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
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 (Salmero 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:

\[
\frac{da}{dt} = -K_a a + b + \rho \left[ \sum_{l=1}^{n} x_l + \sum_{j=1}^{m} x_j \right] - aa - \sum_{j=1}^{m} \phi_j a
\]

\[
\frac{dx_i}{dt} = c_i - x_i (a + \rho) \quad \text{for} \quad i = 1, 2, \ldots, n
\]

\[
\frac{dc_i}{dt} = c_i - x_i (a + \rho) \quad \text{for} \quad i = 1, 2, \ldots, n
\]

\[
\frac{db}{dt} = c_i - x_i (a + \rho) \quad \text{for} \quad i = 1, 2, \ldots, n
\]

\[
\frac{dc_j}{dt} = c_j - x_j (a + \rho) \quad \text{for} \quad j = 1, 2, \ldots, m
\]

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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.

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### Mathematical Model

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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, ..., n) and subscript j to refer to exogenous peptide-derived species (j = 1, 2, ..., 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:

\[
\frac{da}{d\tau} = -K_a a + b + \rho \left[ \sum_{i=1}^{n} x_i + \sum_{j=1}^{m} x_j \right] - a - \sum_{i=1}^{n} \phi a \tag{1}
\]

\[
\frac{dx_i}{d\tau} = c_i - x_i (\alpha + \rho) \quad \text{for} \quad i = 1, 2, ..., n \tag{2}
\]

\[\text{We have previously presented these equations in dimensional form for a one antigen scenario (Singer & Linderman, 1990).}\]
Finally, the time-dependent intracellular accumulation of native proteins \( l_i \) in endosomes can be formulated as follows:

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

Equation (9) represents an exact solution for \( l_i \) as a function of the dimensionless time, \( \tau \), 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; Babbott 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.,...
Finally, the time-dependent intracellular accumulation of native proteins \( (l) \) in endosomes can be formulated as follows:

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

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

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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). Demozt *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 (Demosz 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 μm², 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...
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 $K_a$ 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; Aragnoi 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 recycling 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, \((x_{\text{Ia}} A_{\text{Ag}} / S_{\text{A}})\) (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

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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 IgA complexed with peptide fragments of this protein and macrophage cultured in the presence of 3 μM protein have approximately 25-40% of their IgA 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 IgA 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 IgA to reach endosomes and bind peptides, are shown in Fig. 2(a)-(d). In these figures, we plot stimulatory IgA-Ag complex density (x_Ag/SA) 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 IgA synthesis rate corresponding to the measured 1E synthesis rate (α = 0-0144; Emerson & Cone, 1979; Emerson et al., 1980) and, for comparison, an elevated synthesis rate (α = 0-115) corresponding to an IgA half-life of 2 hr. With the exception of the internalization rate constant, k_i, and the cell surface area, SA, all parameter values are equal for the two cell types. Macrophages exhibit a faster rate of fluid phase internalization (Swanson et al., 1985; Chesnutt 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 α = 0-0144, macrophages exhibit an average complex density of 0-15 IgA-Ag μM⁻², while B cells exhibit an average complex density of 0-30 IgA-Ag μM⁻² over the range of interest of antigen concentrations. Criterion 2 suggests the model should predict a complex density between 1-3 IgA-Ag μM⁻². As can be seen from Fig. 2, maximum complex densities of 0-4 and 1-2 IgA-Ag μM⁻² are predicted for B cells and 0-2 and 1-0 IgA-Ag μM⁻² for macrophages for synthesis rate constants α = 0-0144 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 IgA 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 μM² using flow cyrometric 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 1-A³⁴ 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 IgA 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 IgA 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 IgA for the synthesis model at a self protein concentration of 3-0 μM shows that 2% and 10% of the IgA 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
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 (κ_l-μ_Ag/SA) 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 situ 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 0-01-1-5-0 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 (α = 0-0144; Emerson & Cone, 1979; Emerson et al., 1980) and, for comparison, an elevated synthesis rate (α = 0-115) corresponding to an Ia half-life of 2 hr. With the exception of the internalization rate constant, k_i, and the cell surface area, SA, 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 suggests that both the macrophage and B cell are quite insensitive to the range of competing protein tested.

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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
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 η is equal to 0.0144 [Fig. 3(a) and (c)] and 0.00385 [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.
The synthesis cycle model, which allows 1a 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 1a synthesis rates corresponding to 1-A and 1-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 1-A and 1-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 1a–Ag μM⁻² for macrophage APC and Fig. 3(c) shows a decrease in complex density from 5.4–4.4 1a–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.

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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 1a 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 1a 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 1a molecules over the range of 1.0–5.0 μM of self protein.
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 10 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

![Graph showing complex density as a function of competing protein concentration for macrophage APC.](image)

![Graph showing complex density on the surface for macrophage APC as a function of competing protein concentration with α = 0.0385.](image)
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Fig. 3. Stimulatory la-Ag complex density \((x_{1-A} / A_{0})\) 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 \( \alpha = 0.0385 \).
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 for 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 0-15 min⁻¹ and a half-time of 5 min for the transferrin receptor and an internalization rate constant of approximately 0-004 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 simulations of B cell APC, 0-0075 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 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. It has been reported that there are approximately 110 000 Ia molecules on a B cell or macrophage surface
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.

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responsiveness, 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 the 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


ANTIGEN PROCESSING


APPENDIX A

List of Symbols

- $A_0$ Total number of la molecules (no. cell$^{-1}$)
- $A_V$ Number of free cell surface la molecules (no. cell$^{-1}$)
- $A_{ig}$ Intracellular peptide concentration derived from protein $i$ (M)
- $A_{ig}$ Extracellular peptide concentration derived from peptide $j$ (M)
- $B$ Number of intracellular la molecules (no. cell$^{-1}$)
- $C$ Number of intracellular la complexes derived from fragments of protein $i$ (no. cell$^{-1}$)
- $C_{ig}$ Number of intracellular la complexes derived from peptide $j$ (no. cell$^{-1}$)
- $L_0$ Extracellular concentration of protein $i$ (M)
- $L_i$ Intracellular concentration of protein $i$ (M)
- $N_i$ Number of endosomes containing la molecules (no. cell$^{-1}$)

- $S_A$ Surface area of the cell ($\mu$m$^2$)
- $S_{SA}$ Surface area of an endosome ($\mu$m$^2$)
- $V_0$ Volume of an endosome ($\mu$m$^3$)
- $X_0$ Number of la surface complexes derived from fragments of protein $i$ (no. cell$^{-1}$)
- $X_i$ Number of cell surface la complexes derived from peptide $j$ (no. cell$^{-1}$)
- $A_{A_0}$ Dimensionless number of free cell surface la, $[A_0]/[A_{ig}]$
- $A_{ig}$ Dimensionless intracellular peptide concentration derived from the $i$th protein, $[A_{ig}]/[L_i]$
- $A_{ig}$ Intracellular peptide concentration derived from the $j$th peptide, $[A_{ig}]_{ij}/[L_i]$
- $B_{A_i}$ Dimensionless intracellular peptide free la, $[B_{A_i}]/[A_{ig}]$
- $C_{ig}$ Dimensionless number of intracellular la complexes derived from the $i$th protein, $[C_{ig}]/[A_{ig}]$
- $C_{ig}$ Dimensionless number of intracellular la complexes derived from the $j$th peptide, $[C_{ig}]/[A_{ig}]$
- $V_i$ Component number for protein species
- $k_d$ Rate constant for degradation of native antigen (min$^{-1}$)
- $k_{A_0}$ Association constant for binding of la and peptide (M min$^{-1}$)$^{-1}$
- $k_{A_0}$ Rate constant for routing of la to lysosome (min$^{-1}$)$^{-1}$
- $k_{A_0}$ Dissociation rate constant for la-peptide complex (min$^{-1}$)$^{-1}$
- $k_{A_0}$ Rate constant for synthesis of la molecules (min$^{-1}$)$^{-1}$
- $k_{A_0}$ Rate constant for internalization (min$^{-1}$)$^{-1}$
- $k_{A_0}$ Rate constant for recycle (min$^{-1}$)$^{-1}$

- $k_{A_0}$ Dimensionless intracellular concentration of the $i$th protein, $[L_i]/[L_0]$
- $t_i$ Time (min)
- $X_i$ Dimensionless number of cell surface la complexes derived from the $i$th protein, $[X_i]/[A_0]$
- $X_i$ Dimensionless number of cell surface la complexes derived from the $i$th peptide, $[X_i]/[A_{ig}]$
- $X_i$ Dimensionless synthesis rate constant, $k_{A_0}/k_{A_0}$
- $X_i$ Dimensionless number of la with respect to the $i$th protein concentration, $[A_0]/[N_{A_i}]$
- $N_{A_i}$ Dimensionless number of la with respect to the $j$th peptide concentration, $[A_{ig}]_{ij}/[N_{A_i}]$
- $N_{A_i}$ Dimensionless degradation rate constant, $[k_{A_0}]/[k_{A_0}]$
- $N_{A_i}$ Dimensionless internalization rate constant, $[k_{A_0}]/[k_{A_0}]$
- $N_{A_i}$ Dimensionless dissociation rate constant, $[k_{A_0}]/[k_{A_0}]$
- $N_{A_i}$ Dimensionless internal area, $[S_A]/[S_A]$
- $t_{i}$ Dimensionless time, $[t_i]/[t_i]$
- $k_{A_0}$ Dimensionless association rate constant for the $i$th protein fragment, $[k_{A_0}]/[k_{A_0}]$
- $k_{A_0}$ Dimensionless association rate constant for the $j$th peptide fragment, $[k_{A_0}]/[k_{A_0}]$
- $k_{A_0}$ Dimensionless routing rate constant, $[k_{A_0}]/[k_{A_0}]$
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APPENDIX A

List of Symbols

- $A_0$: Total number of Ia molecules (no. cell$^{-1}$)
- $A_a$: Number of free cell surface Ia molecules (no. cell$^{-1}$)
- $A_g$: Intracellular peptide concentration derived from protein $i$ (m)
- $A_g^0$: Extracellular concentration of peptide $j$ (m)
- $B$: Number of intracellular Ia molecules (no. cell$^{-1}$)
- $C$: 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$^{-1}$)
- $L_i$: Intracellular concentration of protein $i$ (m)
- $N$ : Number of endosomes containing Ia molecules (no. cell$^{-1}$)
- $N_A$: Avogadro’s number
- $S_A$: Surface area of the cell ($\mu$m$^2$)
- $S_A$: Surface area of an endosome ($\mu$m$^2$)
- $V$: Volume of an endosome ($\mu$m$^3$)
- $X_i$: Number of cell surface Ia complexes derived from fragments of protein $i$ (no. cell$^{-1}$)
- $x_i$: Number of cell surface Ia complexes derived from peptide $j$ (no. cell$^{-1}$)
- $a$: Dimensionless number of free cell surface Ia, $[A_A]/[A_a]$
- $a_i$: Dimensionless intracellular peptide concentration derived from the ith protein, $([A_g]/L_i)_{[A_g]}$
- $b_i$: Dimensionless intracellular peptide concentration derived from the jth peptide, $([A_g]/L_{[A_g]})_{[A_g]}$
- $c_i$: Number of intracellular Ia complexes derived from the ith protein, $[C_i]/[A_A]$
- $c_i$: Dimensionless number of intracellular Ia complexes derived from the jth peptide, $[C_i]/[A_A]$
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:

\[
I = \frac{K_s}{\sigma N(\delta + \Phi)} \quad (B.1)
\]

\[
c = \frac{-y_1 \pm \sqrt{y_1^2 - 4y_1y_2}}{2y_1} \quad (B.2)
\]

\[
b = \frac{c(1+\rho)}{\alpha \Phi} \quad (B.3)
\]

\[
x = \frac{c}{(\rho + \alpha)} \quad (B.4)
\]

\[
a = \frac{c(1+\rho)}{\alpha \Phi} \quad (B.5)
\]

\[
Y_1 = \frac{-(\alpha(1+\rho + \alpha) + \rho)}{\alpha(\rho + \alpha)} \quad (B.6)
\]

\[
Y_2 = \frac{\alpha(1+\rho + \alpha) + \rho}{\alpha(\rho + \alpha)} \quad (B.7)
\]

Notation is as defined in Appendix A.

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

\[
c = \frac{-y_1 \pm \sqrt{y_1^2 - 4y_1y_2}}{2y_1} \quad (B.7)
\]

\[
a = \frac{(b + px)}{(\alpha + K_c)} \quad (B.8)
\]

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

\[
Y_1 = \frac{-(\alpha(1+\rho + \alpha) + \rho)}{\alpha(\rho + \alpha)} \quad (B.6)
\]

\[
Y_2 = \frac{\alpha(1+\rho + \alpha) + \rho}{\alpha(\rho + \alpha)} \quad (B.7)
\]

\[
Y_3 = -\delta l \quad (B.8)
\]

It is also known that introns sometimes contain sequences that regulate transcription (Gillies et al., 1983; Mercola et al., 1983). For some genes, introns act after transcription to stabilize RNA (Buchman & Berg, 1988). It has also been suggested that introns may stabilize genes by reducing deleterious recombination between homologous genes and pseudogenes (Milner-White, 1984; Doolittle, 1985). All of these features of introns and splicing provoke questions about their evolution.

There are several theories or models of intron evolution. These can be divided into class I models, which assume that classical introns (pre-mRNA introns) were evolved before class II introns.