–Ia association and dissociation have shown little sensitivity to the Ia haplotype or antigen under investigation for immunogenic systems (Buus *et al.*, 1987; Babbitt *et al.*, 1985). Thus, for most of our simulations, we will use identical binding rate constants for all Ia-binding species. Values for the synthesis rate constants for murine MHC Class II molecules have been measured and shown to be haplotype specific, I–A having a shorter half-life than I–E molecules (Emerson & Cone, 1979; Emerson *et al.*,

1980). Specific parameter values for each simulation are listed in the accompanying figure legends.

In order to address the role of newly synthesized Ia in antigen presentation, we modify the initial set of equations to eliminate any internalization of Ia from the cell surface by removing the term $K_v a$ from eqns (1) and (4). In other words, we allow APC to selectively omit Ia from entering the pinocytic vesicles. There is some evidence that cells are able to select specific surface molecules for internalization. For instance, T cells internalize MHC Class I and omit MHC Class II (Machy *et al.*, 1982; Aragnol *et al.*, 1986), and it has been shown that B cells which bind antigen to surface immunoglobulin selectively internalize only bound immunoglobulin (Drake, 1989). This version of the model is termed the *synthesis model.*

The mathematical model as initially proposed, eqns (1-9), incorporates both synthesis of new Ia, which are transported directly to endosomes, and cycling of free Ia from the APC surface to endosomes. Evidence for internalization of surface Ia has been shown for splenic B cells (Salamero *et al.*, 1990; Pernis, 1985), hybridoma cells and peritoneal macrophages (Harding & Unanue, 1989). Ia–Ag complexes can thus be formed from a larger pool of Ia molecules than in the synthesis model, for both internalization and synthesis deliver Ia molecules to endosomes. This variation of the model is termed the *synthesis/cycle model.*

CRITERIA FOR A SUCCESSFUL MODEL

The goal of our analysis of antigen processing and presentation is to assess APC ability to efficiently present a particular antigen when other proteins and peptides are present and can compete for Ia binding as well as to address the importance of newly synthesized Ia in the process of presenting antigen. In order to ascertain the most appropriate of the two models, the synthesis or the synthesis/cycle model, a set of criteria is established to address these issues. We then select the model which most successfully satisfies this set of criteria.

Criterion 1

Two of the most common types of APC are macrophages and B cells. It has been shown experimentally that macrophages require approximately 60 min of antigen processing for effective antigen presentation (Ziegler & Unanue, 1981), while B cells require approximately 8 hr of antigen processing to obtain the same level of T cell stimulation (Lakey *et al.*, 1988) for approximately the same amount of protein antigen (4–7 μM). These processing times have been shown for a range of antigens (Diment, 1990; Jensen, 1988; Gosselin *et al.*, 1988; Ziegler & Unanue, 1981). We will only be comparing cases in which the same antigen is administered. Previously, we correlated T cell stimulation with the complex density of Ia–Ag on the surface of APC, $(x_{i=1}A_0/SA_c)$ (Singer & Linderman, 1990). In doing so, we assume that the T cell surface will form a finite contact area with the APC surface which will be approximately equal regardless of the APC type. Thus, the number of T cell receptor bonds formed is initially a function of complex density rather than total number of surface complexes, and we assume that to obtain the same degree of T cell stimulation equal complex densities among APC are required. In evaluating

each of the proposed models, then, the first criterion to be satisfied is that macrophages and B cells acquire the same complex density of Ia–Ag in the required processing time, approximately 1 and 8 hr, respectively, over the same range of antigen concentrations (4–7 μM).

Criterion 2

To date few quantitative measures of Ia–Ag complexes on the APC surface have been reported. Previously, researchers have gained insight into the number of Ia–Ag complexes through functional assays, such as the T cell proliferative response. By preincubating antigen specific for surface immunoglobulin with B cell APC and thereby quantitating the total amount of antigen possibly available for presentation, Lanzavecchia *et al.*, predicted that as few as 1000 complexes are needed to stimulate a response (Lanzavecchia *et al.*, 1988). Demotz *et al.* and Harding & Unanue have recently shown in whole cell assays that to elicit a *threshold* level T cell response approximately 300 Ia–Ag complexes are present on the APC (Demotz *et al.*, 1990; Harding & Unanue, 1990). One can also derive from these data that approximately 110–2700 stimulatory Ia–Ag complexes on the APC surface are needed to elicit a *maximal* T cell response. We are interested here in the maximal response. For typically sized B cells with surface area between 300–1000 μm^2, this corresponds to a complex density between 1 and 3 Ia–Ag μm^{-2}. A quantitatively similar prediction of the number of complexes necessary for T cell stimulation can be obtained from the experiments of Watts & McConnell, in which T cells were stimulated by antigen bound to purified Ia embedded in planar membranes (Watts & McConnell, 1986). Thus, criterion 2 will require that for each model the Ia–Ag complex density ranges between 1–3 Ia–Ag μm^{-2} between 4–7 μM of foreign antigen.

Criterion 3

The suggestion that self peptides may occupy the binding pocket of Ia molecules was made during the purification of Ia molecules, when it was found that only a small percentage of purified Ia molecules were able to bind to peptides (Buus *et al.*, 1988). A simple explanation would have been that Ia were no longer conformationally viable for participating in the reaction. However, further investigation showed that a small molecular weight peptide could be acid-washed from the Ia preparation and this accounted for the observed Ia inactivity. Buus *et al.* (1988) then determined that for their A20 cells, a B cell lymphoma, 70% of the Ia molecules were bound to self peptides at the time of purification. It is likely that the primary source of these self peptides is the cell culture medium containing 10% fetal calf serum (FCS), corresponding to a protein concentration of approximately 110 μM (Lorenz *et al.*, 1990).

A similar measurement is not available for macrophages; however, Harding & Unanue have investigated the equilibrium distribution of Ia in peritoneal macrophages (Harding & Unanue, 1989). At concentrations of self protein which are present *in situ* (~3 μM), macrophages distribute their Ia with 25–40% intracellular.

As a third model criterion, then, we require that at steady-state prior to the addition of antigenic protein or peptide, A20 cells cultured in the presence of 110 μM

self protein have approximately 70% of Ia complexed with peptide fragments of this protein and macrophage cultured in the presence of 3 μM protein have approximately 25–40% of their Ia in an intracellular location.

Criterion 4

In order to determine the physiological concentration of competing protein *in situ*, a number of approaches have been taken. For instance, Lorenz & Allen have devised a T cell assay in which a naturally occurring protein, hemoglobin, is identified by a specific T cell clone when presented in the context of Ia on the surface of macrophage APC (Lorenz & Allen, 1988). It was found by an *in vitro* comparison that the concentration of self protein *in situ* is approximately 3·0 μM. This finding led Lorenz & Allen to investigate the effects of protein present in serum. At serum levels commonly used to culture cells, 10%, the decrease in the T cell response as compared to 0% serum is greater than 50% (Lorenz *et al.*, 1990).

In a related observation, Adorini *et al.*, have shown a decrease in T cell stimulation with increasing concentrations of a competing peptide in a B cell hybridoma APC incubation media. Fifty percent inhibition of the control response to 7 μM whole hen egg lysozyme is achieved with 9 μM of a non-stimulatory peptide analog as the competing peptide (Adorini *et al.*, 1989). Criterion 4 is an assessment of each model's validity based on a comparison to these literature findings.

Results

SYNTHESIS MODEL

The results of simulations with the synthesis model, the model allowing only newly synthesized Ia to reach endosomes and bind peptides, are shown in Fig. 2(a)–(d). In these figures, we plot stimulatory Ia–Ag complex density ($x_{i=1}A_0/SA_c$) as a function of protein antigen concentration. Our initial condition for these simulations is that no foreign antigen is present and that the APC has been exposed to the competing protein for enough time to achieve steady-state values of all variables. The synthesis model is solved analytically for these steady-state values. The analytical solution is given in Appendix B. These initial conditions seem most appropriate in order to simulate *in vivo* behavior as well as cultured cell behavior in which the cell is constantly exposed to exogenous proteins present in the media. Each simulation is run for antigen concentrations in the range of 0·1–10·0 μM and competing protein concentrations between 0·01–5·0 μM, concentrations typically used in competition assays. Furthermore, B cell and macrophage simulations are terminated after 8 hr and 60 min, respectively, the reported processing times for maximal T cell stimulation discussed earlier. We use a representative Ia synthesis rate corresponding to the measured I-E synthesis rate ($\alpha = 0{\cdot}0144$; Emerson & Cone, 1979; Emerson *et al.*, 1980) and, for comparison, an elevated synthesis rate ($\alpha = 0{\cdot}115$) corresponding to an Ia half-life of 2 hr. With the exception of the internalization rate constant, k_v, and the cell surface area, SA_c, all parameter values are equal for the two cell types. Macrophages exhibit a faster rate of fluid phase internalization (Swanson *et al.*, 1985; Chesnut *et al.*, 1982; Goud *et al.*, 1984;

Goldmacher *et al.*, 1986) and have a larger surface area than B cells. An examination of simulation results suggest that both the macrophage and B cell are quite insensitive to the range of competing protein tested.

To assess the feasibility of the results of the synthesis model just presented, we proceed through the criteria. Criterion 1 states that macrophages and B cells must exhibit the same complex density for similar ligand concentrations. Over the range of 4·0–7·0 μM, antigen concentrations which typically provide maximal T cell stimulation for fluid phase uptake of antigen, similar complex densities only occur for the larger value of α. For example, for the synthesis rate constant $\alpha = 0{\cdot}0144$, macrophages exhibit an average complex density of 0·15 Ia–Ag μm^{-2}, while B cells exhibit an average complex density of 0·30 Ia–Ag μm^{-2} over the range of interest of antigen concentrations. Criterion 2 suggests the model should predict a complex density between 1–3 Ia–Ag μm^{-2}. As can be seen from Fig. 2, maximum complex densities of 0·4 and 1·2 Ia–Ag μm^{-2} are predicted for B cells and 0·2 and 1·0 Ia–Ag μm^{-2} for macrophages for synthesis rate constants $\alpha = 0{\cdot}0144$ and 0·115, respectively. Thus, our simulations for the synthesis model are only able to satisfy criteria 1 and 2 at an elevated, non-physiological value of the synthesis rate constant.

In order to satisfy the B cell APC portion of criterion 3, parameter values for A20 cells must be utilized to determine the steady-state portion of Ia species which are complexed in the presence of 110 μM competing protein, representing 10% FCS in normal cell culture media. For A20 cells, we have measured a cell surface area of approximately 2600 μm^2 using flow cytometric techniques and an average internalization rate constant of 0·0092 min^{-1} using the radiolabeled probe inulin as a fluid phase marker (data not shown). A20 cells have I–A^d molecules on their cell surface, which exhibit a dimensionless synthesis rate constant of 0·0385 (Emerson & Cone, 1979; Emerson *et al.*, 1980). All other parameter values are assumed to remain the same as in our previous simulations of B cell behavior. Simulations predict that at steady-state in the presence of 110 μM competing protein A20 cells will have approximately 37% of their Ia molecules in a complexed form with a peptide fragment from the protein. This is one-half of the experimentally observed value.

The macrophage portion of criterion 3 applies to the steady-state distribution of Ia in the cell population. Literature data suggest a physiological self protein concentration of approximately 3·0 μM (Lorenz & Allen, 1988) for macrophage APC. Examination of the steady-state distribution of macrophage Ia for the synthesis model at a self protein concentration of 3·0 μM shows that 2% and 10% of the Ia are inside of the APC at the low and high values of α, respectively. Again, this is significantly less than the experimentally observed value and suggests that this model does not meet criterion 3.

Criterion 4 allows a direct comparison to literature data in order to determine the effects of physiological ranges of self protein concentrations as well as the validity of the synthesis model's predictions. Thus far, the synthesis model has not been able to satisfy criteria 1–3 and we will only briefly comment on the other evidence. The data of Adorini *et al.* (1989) and Lorenz *et al.* (1990) have shown that both macrophages and B cells are sensitive to changes of competing protein

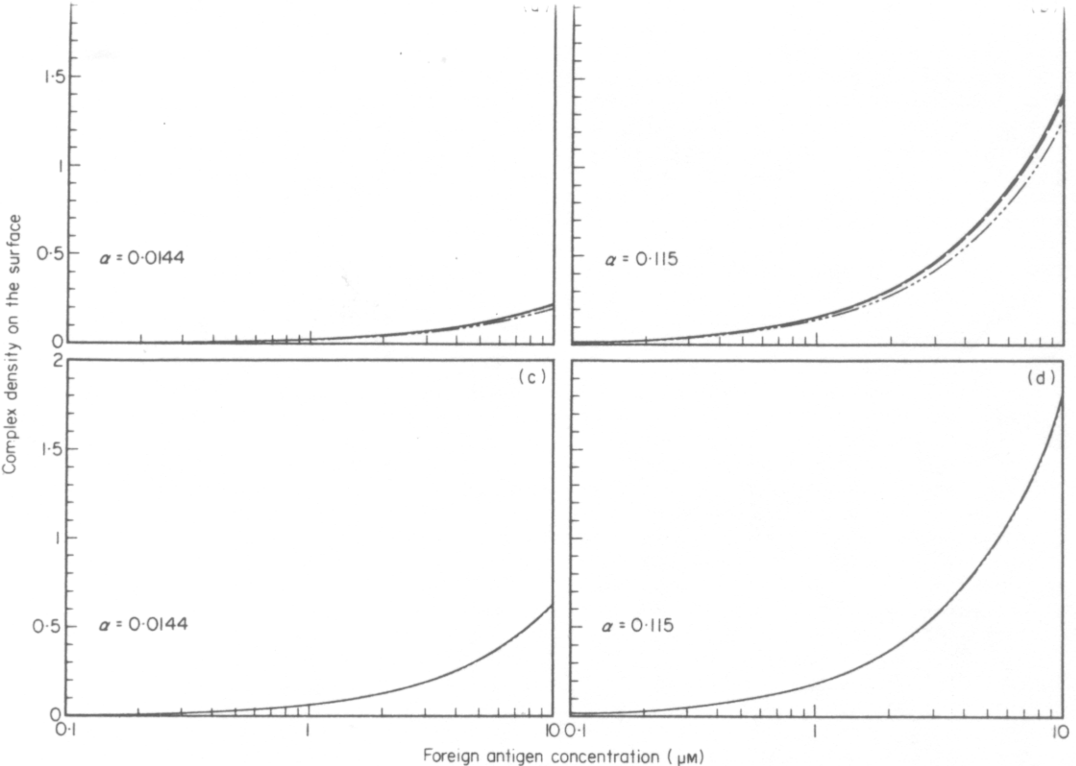

FIG. 2. Stimulatory Ia–Ag complex density ($x_{i=1}A_0/SA_c$) in complexes μm^{-2} as a function of protein antigen concentration for macrophage and B cell APC using the synthesis model. (a) and (b) are for macrophage APC and (c) and (d) are for B cell APC. The following parameters are identical for both cell types: $\rho = 0{\cdot}0084$; $\delta = 0{\cdot}24$; $\phi = 2{\cdot}10$; $N = 10{\cdot}0$ vesicles cell^{-1}; $\varphi_i = 5000 L_{0i}$; $\gamma_i = 4{\cdot}6 \times 10^{-4}/L_{0i}$. The APC specific parameter values are: $K_v = 0{\cdot}84$; $\sigma = 6{\cdot}9 \times 10^{-4}$; $\tau = 3{\cdot}0$ for macrophage APC and $K_v = 0{\cdot}15$; $\sigma = 0{\cdot}0017$; $\tau = 24{\cdot}0$ for B cell APC. (a) and (c) Have $\alpha = 0{\cdot}0144$ and (b) and (d) have $\alpha = 0{\cdot}115$. Competing protein concentrations are shown as (——) for 0·01, (- - -) for 1·0, and (- ···· -) for 5·0 μM. In (c) and (d), these curves fall on top of each other

concentrations over the range of 0·1–10·0 μM. As previously stated and shown in Fig. 2, the synthesis model shows essentially no sensitivity over this range of competing protein. In summary, then, the synthesis model does not satisfy the criteria.

SYNTHESIS/CYCLE MODEL

We now return to the synthesis/cycle model, which allows Ia to reach endosomes following internalization from the APC surface as well as by direct transport of newly synthesized molecules to endosomes. As in the synthesis model, our initial conditions assume that APC are at steady-state with the competing protein prior to encountering foreign antigen. This initial condition is given explicitly in Appendix B. We examine an extended range of competing protein concentrations, 0·01–20·0 μM, two physiological Ia synthesis rates corresponding to I–A and I–E synthesis, and the range 0·1–10·0 μM of antigen concentrations for both macrophage and B cell APC. As stated earlier, for each simulation macrophages and B cells are allowed a processing of time 60 min and 8 hr, respectively.

The results of the synthesis/cycle model simulations are shown in Fig. 3(a)–(d). Figure 3(a) and (b) presents simulations for macrophage APC and Fig. 3(c) and (d) presents simulations for B cell APC. The synthesis rate constant α is equal to 0·0144 [Fig. 3(a) and (c)] and 0·0385 [Fig. 3(b) and (d)] for I–A and I–E, respectively. As in the case of the synthesis model, we proceed through the developed criteria to determine the feasibility of this model in explaining the experimentally observed behavior of antigen processing and presentation. In general, our simulations show that macrophage APC are more sensitive to changes in self protein concentration than are B cell APC in the range of self protein concentrations tested. For example, Fig. 3(a) shows a decrease in complex density from 5·7–0·6 Ia–Ag μm^{-2} for macrophage APC and Fig. 3(c) shows a decrease in complex density from 5·4–4·4 Ia–Ag μm^{-2} for B cell APC at an antigen concentration of 10 μM as the extracellular self protein concentration increases from 0·01–20·0 μM.

Criteria 1 and 2 are easily satisfied by the synthesis/cycle model for both APC types. As shown in Fig. 3(a)–(d), macrophages and B cells can achieve comparable complex densities of 1–3 Ia–Ag μm^{-2} for antigen concentrations between 4·0–7·0 μM in the requisite processing times. The range of competing protein for which criterion 2 is met varies with APC type. Macrophages can maintain the required complex density for concentrations of competing protein less than approximately 10·0 μM, while the B cell can withstand at least 20·0 μM of competing protein.

The synthesis/cycle model is able to satisfy criterion 3. First, steady-state conditions for A20 cells in the presence of 110 μM of self protein for the synthesis/cycle model result in 74% of the Ia molecules being complexed, in good agreement with reported value of 70%. To apply the second portion of criterion 3, we examine the steady-state distribution of Ia molecules in peritoneal macrophages exposed to self proteins. As Lorenz & Allen have determined an *in situ* self protein concentration of approximately 3·0 μM for peritoneal macrophages (Lorenz & Allen, 1988), we look at the distribution of Ia molecules over the range of 1·0–5·0 μM of self protein.

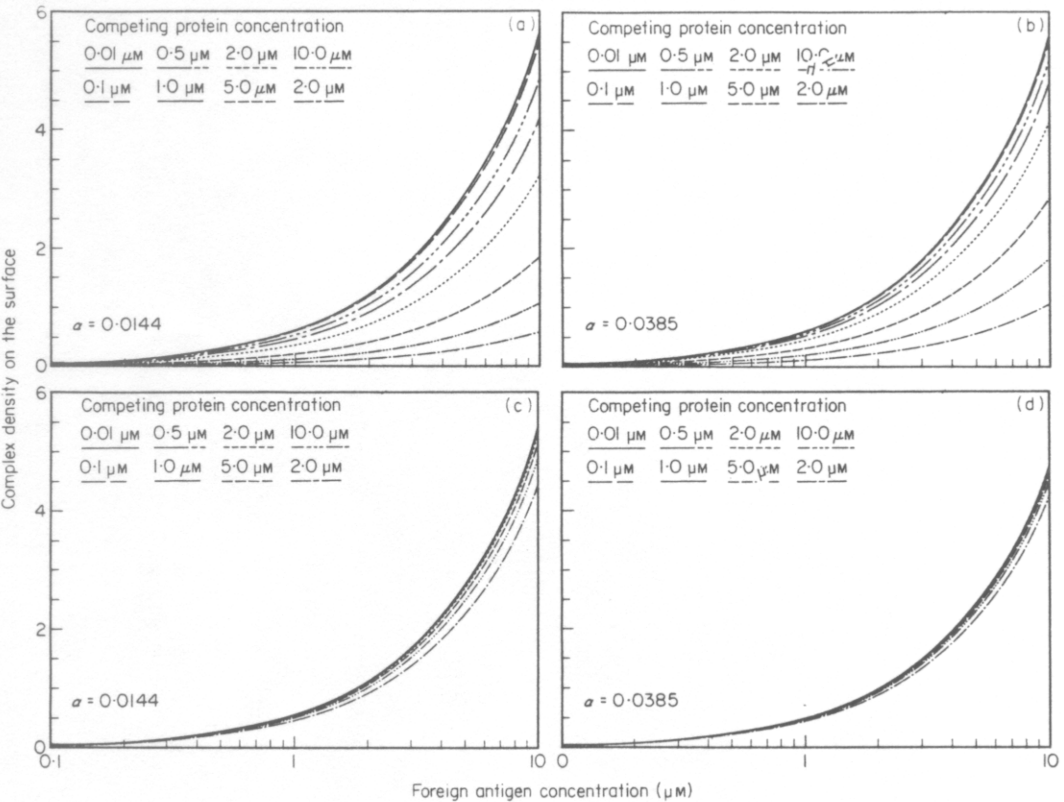

FIG. 3. Stimulatory Ia–Ag complex density ($x_{i=1}A_0/SA_c$) in complexes μm^{-2} as a function of protein antigen concentration for macrophage and B cell APC using the synthesis/cycle model. (a) and (b) Are for macrophage APC and (c) and (d) are for B cell APC. The parameter values are as stated for Fig. 2 except for the values of α. (a) and (c) Have $\alpha = 0{\cdot}0144$ and (b) and (d) have $\alpha = 0{\cdot}0385$. Competing protein concentrations are 0·01, 0·05, 0·1, 1·0, 2·0, 5·0, 10·0, 20·0 μM, as shown in the figures.

For Ia molecules which are I–A, with a synthesis rate constant of 0·0385, macrophages distribute 39% and 23% of their Ia intracellularly at 1·0 and 5·0 μM of competing protein, respectively. This is in good agreement with the observed range of 25–40%.

Our final test of the validity of the synthesis/cycle model in describing the time-dependent behavior of APC, criterion 4, applies when antigen must compete with other protein molecules for binding to Ia molecules. Lorenz *et al.* (1990) addressed the sensitivity of APC to changes in self protein levels by adjusting the percentage of serum in their assay medium. In Fig. 4 simulation results showing complex density as a function of the concentration of competing protein for a fixed antigen concentration of 1·0 μM are shown for macrophage APC. Our simulations are consistent with Lorenz & Allen's data, which show a 50% inhibition of the T cell response at a competing protein concentration of 5·0 μM as compared to the response with no competing protein present.

Finally, Adorini *et al.* (1990) have done a series of assays in which they incubate B cells with foreign antigen and competing peptide together in the presence of 10% FCS. Our model is easily manipulated to simulate this assay by setting $n = 2$ and $m = 1$. In this case, we assume our initial conditions to be the steady-state for cells cultured in 10% FCS. B cell parameters are used; the Ia synthesis rate constant corresponding to I–A is chosen in order to describe the cell phenotype used by Adorini *et al.* In order to simulate the data of Adorini *et al.*, it is necessary to set

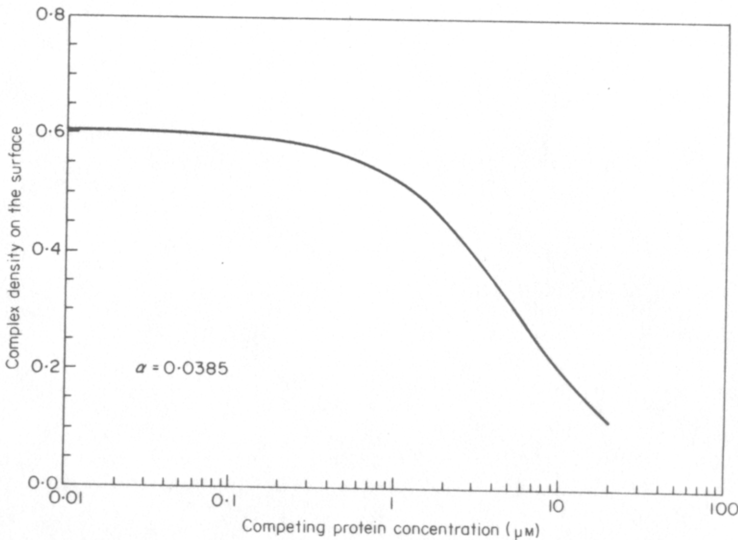

FIG. 4. Stimulatory Ia–Ag complex density ($x_{i=1}A_0/SA_c$) in complexes μm^{-2} for macrophage APC as a function of competing protein concentration for a fixed foreign antigen concentration of 1·0 μM. All parameter values are the same as stated for macrophages in Fig. 2 except that $\alpha = 0·0385$.

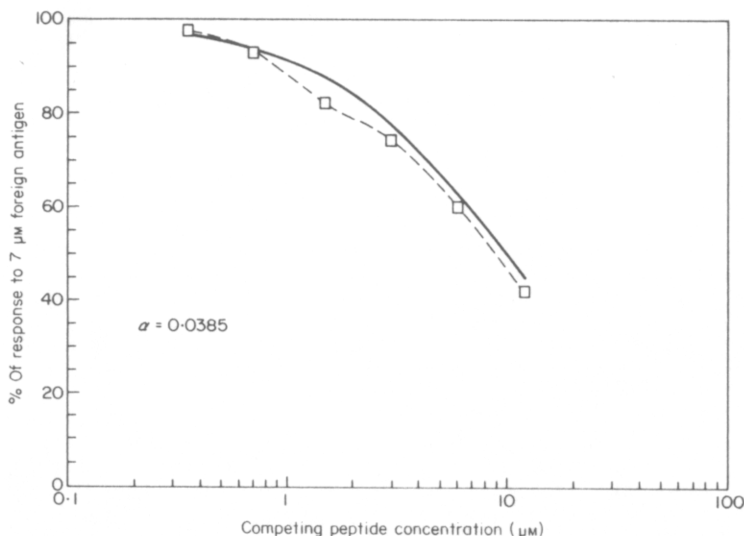

FIG. 5. Percent of response to protein antigen in the presence of 10% FCS (110 μM) and competing peptide. The (□) symbols represent the data of Adorini and co-workers (Adorini *et al.*, 1989) and the solid line represents our simulated response. The parameter values for the protein species are the same as for B cells in Fig. 2 except that $\alpha = 0{\cdot}0385$ and for the peptide species $\varphi_j = 1{\cdot}0\times10^4 Ag_{0j}$ and $\gamma_j = 4{\cdot}6\times10^{-4}/Ag_{0j}$.

the forward association rate constant between competing peptide and Ia to approximately twice that for the antigenic fragment and Ia. We note that the exact experimental values of these rate constants are not known. Our results are presented in Fig. 5. We use the predicted complex density in the presence of no competing peptide as a measure of 100% stimulation. Shown are Adorini's results for simultaneous addition of competing peptide and antigenic protein as the square points and our simulation as the smooth curve. As can be seen in the figure, our results indicate a strong correlation between the experimentally measured T cell response and our prediction of the number of Ia–Ag complexes on the surface of APC. Furthermore, our simulations show that APC can effectively present Ia–Ag complexes even if the competing protein has a kinetic advantage. The successful comparison of our model predictions to the Lorenz & Allen data and the Adorini data indicates that the synthesis/cycle model satisfies criterion 4.

Discussion

We are able to quantitatively analyze two distinct models by examining each model's ability to meet a set of criteria we formulate and base upon experimental evidence. Our set of criteria consider conditions such as APC type, reported antigen processing times, complex density, and steady-state Ia distribution. We draw two major conclusions from our results.

First, given experimentally determined parameters for the various transport and reaction steps occurring during antigen processing and presentation as well as rates for Ia synthesis, we show that it is likely that APC, both B cells and macrophages, need to cycle their Ia molecules from the cell surface. As described by the synthesis/cycle model, internalized surface Ia are transported to endosomes where Ia–Ag binding occurs and are then transported back to the cell surface for presentation. Newly synthesized Ia molecules contribute only 1–20% of the total number of Ia–Ag complexes as the concentration of self protein varies from 0·01–5·0 μM. Allowing only newly synthesized Ia to participate in Ia–Ag complex formation, as described by the synthesis model, did not satisfy our set of criteria given physiological parameter values for the cell types under consideration. The measured rates of Ia synthesis are simply too low to provide substantial opportunity for antigen binding and thus antigen presentation. This low measured rate of Ia synthesis is supported by the data of Harding and co-workers, who have shown that the total number of Ia is unaffected by the protein synthesis inhibitor cyclohexamide under their experimental conditions (Harding & Unanue, 1989; Harding *et al.*, 1990). Given a novel APC which exhibits an increased rate of Ia synthesis or an APC in which the loss of functional Ia is significantly slower than the rate of synthesis of new Ia, the results for the synthesis model may very well differ. In most cases, however, we argue that Ia cycling is necessary for sufficient Ia–Ag complex presentation.

Experimental determination of the rate of Ia internalization can be deceiving. The rate of MHC Class II internalization is often measured in comparison to receptor molecules such as transferrin (Guagliardi *et al.*, 1990). The transferrin receptor enters the cell through receptor-mediated endocytosis, but MHC Class II molecules are believed to be internalized by default as the cell membrane invaginates during fluid phase pinocytosis. It can be shown that the constitutive uptake of cell surface molecules is very slow in comparison to receptor-mediated endocytosis, in which cell surface molecules are typically concentrated in coated pits prior to internalization. Guagliardi *et al.* (1990) have measured an internalization rate constant of $0{\cdot}15\ \mathrm{min}^{-1}$ and a half-time of 5 min for the transferrin receptor and an internalization rate constant of approximately $0{\cdot}004\ \mathrm{min}^{-1}$ and a half-time of approximately 170 min for the MHC Class II (Ia) molecule in IM-9 cells, a human tumor cell line. Although the rate constant for MHC Class II internalization is almost negligible in comparison to the transferrin receptor, it is of the same order of magnitude as the rate constant for Ia internalization used in our simmulations of B cell APC, $0{\cdot}0075\ \mathrm{min}^{-1}$. Significantly, we have shown that the rate constant for Ia internalization need not be very large to satisfy model criteria with the synthesis/cycle model.

In this paper, we address the specific cases of a protein antigen competing with one self protein and a protein antigen competing with both a serum protein and a non-antigenic peptide. Our framework, however, is extremely general and can describe the interactions of many proteins and peptides in the same system. The second major conclusion that can be drawn from our simulations is that both macrophages and B cells are easily able to present between 1–3 Ia–Ag complexes $\mu\mathrm{m}^{-2}$ for antigen concentrations in the range of 4–7 μM while in the presence of approximately 0–10 μM competing proteins or peptides. It has been reported that there are approximately 110 000 Ia molecules on a B cell or macrophage surface

(Lakey *et al.*, 1988; Demotz *et al.*, 1990). Thus, the requirement of a surface Ia–Ag complex density of 1–3 Ia–Ag μm^{-2} means that only 2·0% and 4·0% of B cell and macrophage surface Ia molecules, respectively, are complexed with antigen. Our simulations show that competition from self antigens is unlikely to significantly diminish the ability of the APC to present foreign antigens. Our simulations can also be interpreted as though 10 μM of competing protein is actually composed of two additional antigens at concentrations of 5 μM each. In this scenario each of the antigen species would be presented at the complex density necessary to elicit a significant T cell response. This seems an optimal means of utilizing the available Ia on an APC to present a large number of antigen species.

The synthesis/cycle model shows that the macrophage APC is more sensitive to self protein than the B cell APC. This prediction has not yet been experimentally tested, although Lorenz & Allen have shown significant sensitivity of macrophages to competing protein (Lorenz & Allen, 1988). As previously shown, the difference in processing times between the APC types is due to the difference in the internalization rate constant for antigen and Ia (Singer & Linderman, 1990); macrophages have greater internalization rate constants. The faster the Ia are internalized, the more quickly they are returned to the cell surface where they may be shed or routed to degradation pathways. Therefore, macrophages can withstand less competing protein due to the balance of lost Ia species to newly synthesized Ia molecules. This sensitivity is directly related to our assumption of a constant number of Ia in the APC for all times. There is evidence that macrophages and dendritic cells, an additional type of APC, can regulate the number of Ia in response to lymphokines such as γ-interferon, which is secreted by activated T cells (Adams & Hamilton, 1987; Borashi *et al.*, 1979). Our model assumption is valid for the assay system in which the APC are prepulsed with antigen before incubation with T cells. In such a system, the macrophage may increase the number of Ia on the surface of its cell but only after contact with the T cell, which we do not consider in this simulation.

Our conclusions are based on the use of our model with parameter values derived from reported experimental data. We do not consider significant variations in the antigen degradation rate constant, k_d, and the Ia–Ag binding and dissociation rate constants, k_f and k_r. Our mathematical description of the events of antigen processing and presentation allows us to analyze cases in which the antigen must be thought to have a disadvantage due to presence of self proteins, which bind to Ia molecules at least as well as immunogenic antigen. Surprisingly, our results show antigen is clearly able to be presented under the conditions we investigate. Although we are not aware of any data which suggest significant differences in the binding rate constants between peptides which bind a specific Ia haplotype (Babbitt *et al.*, 1985; Buus *et al.*, 1987), more thorough experimental investigation into these parameter values is certainly warranted.

Further, we note that only one estimate of the self protein concentration *in vivo*, 3 μM (Lorenz & Allen, 1988), has been reported. This number is based on an assay in which a reactive T cell is used to titrate the amount of naturally presented protein. Clearly, more thorough experimental investigation needs to be made to determine the range of self-protein concentration found within the body. In addition, we note that the concentration of antigen *in situ* varies over the course of an immune

response, and this variation should be investigated as well before our model can be used confidently for *in vivo* as well as *in vitro* predictions.

Our model allows the *a priori* determination of the ability of a competing protein or peptide to inhibit an immune response based upon the knowledge of a number of parameters, such as binding constants and the rate of protein internalization. Such knowledge may have application in the treatment of disease. For example, in the case of an autoimmune disorder, a non-immunogenic protein analog could be administered that would compete with the autologous stimuli. If administered at the proper dose, i.e. greater than 10 μM as shown for macrophage APC, the number of antigenic complexes could be sufficiently decreased so as to prevent the stimulation of autoreactive T cells. Thus, our mathematical modeling can be used to make quantitative predictions as to methods for manipulating the immune response at the level of antigen processing and presentation.

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REFERENCES

ADAMS, D. O. & HAMILTON, T. A. (1987). Molecular transductional mechanisms by which IFNγ and other signals regulate macrophage development. *Immunol. Rev.* **97,** 5–27.

ADORINI, L., APPELLA, E., DORIA, G., CARDINAUX, F. & NAGY, Z. A. (1989). Competition for antigen presentation in living cells involves exchange of peptides bound by Class II MHC molecules. *Nature, Lond.* **342,** 800–803.

ALLEN, P. M. (1987). Antigen processing at the molecular level. *Immunol. Today* **8,** 270–273.

ALLEN, P. M., BABBIT, B. P. & UNANUE, E. R. (1987). T-cell recognition of lysozyme: the biochemical basis of presentation. *Immunol. Rev.* **98,** 170–187.

ARAGNOL, D., MALISSEN, B., SCHOFF, C., PIRON, M. & LESERMAN, L. D. (1986). Endocytosis of MHC molecules by B-cell-B lymphoma and B-cell-T lymphoma hybrids. *J. Immunol.* **137,** 3347–3353.

BABBIT, B. P., ALLEN, P. M., MATSUEDA, G., HABER, E. & UNANUE, E. R. (1985). Binding of immunogenic peptides to Ia histocompatibility molecules. *Nature, Lond.* **317,** 359–361.

BORASHI, D., PASQUALETTO, E., GHEZZI, P., SALMONA, M., NIEDERHUBER, J. E. & TAGLIABUE, A. (1979). Regulation of macrophage functions by interferon. *Adv. exp. Med. Biol.* **121B,** 519–524.

BUUS, S., SETTE, A., COLON, S. M. & GREY, H. M. (1988). Autologous peptides constitutively occupy the antigen binding site on Ia. *Science* **242,** 1045–1047.

BUUS, S., SETTE, A. & GREY, H. M. (1987). The interaction between protein-derived immunogenic peptides and Ia. *Immunol. Rev.* **98,** 115–141.

CASTEN, L. A. & PIERCE, S. K. (1988). Receptor-mediated B cell antigen processing. *J. Immunol.* **140,** 404–410.

CHESNUT, R. W., COLON, S. M. & GREY, H. M. (1982). Antigen presentation by normal B cells, B cell tumors, and macrophages: functional and biochemical comparison. *J. Immunol.* **128,** 1764–1768.

CHESNUT, R. W. & GREY, H. M. (1985). Antigen presenting cells and mechanisms of antigen presentation. *CRC Crit. Rev. Immunol.* **5**(3), 263–316.

CRESSWELL, P. (1985). Intracellular Class II HLA antigens are accessible to transferrn-neuraminidase conjugates internalized by receptor-mediated endocytosis. *Proc. natn. Acad. Sci. U.S.A.* **82,** 8188–8192.

DEMOTZ, S., GREY, H. M. & SETTE, A. (1990). The minimal number of Class II MHC-antigen complexes needed for T cell activation. *Science.* **249,** 1028–1030.

DIMENT, S. (1990). Different roles for thiol and aspartyl proteases in antigen presentation of ovalbumin. *J. Immunol.* **145,** 417–422.

DIMENT, S. & STAHL, P. (1985). Macrophage endosomes contain proteases which degrade protein ligands. *J. biol. Chem.* **260,** 15311–15317.

DRAKE, J. R., REPASKY, E. A. & BANKERT, R. B. (1989). Endocytosis of antigen, anti-idiotype, and anti-immunoglobin antibodies and receptor re-expression by murine B cells. *J. Immunol.* **143**, 1768-1776.

EMERSON, S. G. & CONE, R. E. (1979). Turnover and shedding of Ia antigens by murine spleen cells in culture. *J. Immunol.* **122**, 892-899.

EMERSON, S. G., MURPHY, D. B. & CONE, R. E. (1980). Ia antigen turnover. *J. Immunol.* **125**, 406-410.

FERGUSON, J. B., ANDREWS, J. R., VOYNICK, I. M. & FRUTON, J. S. (1973). The specificity of Cathepsin D. *J. biol. Chem.* **348**, 6701-6708.

GERMAIN, R. N. (1986). The ins and outs of antigen processing and presentation. *Nature, Lond.* **322**, 687-689.

GOLDMACHER, V. S., TINNEL, N. L. & NELSON, B. C. (1986). Evidence that pinocytosis in lymphoid cells has a low capacity. *J. Cell Biol.* **102**, 1312-1319.

GOSSELIN, E. J., TONY, H. & PARKER, D. C. (1988). Characterization of antigen processing and presentation by resting B lymphocytes. *J. Immunol.* **140**, 1408-1413.

GOUD, B., JOUANNE, C. & ANTOINE, J. (1984). Reversible pinocytosis of horseradish peroxidase in lymphoid cells. *Exp. Cell Res.* **153**, 218-235.

GUAGLIARDI, L. E., KOPPLEMAN, B., BLUM, J. S., MARKS, M. S., CRESSWELL, P. & BRODSKY, F. M. (1990). Co-localization of molecules involved in antigen processing and presentation in an early endocytic compartment. *Nature, Lond.* **343**, 133-139.

HARDING, C. V. & UNANUE, E. R. (1990). Quantitation of antigen-presenting cell MHC Class II/peptide complexes necessary for T-cell stimulation. *Nature, Lond* **346**, 574-576.

HARDING, C. V. & UNANUE, E. R. (1989). Antigen processing and intracellular Ia. *J. Immunol.* **142**, 12-19.

HARDING, C. V., UNANUE, E. R., SLOT, J. W., SCHWARTZ & GEUZE, H. J. (1990). Functional and ultrastructural evidence for intracellular formation of major histocompatibility complex class II-peptide complexes during Antigen Processing. *Proc. natn. Acad. Sci. U.S.A.* **87**, 5553-5557.

JENSEN, P. (1988). Protein synthesis in antigen processing. *J. Immunol.* **141**, 2545-2550.

LAKEY, E. K., CASTEN, L. A., NIEBLING, W. L., MARGOLIASH, E. & PIERCE, S. K. (1988). Time dependence of B-cell processing and presentation of peptide and native protein antigens. *J. Immunol.* **140**, 3309-3314.

LANZAVECCHIA, A., SIERVO, S. & SCHEIDEGGER, D. (1988). On the role of surface Ig in antigen presentation to T cells In: *Processing and Presentation of Antigens*. pp. 215-219. New York: Academic Press.

LORENZ, R. G. & ALLEN, P. M. (1988). Direct evidence for functional self-protein/Ia-molecule complexes *in vivo*. *Proc. natn. Acad. Sci. U.S.A.* **85**, 5220-5223.

LORENZ, R. G., BLUM, J. S. & ALLEN, P. M. (1990). Constitutive competition by self proteins for antigen presentation can be overcome by receptor-enhanced uptake. *J. Immunol.* **144**, 1600-1606.

MACHY, P., BARBET, J., & LESERMAN, L. D. (1982). Differential endocytosis of T and B lymphocyte surface molecules evaluated with antibody-bearing fluorescent liposomes containing methotrexate. *Proc. natn. Acad. Sci. U.S.A.* **79**, 4148-4152.

MURPHY, R. F. & ROEDERER, M. (1986). Flow cytometric analysis of endocytic pathways. In: *Applications of Fluorescence in the Biomedical Sciences*. pp. 545-566. New York: Alan R. Liss.

PERNIS, B. (1985). Internalization of lymphocyte membrane components. *Immunol. Today* **6**, 45-49.

PHILLIPS, M. L., YIP, C. C., SHEVACH, E. M. & DELOVITCH, T. L. (1986). Photoaffinity labelling demonstrates binding between Ia molecules and nominal antigen on antigen-presenting cells. *Proc. natn. Acad. Sci., U.S.A.* **83**, 5634-5638.

ROOF, R. W., LUESCHER, I. F. & UNANUE, E. R. (1990). Phospholipids enhance the binding of peptides to Class II major histocompatibility molecules. *Proc. natn. Acad. Sci. U.S.A.* **87**, 1735-1739.

ROSENTHAL, A. S. & SHEVACH, E. M. (1973). Function of macrophage in antigen recognition by guinea pig T lymphocytes. *J. exp. Med.* **138**, 1194-1212.

ROSLONIEC, E., VITEZ, L. J., BUUS, S. & FREED, J. H. (1990). MHC Class II-derived peptides can bind to Class II molecules, including self molecules, and prevent antigen presentation. *J. exp. Med.* **171**, 1419-1430.

SALAMERO, J., HUMBERT, M., COSSON, P. & DAVOUST, J. (1990). Mouse B lymphocyte specific endocytosis and recycling of MHC Class II molecules. *EMBO J.* **91**, 3489-3496.

SCHMIDTKE, J. R. & UNANUE, E. R. (1971). Macrophage antigen interaction: uptake, metabolism and immunogenicity of foreign albumin. *J. Immunol.* **107**, 331-338.

SCHMIDTKE, J. R. & UNANUEE, E. R. (1971). Macrophage antigen interaction: uptake, metabolism and immunogenicity of foreign albumin. *J. Immunol.* **107**, 331-338.

SINGER, D. F. & LINDERMAN, J. J. (1990). The relationship between antigen concentration, antigen internalization, and antigenic complexes: modeling insights into antigen processing and presentation. *J. Cell Biol.* **111**, 55-68.

SNIDER, D. P. & SEGAL, D. M. (1989). Efficiency of antigen presentation after antigen targeting to surface IgD, IgM, MHC, FcγRII, and B220 molecules on murine splenic B cells. *J. Immunol.* **143**, 59-65.

SWANSON, J. A., YIRINEC, B. D. & SILVERSTEIN, S. C. (1985). Phorbol esters and horseradish peroxidase stimulate pinocytosis and redirect the flow of pinocytosed fluid in macrophages. *J. Cell Biol.* **100**, 851-859.

TYCKO, B. & MAXFIELD, F. R. (1982). Rapid acidification of endocytic vesicles containing α_2-microglobulin. *Cell* **28**, 643-651.

UNANUE, E. R. & CEROTTINI, J. (1989). Antigen presentation. *FASEB J.* **3**, 2496-2502.

WATTS, T. H. & MCCONNELL, H. M. (1986). High-affinity fluorescent peptide binding to I-A^d in lipid membranes. *Proc. natn. Acad. Sci. U.S.A.* **83**, 9660-9664.

ZIEGLER, K. & UNANUE, E. R. (1981). Identification of a macrophage antigen-processing event required for I-region-restricted antigen presentation to T lymphocytes. *J. Immunol.* **127**, 1869-1875.

APPENDIX A

List of Symbols

A_0	Total number of Ia molecules (no. $cell^{-1}$)
A	Number of free cell surface Ia molecules (no. $cell^{-1}$)
Ag_i	Intracellular peptide concentration derived from protein i (M)
Ag_j	Intracellular peptide concentration derived from peptide j (M)
Ag_{0j}	Extracellular concentration of peptide j (M)
B	Number of intracellular Ia molecules (no. $cell^{-1}$)
C_i	Number of intracellular Ia complexes derived from fragments of protein i (no. $cell^{-1}$)
C_j	Number of intracellular Ia complexes derived from peptide j (no. $cell^{-1}$)
L_{0i}	Extracellular concentration of protein i (M)
L_i	Intracellular concentration of protein i (M)
N	Number of endosomes containing Ia molecules (no. $cell^{-1}$)
N_A	Avogadro's number
SA_c	Surface area of the cell (μm^2)
SA_v	Surface area of an endosome (μm^2)
V_v	Volume of an endosome (μm^3)
X_i	Number of surface Ia complexes derived from fragments of protein i (no. $cell^{-1}$)
X_j	Number of cell surface Ia complexes derived from peptide j (no. $cell^{-1}$)
a	Dimensionless number of free cell surface Ia, $[A/A_0]$
ag_i	Dimensionless intracellular peptide concentration derived from the ith protein, $[(Ag)_i/L_{0i}]$
ag_j	Dimensionless intracellular peptide concentration derived from the jth peptide, $[(Ag)_j/Ag_{0j}]$
b	Dimensionless number of intracellular free Ia, $[B/A_0]$
c_i	Dimensionless number of intracellular Ia complexes derived from the ith protein, $[C_i/A_0]$
c_j	Dimensionless number of intracellular Ia complexes derived from the jth peptide, $[C_j/A_0]$
i	Component number for protein species
j	Component number for peptide species
k_d	Rate constant for degradation of native antigen (min^{-1})
k_f	Association rate constant for binding of Ia and peptide $(M\ min)^{-1}$
k_p	Rate constant for routing of molecules to lysosome (min^{-1})
k_r	Dissociation rate constant for Ia-peptide complex (min^{-1})
k_{syn}	Rate constant for synthesis of Ia molecules (min^{-1})
k_v	Rate constant for internalization (min^{-1})
k_{-v}	Rate constant for recycle (min^{-1})
l_i	Dimensionless intracellular concentration of the ith protein, $[L_i/L_{0i}]$
t	Time (min)
x_i	Dimensionless number of cell surface Ia complexes derived from the ith protein, $[X_i/A_0]$
x_j	Dimensionless number of cell surface Ia complexes derived from the jth peptide, $[X_j/A_0]$
α	Dimensionless synthesis rate constant, $[k_{syn}/k_{-v}]$
γ_i	Dimensionless number of Ia with respect to the ith protein concentration, $[A_0/NL_{0i}N_AV_v]$
γ_j	Dimensionless number of Ia with respect to the jth peptide concentration, $[A_0/N(Ag)_{0j}N_AV_v]$
δ	Dimensionless degradation rate constant, $[k_d/k_{-v}]$
K_v	Dimensionless internalization rate constant, $[k_v/k_{-v}]$
ρ	Dimensionless dissociation rate constant, $[k_r/k_{-v}]$
σ	Dimensionless endosomal area, $[SA_v/SA_c]$
τ	Dimensionless time, $[tk_{-v}]$
φ_i	Dimensionless association rate constant for the ith protein fragment, $[k_fL_{0i}/k_{-v}]$
φ_j	Dimensionless association rate constant for the jth peptide fragment, $[k_f(Ag)_{0j}/k_{-v}]$
Φ	Dimensionless routing rate constant, $[k_p/k_{-v}]$

APPENDIX B

For the model simulations reported here, we used as an initial condition the steady-state found when APC are incubated with only a single species of self protein. For the synthesis model, this steady-state is a function of the relevant rate constants and self protein concentration and is given by:

$$l=\frac{K_v}{\sigma N(\delta+\Phi)} \tag{B.1}$$

$$c=\frac{-Y_2\pm\sqrt{Y_2^2-4Y_1Y_3}}{2Y_1} \tag{B.2}$$

$$b=\frac{c(1+\rho)}{ag\varphi} \tag{B.3}$$

$$x=\frac{c}{(\rho+\alpha)} \tag{B.4}$$

$$ag=\frac{(\delta l-\gamma c)}{\Phi} \tag{B.5}$$

$$a=\frac{(b+\rho x)}{\alpha}, \tag{B.6}$$

where

$$Y_1=\frac{-\gamma[(\alpha(1+\rho+\alpha)+\rho]}{\alpha(\rho+\alpha)}$$

$$Y_2=\frac{\alpha(1+\rho+\alpha)\delta l\varphi+\Phi\alpha(\rho+\alpha)(1+\rho)+\gamma\varphi\alpha(\rho+\alpha)+(\rho+\alpha)(1+\rho)\Phi+\varphi\delta lp}{\varphi\alpha(\rho+\alpha)}$$

$$Y_3=-\delta l.$$

Notation is as defined in Appendix A.

For the synthesis/cycle model, the initial condition is given by:

$$c=\frac{-Y_2\pm\sqrt{Y_2^2-4Y_1Y_3}}{2Y_1} \tag{B.7}$$

$$a=\frac{(b+\rho x)}{(\alpha+K_v)} \tag{B.8}$$

where l, b, x, and ag are as given in eqns (B.1), (B.3), (B.4) and (B.5) for

$$Y_1=\frac{-\gamma[(\alpha+K_v)(1+\rho+\alpha)+\rho]}{(\alpha+K_v)(\rho+\alpha)},$$

$$Y_2=\frac{[(1+\rho+\alpha)\delta l\varphi+\Phi(\rho+\alpha)(1+\rho)+\gamma\varphi(\rho+\alpha)+\dfrac{(\rho+\alpha)(1+\rho)\Phi}{(\alpha+K_v)}+\varphi\delta l\rho]}{\varphi(\rho+\alpha)},$$

$$Y_3=-\delta l.$$