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Exploiting the Diversity of Time Scales in the Immune System: A B-Cell Antibody Model

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Using the continuous shape space formalism, we develop an immune system model involving both B lymphocytes and antibody molecules. The binding and cross-linking of receptors on B cells stimulates the cells to divide and, with a lag, to secrete antibody. Using the method of multiple scales, we show how to correctly formulate long-time-scale equations for the population dynamics of B cells, the total antibody concentration, and rate of antibody secretion. We compare our model with previous phenomenological formulations.

KEY WORDS: Immune system modeling; shape space.

1. INTRODUCTION

The immune system is a complex system of interacting cells and molecules. The cells of the immune system are a class of white blood cells called lymphocytes. Chemical interactions between solution-phase molecules and cell surface receptors allow lymphocytes to sense their environment. Ultimately such ligand–receptor interactions largely determine the activity of the immune system. Thus, for example, the binding of antigen or anti-idiotypic antibody to the immunoglobulin receptors on a B cell can stimulate the cell into proliferation and antibody secretion.

Most models of the immune system have ignored the detailed chemical interactions between receptors and ligands and have attempted to characterize in a purely phenomenological way the behavior of immune system cells. Antibody is frequently neglected and implicitly assumed to have a

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concentration proportional to the B-cell concentration.[14] We have previously shown[4] that this can be an unrealistic assumption that leads to significantly different dynamics than models in which antibody is explilcitly present. For example, models that incorporate both antibody and B cells have been shown to exhibit complex dynamics characterized by oscillatory and chaotic behavior[3,10,12,17] similar to that seen in recent experiments.[8] When antibody and B cells have the same lifetime or if antibody is eliminated from the model, such complex behavior is eliminated.[3,12]

We recognize that in modeling, some simplifications must be made. Otherwise the models are so complicated that efficient analysis is very difficult and understanding of the results essentially impossible. As a contribution to the simplification process, we show in an immune system model involving both B cells and antibody molecules how to reduce greatly the number of differential equations and also how to bypass stiffness problems in numerical integration. This is accomplished by properly taking into account the fact that chemical reactions take place on a time scale (milliseconds to seconds, perhaps minutes) that is much shorter than the time scales characteristic of cellular changes (e.g., activation, proliferation—hours to days). We provide formal justification of our simplifications by means of the method of multiple scales. This method was introduced by Segel and Perelson[15] in the context of a simpler chemical model in which receptor cross-linking was ignored.

B cells are the class of lymphocytes in the immune system that secrete antibody molecules. Each B cell has on its surface approximately 10^5 receptor molecules, each receptor having an identical variable (V) region or antigen-binding site. The population of B cells is very diverse, with different B cells expressing receptors with different V regions. The repertoire, or number of different receptor V regions, is estimated to be of order 10^7 in mammals. In the absence of stimulation, B cells are small nondividing cells. The binding and cross-linking of receptors on the surface of a B cell by a ligand, such as the polysaccharide on the surface of a bacterium or an antibody in the serum complementary in shape to the receptor, can cause the B cell to become activated, i.e., to enlarge, proliferate, and secrete antibody molecules with the same V region as the cell's receptors.

Modeling systems with 10^7 distinct B-cell species, with behavior governed by chemical reactions occurring on the surface of the cells, is the challenging rask that we approach in this paper. To this end we employ the continuous shape space formalism. This was introduced by Segel and Perelson[14] in the context of a highly schematic model that was designed to illustrate certain general principles, notably the assertion that the immune system should be poised in a state of stability, but not excessive

stability. A similar schematic model was introduced by Percus,[9] who exploted the equilibrium behavior of a class of immune network models using potential methods. The model considered here is an extension of that of Segel and Perelson,[14] but is considerably more realistic in its explicit consideration of antibody molecules and their chemical reaction with each other and with receptors. The underlying chemical model follows that introduced by Perelson[10] for a restricted case in which only two B-cell clones and the antibodies they produced were considered. Analytic and numerical solutions of the model equations will be presented elsewhere.

2. FORMULATION OF THE MODEL

We introduce the following notation.

$A_0(y, t)\,dy$: concentration of free antibodies (at time t) with shapes in the range $(y, y+dy)$, for very small dy.

$A_1(x, y, t)\,dy$: number of antibodies of shapes in the range $y, y+dy$ bound at one site to receptors on a cell of type x.

$A_2(x, y, t)\,dy$: number of antibodies of shapes in the range $y, y+dy$ bound at two sites to receptors on a cell of type x.

$P(x, t)$: total number of receptor sites per cell, shape x.

$P_0(x, t)$: corresponding number of free receptor sites per cell.

$C(y, y', t)\,dy\,dy'$: concentration of complexes formed of antibodies having shapes in the range $(y, y+dy)$ with antibodies having shapes in the range $(y', y'+dy')$.

$B(x, t)\,dx$: concentration of cells that bear receptors with shapes in range $(x, x+dx)$.

By "cell" we always mean "B cell." By "receptor" we mean an immunoglobulin (antibody) receptor on the surface of a B cell. The subscript 0 denotes a free molecule; the subscript 1 a molecule bound at one site; the subscript 2 a molecule bound at two sites.

In the present model we ignore the possibility that an antibody bound at two sites is bound to a single receptor. Such states, called monogamous bivalent attachments, are possible for very flexible antibodies, and can be includded in more detailed theories. Here we assume that all doubly-bound antibody is a cross-link between two receptors.

We must document the reversible binding of free antibody A_0 to free receptor sites P_0, to form single bound antibody,

$$A_0(y, t) + B(x, t)\,P_0(x, t) \underset{k_{-1}(x, y)}{\overset{vk_1(x, y)}{\rightleftharpoons}} A_1(x, y, t)\,B(x, t) \tag{1a}$$

and the binding of singly bound antibody to a second receptor site to form a cross-link,

$$A_1(x, y, t) + P_0(x, t) \underset{2\bar{k}_{-2}(x, y)}{\overset{(v-1)\bar{k}_2(x, y)}{\rightleftharpoons}} A_2(x, y, t) \tag{1b}$$

In (1), $\bar{k}_1(x, y)$ and $\bar{k}_{-1}(x, y)$ denote the shape-dependent rate constants of the reaction for antibody binding from solution to a cell, and $\bar{k}_2(x, y)$ and $\bar{k}_{-2}(x, y)$ denote the shape-dependent rate constants for receptor cross-linking. In (1) and (2) the statistical factors v and v^2 appear, since the rate constants refer to individual sites that are assumed to be equivalent and characterized by the same forward and reverse rate constants. Each antibody has v sites, with valence $v=2$ for IgG and $v=10$ for IgM antibodies.

Similar to (1a) and (1b), we symbolize the formation of antibody–antibody complexes by

$$A_0(y, t) + A_0(y', t) \underset{\bar{m}_-(y, y')}{\overset{v^2\bar{m}_+(y, y')}{\rightleftharpoons}} C(y, y', t) \tag{2}$$

In the present study we shall neglect receptor internalization and synthesis, so that the number of immunoglobulin receptor sites per cell is constant. Also, we assume that all cells have the same number of receptor sites, i.e.,

$$P(x, t) = P = \text{const} \tag{3}$$

$$P_0(x, t) + \int A_1(x, y, t)\, dy + 2\int A_2(x, y, t)\, dy = P \tag{4}$$

All integrals will be taken over a symmetric shape interval of "length" $2L$,

$$-L < x < L, \qquad -L < y < L \tag{5}$$

From (1), the differential equations for singly and doubly bound antibody are

$$\begin{aligned}\frac{\partial A_1}{\partial t}(x, y, t) = {} & v\bar{k}_1(x, y)\, A_0(y, t)\, P_0(x, t) - \bar{k}_{-1}(x, y)\, A_1(x, y, t) \\ & - (v-1)\, \bar{k}_2(x, y)\, A_1(x, y, t)\, P_0(x, t) \\ & + 2\bar{k}_{-2}(x, y)\, A_2(x, y, t)\end{aligned} \tag{6a}$$

$$\begin{aligned}\frac{\partial A_2}{\partial t}(x, y, t) = {} & (v-1)\, \bar{k}_2(x, y)\, A_1(x, y, t)\, P_0(x, t) \\ & - 2\bar{k}_{-2}(x, y)\, A_2(x, y, t)\end{aligned} \tag{6b}$$

Implicit in these equations is the simplifying assumption that when cells are born or die, they have on their surface the current average number of singly and doubly bound antibodies. Thus changes in $B(x, t)$ will not affect the number of singly or doubly bound antibodies per cell.

For free antibody we have the following kinetic equation, given schemes (1) and (2) and taking into account antibody secretion at rate S_A per cell and degradation of free antibody with rate constant d_A:

$$\begin{aligned}\frac{\partial A_0(y, t)}{\partial t} = &\int \bar{m}_-(y, y')\, C(y, y', t)\, dy' \\ &- A_0(y, t) \int v^2 \bar{m}_+(y, y')\, A_0(y', t)\, dy' \\ &- A_0(y, t) \int v \bar{k}_1(x, y)\, P_0(x, t)\, B(x, t)\, dx \\ &+ \int \bar{k}_{-1}(x, y)\, A_1(x, y, t)\, B(x, t)\, dx \\ &- d_A A_0(y, t) + S_A(y, t)\, B(y, t) \end{aligned} \tag{7}$$

Antibody–antibody complex is governed by the kinetics of scheme (2), together with a destruction term (proportional to the factor d_C) that combines nonspecific losses and macrophage endocytosis:

$$\begin{aligned}\partial C(y, y', t)/\partial t = &v^2 \bar{m}_+(y, y')\, A_0(y, t)\, A_0(y', t) \\ &- \bar{m}_-(y, y')\, C(y, y', t) - d_C C(y, y', t)\end{aligned} \tag{8}$$

The antibody secretion rate for y-cells [that appears in (7)] will be determined by

$$\partial S_A(y, t)/\partial t = k_A[S_M q_A(y, t) - S_A(y, t)] \tag{9a}$$

where the constant S_M represents the maximal secretion rate. The expression

$$q_A(y, t) = q_A\left[P^{-1} \int A_2(y, x, t)\, dx\right] \tag{9b}$$

where q_A is defined to have a maximal value of unity, states the functional dependence of the secretion rate on the fraction of cross-linked receptors. The function q_A may also depend on various cytokine concentrations. These factors, secreted by helper T cells and other cells, are regarded as at some suitable constant level in this B-cell model. Thus, no dependence on cytokine concentrations is written into the present version of our equations.

Equations (9a) and (9b) have the property that if A_2 were independent of time, then q_A would be time-independent and the secretion rate S_A would reach a steady value on a time scale determined by k_A. Hence, at steady state the dimensionless quantity q_A gives the fraction of maximal secretion that occurs as a function of receptor occupancy. By using a differential equation to determine $S_A(y, t)$, we account in a crude fashion for the delays involved in lymphocyte activation and the "gearing up" for antibody secretion. This generally takes several days.

Finally, B cells proliferate at a rate $k_B r_B(x, t)$, where k_B is a representative time constant for proliferation (so that the function r_B is dimensionless). There is an influx m_B from the bone marrow and a per capita death rate d_B:

$$\partial B(x, t)/\partial t = k_B r_B(x, t)\, B(x, t) + m_B - d_B B(x, t) \tag{10a}$$

Both m_B and d_B will be assumed constant. The dimensionless proliferation rate

$$r_B(x, t) = r_B\left[P^{-1}\int A_2(x, y, t)\, dy, \int B(y, t)\, dy, B(x, t)\right] \tag{10b}$$

depends on the fraction of cross-linked receptors, the total B-cell population, and the number of cells of type x. [Note that on the left and right sides of (10b) there appear two different functions $r_B(\cdot, \cdot)$ and $r_B(\cdot, \cdot, \cdot)$. A similar notational practice is employed in (9b).] In a full model, cytokine concentrations would also affect r_B, but here the concentration of such nonspecific growth factors is assumed to be proportional to the total population size $\int B(y, t)\, dt$. The dependence of r_B on the specific clone size $B(x, t)$ models such effects as crowding in the lymph nodes and in the spleen.

Equations (6)–(10) constitute our mathematical model: there are six equations for A_2, A_1, A_0, C, B, and S_A—with the conservation equation (4) permitting the expression of P_0 in terms of A_1 and A_2.

3. DIMENSIONLESS EQUATIONS

We now introduce scaled dimensionless variables. As a time scale we employ d_B^{-1}, the half-life of unstimulated B cells [see (10a)]. Accordingly, the dimensionless time T is given by

$$T = t/d_B^{-1} \tag{11}$$

"Spatial variables" (in shape space) x and y are nondimensionalized in terms of the shape range $2L$:

$$\tilde{x} = x/(2L), \qquad \tilde{y} = y/(2L) \tag{12a}$$

However, we immediately drop the tildes; from now on all spatial variables are dimensionless.

We run into difficulty with respect to the scale $\hat{B}$ for the B-cell concentration. $\hat{B}$ may depend strongly on the parameter values in a way that is hard to predict in advance. More vexing is the fact that for a given set of parameters the scale of B may vary markedly for different clones. We thus leave $\hat{B}$ unspecified for the present in the scaling,

$$b = 2LB/\hat{B} \tag{12b}$$

In writing (12b) we have taken cognizance of the fact that, by definition, B is a concentration per unit distance in shape space. Thus the total B-cell concentration has the magnitude of $2L$ times a typical value of B. Similarly, the total source of B cells is

$$\bar{m}_B = 2Lm_B \tag{12c}$$

The secretion rate will be scaled by its maximal value S_M:

$$s = S_A/S_M \tag{12d}$$

We denote the equilibrium association constants for antibody-receptor site, receptor cross-linking, and antibody–antibody binding by $\bar{K}_1$, $\bar{K}_2$, and $\bar{M}$:

$$\bar{K}_1(x, y) \equiv \bar{k}_1(x, y)/\bar{k}_{-1}(x, y) \tag{13a}$$

$$\bar{K}_2(x, y) \equiv \bar{k}_2(x, y)/\bar{k}_{-2}(x, y) \tag{13b}$$

$$\bar{M}(y, \bar{y}) = \bar{m}_+(y, y')/\bar{m}_-(y, y') \tag{13c}$$

We write

$$K_1(x, y) = v\bar{K}_1(x, y)/\kappa_1 \tag{14a}$$

$$K_2(x, y) = (v-1)\,\bar{K}_2(x, y)/(2\kappa_2) \tag{14b}$$

$$M(y, y') = v^2\bar{M}(y, y')/\mu \tag{14c}$$

where the normalization factors κ_1, κ_2, and μ are chosen so that

$$\int K_1(x, y)\, dy = 1, \tag{15a}$$

$$\int K_2(x, y)\, dy = 1, \tag{15b}$$

$$\int M(y, y')\, dy' = 1 \tag{15c}$$

Note that κ_1 and μ have the dimensions of inverse concentration and that $K_1(x, y)$, $K_2(x, y)$, and $M(y, y')$ are dimensionless.

Let the constants k_{off} and m_{off} be typical values of $\bar{k}_{-1}(x, y)$ and $\bar{m}_-(x, y)$, respectively. We assume that the rate of dissociation of a site on a doubly bound antibody, $\bar{k}_{-2}$, is of the same order of magnitude as that of a site on a singly bound antibody, $\bar{k}_{-1}$, so that k_{off} provides a proper scaling for both. With these, we define dimensionless rate constants as follows:

$$k_{-1} \equiv \bar{k}_{-1}/k_{\text{off}}, \qquad k_1 \equiv \bar{k}_1/k_{\text{off}}\kappa_1, \qquad k_{-2} \equiv \bar{k}_{-2}/k_{\text{off}}, \qquad k_2 \equiv \bar{k}_2/k_{\text{off}}\kappa_2,$$

$$m_- = \bar{m}_-/m_{\text{off}}, \qquad m_+ = \bar{m}_+/m_{\text{off}}\mu$$

so that

$$K_1 = vk_1/k_{-1} \tag{16a}$$

$$K_2 = (v-1)k_2/2k_{-2} \tag{16b}$$

$$M = v^2 m_+/m_- \tag{16c}$$

The free antibody concentration A_0 will be scaled with the aid of a balance between antibody secretion by $\hat{B}$ B cells at maximal rate S_M, and destruction of antibody–antibody complexes at rate $d_C C$. [See Eqs. (7) and (8).] This gives $S_M \hat{B} \sim d_C C$. The relation between C and A_0 can be estimated from steady-state conditions. We assume that $A_0(y)$ and $\int A_0(y')\, dy'$ can be estimated by the scale for A_0 and that $\int C(y, y')\, dy'$ can be estimated by the scale for C. We obtain $C \approx \mu A_0^2$ from (8), with $d_C = 0$, and (13c), (14c), and (15c). Hence $A_0 \approx (S_M \hat{B}/\mu d_C)^{1/2}$. The free antibody concentration "per unit shape" A_0 must be multiplied by the shape range $2L$ to estimate the actual antibody concentration. We therefore take

$$a_0 = 2LA_0/(S_M \hat{B}/\mu d_C)^{1/2} \tag{17}$$

Similarly, because $C(y, y', t)$ depends on both y and y', the transition from C to c requires multiplication by $(2L)^2$. Upon incorporating our earlier

estimate for C, we are led to the following scaled dimensionless version of the complex concentration:

$$c = \frac{(2L)^2 C}{S_M \hat{B}/d_C} \tag{18a}$$

Finally, let the dimensionless concentrations of free receptor sites, singly bound antibody, and doubly bound antibody be

$$p_0 = P_0/P, \qquad a_1 = 2LA_1/P, \qquad a_2 = 2LA_2/P \tag{18b}$$

Upon substituting the above variables, we find that the dimensionless versions of equations (4), (6)–(8), (9a), and (10a) are

$$p_0(x, T) + \int a_1(x, y, T)\, dy + 2\int a_2(x, y, T)\, dy = 1 \tag{19}$$

$$\begin{aligned}\varepsilon \frac{\partial a_1(x, y, T)}{\partial T} = {} & v\alpha k_1(x, y)\, a_0(y, T)\, p_0(x, T) - k_{-1}(x, y)\, a_1(x, y, T) \\ & - (v-1)\, \omega k_2(x, y)\, a_1(x, y, T)\, p_0(x, T) \\ & + 2k_{-2}(x, y)\, a_2(x, y, T)\end{aligned} \tag{20a}$$

$$\begin{aligned}\varepsilon \frac{\partial a_2(x, y, T)}{\partial T} = {} & (v-1)\, \omega k_2(x, y)\, a_1(x, y, T)\, p_0(x, T) \\ & - 2k_{-2}(x, y)\, a_2(x, y, T)\end{aligned} \tag{20b}$$

$$\begin{aligned}\varepsilon \frac{\partial a_0(y, T)}{\partial T} = {} & v\rho\alpha \int m_-(y, y')\, c(y, y', T)\, dy' \\ & - v\rho\alpha a_0(y, T) \int v^2 m_+(y, y')\, a_0(y', T)\, dy' \\ & + \zeta\alpha^{-1} \int k_{-1}(x, y)\, a_1(x, y, T)\, b(x, T)\, dx \\ & - \zeta a_0(y, T) \int v k_1(x, y)\, p_0(x, T)\, b(x, T)\, dx \\ & + \varepsilon\alpha\rho\psi_C s(y, T)\, b(y, T) - \varepsilon\psi_A a_0(y, T)\end{aligned} \tag{21}$$

$$\begin{aligned}\varepsilon \frac{\partial c(y, y', T)}{\partial T} = {} & v^2 v m_+(y, y')\, a_0(y, T)\, a_0(y', T) \\ & - v m_-(y, y')\, c(y, y', T) - \varepsilon\psi_C c(y, y', T)\end{aligned} \tag{22}$$

$$\frac{\partial s(y, T)}{\partial T} = \eta[q_A(y, T) - s(y, T)] \tag{23}$$

$$\frac{\partial b(x, T)}{\partial T} = [\gamma r_B(x, T) - 1]\, b(x, T) + \xi \tag{24}$$

The dimensionless parameters are listed in Table I. Particularly important, as we shall see shortly, is $\varepsilon \equiv k_{\text{off}}^{-1}/d_{\text{B}}^{-1}$; ε measures the ratio of a typical chemical half-life to the half-life of a B cell. Clearly $\varepsilon \ll 1$. Parameters ν, γ, η^{-1}, ψ_A, and ψ_C have analogous interpretations. From (14) and (15) we see that ρ gives a measure of the ratio of antibody–antibody to antibody–receptor binding affinities. The parameter α estimates the ratio of antibody concentration to the half-saturation constant of ligand–receptor binding κ_1^{-1}. The parameter ζ estimates the ratio of receptor concentration to κ_1^{-1}. As for ξ, from (10a) it follows that $\bar{m}_{\text{B}}/d_{\text{B}}$ is the B-cell concentration in the virgin state. If we take $\hat{B}$ at the virgin state, $\xi = 1$. If $\hat{B}$ is an estimate of the much higher B-cell concentrations attained after stimulation, then ξ will be a small parameter and therefore negligible.

4. MULTIPLE-SCALE ANALYSIS

To exploit the simultaneous presence of different time scales, we employ a version of the method of multiple scales (see Lin and Segel[7] for an elementary exposition). To this end, we introduce the fast time τ:

$$\tau \equiv \varepsilon^{-1} T \tag{25}$$

We assume

$$a_0(y, T) = a_0^{(0)}(y, \tau, T) + \varepsilon a_0^{(1)}(y, \tau, T) + \cdots \tag{26}$$

$$c(y, y', T) = c^{(0)}(y, y', \tau, T) + \varepsilon c^{(1)}(y, y', \tau, T) + \cdots \tag{27}$$

Table I. Dimensionless Parameters

Parameter	Definition
ε	$d_{\text{B}}/k_{\text{off}}$
α	$\kappa_1(S_M \hat{B}/\mu d_C)^{1/2}$
ν	$m_{\text{off}}/k_{\text{off}}$
ρ	μ/κ_1
ζ	$P\hat{B}\kappa_1$
ψ_A	d_A/d_{B}
ψ_C	d_C/d_{B}
η	k_A/d_{B}
ξ	$\bar{m}_{\text{B}}/\hat{B}d_{\text{B}}$
γ	$k_{\text{B}}/d_{\text{B}}$
ω	$\kappa_2 P$

with similar equations for other dependent variables. Note, for example, that $a_0^{(0)}$ is a function of three variables. Since τ is a function of T, we have by the chain rule

$$\varepsilon \frac{\partial a_0(y, T)}{\partial T} = \frac{\partial a_0^{(0)}(y, \tau, T)}{\partial \tau} + \varepsilon \left[\frac{\partial a_0^{(0)}(y, \tau, T)}{\partial T} + \frac{\partial a_0^{(1)}(y, \tau, T)}{\partial \tau} \right] + \cdots \tag{28}$$

We perform analogous calculations in substituting our series into the governing equations (19)–(24).

We expect some sort of generalized conservation of antibody. Thus we combine (21), (20), and (22) to obtain

$$\frac{\partial a_0(y, T)}{\partial T} + \zeta \alpha^{-1} \int (a_1(x, y, T) + a_2(x, y, T)) \frac{\partial b(x, T)}{\partial T} dx + \rho \alpha \int \frac{\partial c(y, y', T)}{\partial T} dy'$$

$$= \alpha \rho \psi_C s(y, T)\, b(y, T) - \psi_A a_0(y, T) - \rho \alpha \psi_C \int c(y, y', T)\, dy' \tag{29}$$

which is an equation describing the change with time in the total concentration of antibody y.

Upon inserting appropriate series into the secretion equation (23) and the B-cell equation (24) and expanding, we find at lowest order simply

$$\partial s^{(0)}(\chi, \tau, T)/\partial \tau = 0,\ \partial b^{(0)}(\chi, \tau, T)/\partial \tau = 0 \tag{30a,b}$$

Thus $s^{(0)}$ and $b^{(0)}$ are functions only of the slow time T, not of τ. Moreover, employing (30b) we find that the lowest order terms in (29) add to zero. We reach the important conclusion that the total amount of antibody of shape y is a slowly varying function of time, $A_T(y, T)$:

$$a_0^{(0)}(y, \tau, T) + \zeta \alpha^{-1} \int b^{(0)}(x, T)[a_1^{(0)}(x, y, \tau, T) + a_2^{(0)}(x, y, \tau, T)]\, dx + \rho \alpha \int c^{(0)}(y, y', \tau, T)\, dy' = A_T(y, T) \tag{31}$$

(Note that the factors $\zeta \alpha^{-1}$ and $\rho \alpha$ appear because different parameters have been used to normalize the various concentrations.) All we can say about A_T at present is that initially

$$A_T(y, 0) = A_T^{\text{in}}(y) \tag{32}$$

where $A_T^{\text{in}}(y)$ is known in terms of the initial values of a_0, b, a_1, and c. One possibility is

$$A_T^{\text{in}}(y) = a_0(y, 0) \tag{33}$$

i.e., all antibody is initially free in solution.

It is the time scale T that interests us. We do not care to follow the details of the chemical reactions (that typically occur on a fast "k^{-1}" scale of milliseconds, perhaps ranging up to minutes in the case of very slow back reactions). What are of primary immunological interest are changes in such variables as the B-cell population or the total antibody concentration that occur over hours or days. Our small parameter ε is the ratio of fast to slow time scales. The remarks just given imply that we should calculate various functions in the limit

$$\varepsilon \to 0, \qquad T = \varepsilon\tau \text{ fixed} \quad (\text{so } \tau \to \infty) \tag{34}$$

Let us examine the lowest order approximation of (20) in the limit (34).

$$\begin{aligned} &\partial a_1^{(0)}(x, y, \tau, T)/\partial\tau \\ &\quad = v\alpha k_1(x, y)\, a_0^{(0)}(y, \tau, T)\, p_0(x, \tau, T) - k_{-1}(x, y)\, a_1^{(0)}(x, y, \tau, T) \\ &\qquad - (v-1)\, \omega k_2(x, y)\, a_1^{(0)}(x, y, \tau, T)\, p_0(x, \tau, T) \\ &\qquad + 2k_{-2}(x, y)\, a_2^{(0)}(x, y, \tau, T) \end{aligned} \tag{35a}$$

$$\begin{aligned} &\partial a_2^{(0)}(x, y, \tau, T)/\partial\tau \\ &\quad = (v-1)\, \omega k_2(x, y)\, a_1^{(0)}(x, y, \tau, T)\, p_0(x, \tau, T) - 2k_{-2}(x, y)\, a_2^{(0)}(x, y, \tau, T) \end{aligned} \tag{35b}$$

As prescribed by (34), we ignore τ-transients and consider the *quasi-steady limit* $\tau \to \infty$, T fixed—in which $a_1^{(0)}$ and $a_2^{(0)}$ are essentially in chemical equilibrium. We shall use the notation

$$\lim_{\substack{\tau \to \infty \\ T \text{ fixed}}} a_1^{(0)}(x, y, \tau, T) = a_1^{(0)}(x, y, T) \tag{36}$$

with analogous notation for similar variables.

In order to obtain cleaner notation, *from now on we drop the superscript zero* that should appear on P_0, a_0, a_1, a_2, b, c, and s in equations (37)–(44). Thus, from (35) and (16)

$$a_1(x, y, T) = \alpha K_1(x, y)\, a_0(y, T)\, p_0(x, T) \tag{37a}$$

$$a_2(x, y, T) = \omega K_2(x, y)\, a_1(x, y, T)\, p_0(x, T) \tag{37b}$$

Employing (37), we obtain from (19) a quadratic equation with one real positive solution, written in a form analogous to that given in Perelson and DeLisi[11] as

$$p_0(x, T) = \frac{-1 + [1 + 4\delta(x, T)]^{1/2}}{2\delta(x, T)[1 + \alpha \int K_1(x, y)\, a_0(y, T)\, dy]} \tag{38a}$$

where

$$\delta(x, T) = \frac{2\alpha\omega \int K_2(x, y)\, K_1(x, y)\, a_0(y, T)\, dy}{[1 + \alpha \int K_1(x, y)\, a_0(y, T)\, dy]^2} \tag{38b}$$

Of more importance to us are the corresponding expressions for the number of singly bound antibodies and the number of cross-links in terms of the free antibody concentration. From (37) and (38)

$$a_1(x, y, T) = \frac{\alpha K_1(x, y)\, a_0(y, T)}{1 + \alpha \int K_1(x, z)\, a_0(z, T)\, dz} \frac{-1 + [1 + 4\delta(x, T)]^{1/2}}{2\delta(x, T)} \tag{39a}$$

$$a_2(x, y, T) = \frac{K_2(x, y)\, K_1(x, y)\, a_0(y, T)}{\int K_2(x, z)\, K_1(x, z)\, a_0(z, T)\, dz} \times \frac{1 + 2\delta(x, T) - [1 + 4\delta(x, T)]^{1/2}}{4\delta(x, T)} \tag{39b}$$

At lowest order the quasi-steady-state version of (22) yields

$$c(y, y', T) = M(y, y')\, a_0(y, T)\, a_0(y', T) \tag{40}$$

where M is the association constant for antibody–antibody binding defined in (14). In the quasi-steady limit the conservation equation (30) reads

$$a_0(y, T) + \zeta\alpha^{-1} \int b(x, T)[a_1(x, y, T) + a_2(x, y, T)]\, dx + \rho\alpha \int c(y, y', T)\, dy' = A_T(y, T) \tag{41}$$

where $a_1(x, y, t)$ and $a_2(x, y, T)$ are given by (39) and $c(y, y', T)$ is given by (40).

As is common in multi-scale expansions, to complete the equations for the lowest order terms, we must consider certain higher-order equations. Employing (41) and the quasi-steady limit, we obtain from the $O(\varepsilon)$ terms in the expansion of (29)

$$\frac{\partial A_T(y, T)}{\partial T} = \alpha\rho\psi_C s(y, T)\, b(y, T) - \psi_A a_0(y, T) - \psi_C \rho\alpha \int c(y, y', T)\, dy' + \int \alpha^{-1} \int (a_1(x, y, T) + a_2(x, y, T)) \frac{\partial b(x, T)}{\partial T}\, dx \tag{42}$$

This equation describes the slow change in the total antibody concentration owing to antibody secretion, together with the loss of free antibody and complex. The $O(\varepsilon)$ terms in (24) give an equation for the change in the B-cell concentration:

$$\frac{\partial b}{\partial T}(y, T) = [\gamma r_B(y, T) - 1]\, b(y, T) + \xi \tag{43}$$

Finally, the $O(\varepsilon)$ terms in Eq. (23) give for the secretion rate

$$\frac{\partial s}{\partial T}(y, T) = \eta[q_A(y, T) - s(y, T)] \tag{44}$$

Recapitulating, equations (42)–(44) are three equations for the development in "slow" time T of the (lowest order approximations) to the total antibody concentration A_T, the B-cell concentration b, and the secretion rate s. The various required chemical concentrations, ρ_0, a_1, a_2, and c, are given in terms of the free antibody concentration a_0 by (38)–(40). At any given instant a_0 can be determined from b and A_T by the nonlinear integral equation (41). The formulation is completed by suitable initial and boundary conditions and specification of the various equilibrium constants. The initial conditions are the conditions at the end of the fast transient; they can be ascertained as in the usual approach to quasi-steady-state assumptions (see, for example, Segel[13]). For boundary conditions one can use periodic boundary conditions as in Segel and Perelson[14] or deal explicitly with a finite-dimensional shape space, as in De Boer *et al.*[5] As in Segel and Perelson,[14] we take the binding affinity (dimensionless) of shape y to shape x to be a Gaussian centered about the perfectly complementary shape $-x$:

$$\bar{K}_1(x, y) = K_{\max} \exp[-(x + y)^2/2\sigma_1^2] \tag{45a}$$

$$\bar{K}_2(x, y) = K_{\max} \exp[-(x + y)^2/2\sigma_2^2] \tag{45b}$$

$$\bar{M}(y, y') = M_{\max} \exp[-(x + y)^2/2\sigma_M^2] \tag{45c}$$

It remains to specify the secretion and proliferation functions q_A and r_B. We shall assume for simplicity that there is a linear dependence on the fraction of receptors cross-linked. In particular, for some dimensionless parameter q_0

$$q_A(y, T) = q_0 \int a_2(y, x, T)\, dx \tag{46}$$

Similarly, but with further assumptions concerning the other factors of (10b), we take for the dimensionless proliferation function in (43)

$$r_B(y, T) = \int a_2(y, x, T)\, dx \cdot \exp\left[-\lambda_2 \left(\int b(y, T)\, dy \right)^n - \lambda_1 b(y, T) \right] \tag{47}$$

No premultiplicative factor is necessary here [such as q_0 in (46)], since we have already introduced the factor k_B in (10a).

As we have mentioned, immunological evidence suggests that the growth rate of B cells and the rate of antibody secretion depend on the degree of receptor cross-linking. Here, using the method of multiple time scales, we have shown how to compute the average number of cross-links per cell and then employ that information to formulate B-cell growth and antibody secretion rate equations. However, this approach, even though accurate on the long time scale of interest in predicting B-cell growth and serum antibody concentrations, is still quite complex, especially when formulated in the context of a continuous shape space model. In order to gain additional insights, we present a simple example using only two B cells.

5. A TWO-B-CELL EXAMPLE

Consider a system composed of two B-cell populations, $B^{(1)}$ and $B^{(2)}$, where $B^{(1)}$ has receptors complementary to those on $B^{(2)}$. Assume that the antibodies $A^{(1)}$ secreted by $B^{(1)}$ are complementary to antibodies $A^{(2)}$ secreted by $B^{(2)}$, and are able to bind and cross-link receptors on $B^{(2)}$ and form complexes C with $A^{(2)}$. As before, we use the subscripts 0, 1, and 2 to distinguish molecules that are bound at 0, 1, or 2 sites. The specification of this system is then given by following equations:

$$\frac{dA_0^{(1)}}{dt} = S_A B^{(1)} - d_A A_0^{(1)} - v\bar{k}_1 A_0^{(1)} P_0^{(2)} B^{(2)} + \bar{k}_{-1} A_1^{(1)} B^{(2)}$$
$$- v^2 \bar{m}_+ A_0^{(1)} A_0^{(2)} + \bar{m}_- C \tag{48a}$$

$$\frac{dA_1^{(1)}}{dt} = v\bar{k}_1 A_0^{(1)} P_0^{(2)} - \bar{k}_{-1} A_1^{(1)} - (v-1)\,\bar{k}_2 A_1^{(1)} P_0^{(2)} + 2\bar{k}_{-2} A_2^{(1)} \tag{48b}$$

$$\frac{dA_2^{(1)}}{dt} = (v-1)\,\bar{k}_2 A_1^{(1)} P_0^{(2)} - 2\bar{k}_{-2} A_2^{(1)} \tag{48c}$$

$$\frac{dB^{(1)}}{dt} = m_B + k_B r_B(A_2^{(2)}) B^{(1)} - d_B B^{(1)} \tag{48d}$$

$$\frac{dS_A^{(1)}}{dt} = k_A[S_M q_A(A_2^{(2)}) - S_A^{(1)}] \tag{48e}$$

$$\frac{dC}{dt} = v^2 \bar{m}_+ A_0^{(1)} A_0^{(2)} - \bar{m}_- C - d_C C \tag{48f}$$

and a set of five equations analogous to (48a)–(48e), but with the superscripts (1) and (2) interchanged. In (48d) and (48e) the functional dependence of the B-cell growth rate and the antibody secretion rate on the degree of cross-linking is explicitly indicated, but, for simplicity, other factors such as clone size dependence are not. In addition, the number of free receptor sites $P_0^{(i)}$, $i = 1, 2$, is given by the conservation equation

$$P_0^{(i)} + A_1^{(i')} - 2A_2^{(i')} = P, \qquad i = 1, 2 \tag{49}$$

where i' is the index of the cell that antibody i binds, i.e., $i' = 2$ if $i = 1$ and $i' = 1$ if $i = 2$.

This system of 11 ordinary differential equations was studied numerically by Perelson.[10] However, on the time scale of interest, days to months, essentially the same results can be obtained in a much more efficient manner by solving the reduced set of six differential equations that result from the multiple-time-scale method. In this example, the analog of equations (42)–(44), left in dimensional form, are

$$\frac{dA_T^{(i)}}{dt} = S_A^{(i)} B^{(i)} - d_A A_0^{(i)} - d_C M A_0^{(1)} A_0^{(2)} + (A_1^{(i)} + A_2^{(i)}) \frac{dB^{(i')}}{dt}, \quad i = 1, 2 \tag{50a}$$

$$\frac{dB^{(i)}}{dt} = m_B + k_B r_B(A_2^{(i')}) B^{(i)} - d_B B^{(i)}, \qquad i = 1, 2 \tag{50b}$$

$$\frac{dS_A^{(i)}}{dt} = k_A[S_M q_A(A_2^{(i')}) - S_A^{(i)}], \qquad i = 1, 2 \tag{50c}$$

$$A_T^{(i)} = A_0^{(i)} + (A_1^{(i)} + A_2^{(i)})\, B^{(i')} + M A_0^{(1)} A_0^{(2)}, \qquad i = 1, 2 \tag{50d}$$

The number of singly bound antibodies and the number of cross-links per cell are determined on the fast chemical scale to be

$$A_1^{(i)} = K_1 A_0^{(i)} P_0^{(i')}, \qquad i = 1, 2 \tag{51a}$$

$$A_2^{(i)} = K_2 K_1 A_0^{(i)} [P_0^{(i')}]^2, \qquad i = 1, 2 \tag{51b}$$

where $K_1 = v\bar{k}_1/\bar{k}_{-1}$, $K_2 = (v-1)\bar{k}_2/(2\bar{k}_{-2})$, and where $P_0^{(i')}$ is specified by substituting (51) into (49) and solving the resulting quadratic equations. Once $P_0^{(i')}$ is found as a function of $A_0^{(i)}$, $A_0^{(i)}$ is determined by the slowly varying conservation equation

$$A_0^{(i)} + K_1 A_0^{(i)} P_0^{(i')} B^{(i')} + K_2 K_1 A_0^{(i)} [P_0^{(i')}]^2 B^{(i')} + M A_0^{(1)} A_0^{(2)} = A_T^{(i)}(t), \qquad i = 1, 2 \tag{52}$$

where $M = v^2 \bar{m}_+ / \bar{m}_-$. Note that this equation is analogous to (41) and the analogue of (40) has been used so that C does not appear.

The advantages of this system of equations over the original 11-equation model are that (i) rates constants for the various forward and reverse chemical reactions need not be specified, only the approporiate equilibrium constants, (ii) the number of equations is reduced, and (iii) numerical integration is more rapid, since the equations are not as stiff. The major disadvantage is that one has to solve a mixed system of differential and algebraic equations.

In a variety of other models (e.g., De Boer,[1] De Boer and Hogeweg,[2] Stewart and Varela,[16,17] Weisbuch *et al.*,[18] De Boer and Perelson[4]) in which the chemical reactions of antibody binding and cross-linking cellular receptors were not included and in which a rigorous separation of time scales was not carried out, the disadvantage of dealing with a mixed differential algebraic system was unknowingly sidetracked. The various authors implicitly assumed that they were working on the long time scale and thus neglected fast chemical reactions. With the chemistry neglected, no distinction was made between the total amount of antibody $A_T^{(i)}$ and the amount free $A_0^{(i)}$. Thus, the algebraic equations (52) that determine $A_0^{(i)}$ from $A_T^{(i)}$ were not utilized. Our results can be used to show under what conditions these previous analyses are justified. For example, in situations where the number of antibodies greatly exceeds the total number of receptor sites, one is justified in ignoring the loss of free antibody by binding to cellular receptors. Under such circumstances, one can approximate $A_0^{(i)}$ by $A_T^{(i)}$ and use (49) and (51) to compute the number of cross-links as a function of $A_T^{(i)}$. For the two-B-cell model, one finds, as in Perelson and DeLisi,[11] that

$$A_2^{(i)} = P\left[\frac{1 + 2\delta - (1+4\delta)^{1/2}}{4\delta}\right] \tag{53a}$$

where

$$\delta = \frac{2K_2K_1A_T^{(i)}P}{[1+K_1A_T^{(i)}]^2} \tag{53b}$$

In analytical work, to bypass the algebraic complexities inherent in using an expression containing a radical, Dembo and Goldstein[6] derived a simple rational (Padé) approximate for $A_2^{(i)}$. The previously mentioned models of De Boer,[1] De Boer and Hogeweg,[2] Weisbuch *et al.*,[18] De Boer *et al.*,[3] and De Boer and Perelson[4] did not employ (53) for the number of cross-links per cell. Rather, they utilized the following phenomenological function, which is equivalent to the Dembo and Goldstein Padé approximant:

$$A_2^{(i)} = \left(\frac{A_T^{(i)}}{\theta_1 + A_T^{(i)}}\right)\left(\frac{\theta_2}{\theta_2 + A_T^{(i)}}\right) \tag{54}$$

where θ_1 and θ_2 are constants. The functions given by (53) and (54) are both "bell-shaped" and symmetric about their maximum when plotted on a logarithmic scale, as is appropriate when dealing with antibody concentrations. Thus, the approximations capture the main features of a cross-linking curve.

To summarize, in cases where $A_0^{(i)} \approx A_T^{(i)}$, the original system of 11 differential equations can be reduced to a set of 6 differential equations. Clearly, in systems containing more than two B cells, a similar but substantially greater reduction in the number of equations is possible. However, the validity of this full reduction depends on $A_0^{(i)}$ being approximately equal to $A_T^{(i)}$ throughout the dynamics. If the B-cell populations expand, so that binding to cells becomes substantial, this need not be the case. In this paper we have shown that by solving (52), in the case of the two-B-cell model, or (41) in the continuous shape space model, one can rigorously determine the validity of the approximation $A_0^{(i)} \approx A_T^{(i)}$. We have also shown rigorously how to deal with the long-time behavior of the system when $A_0^{(i)}$ is not approximately equal to $A_T^{(i)}$.

6. CONCLUSION

Our final model consists of Eqs. (42)–(44) for the development in time of the total antibody A_T, the B-cell population b, and the secretion rate s—all functions of the shape variable y and the time T. Equations (46) and (47) define q_A and r_B. The basic equations are supplemented by (39)–(41), which must be solved for a_0, a_1, a_2, and c in terms of the three fundamental unknowns.

For the case of two B cells, solutions have been provided by Perelson[10] and De Boer *et al.*[3] and exhibit a wide range of behaviors depending upon parameter choices. The formidable task of analyzing solutions to the general model is underway. Results will be reported in a future publication.

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