

Cross-Linking Reconsidered: Binding and Cross-Linking Fields and the Cellular Response

Bernhard Sulzer,* Rob J. De Boer,[†] and Alan S. Perelson*

*Theoretical Biology and Biophysics, Los Alamos National Laboratory, Los Alamos, New Mexico 87545 USA, and [†]Theoretical Biology, Utrecht University, Padualaan 8, 3584 CH Utrecht, The Netherlands

ABSTRACT We analyze a model for the reversible cross-linking of cell surface receptors by a collection of bivalent ligands with different affinities for the receptor as would be found in a polyclonal anti-receptor serum. We assume that the amount of cross-linking determines, via a monotonic function, the rate at which cells become activated and divide. In addition to the density of receptors on the cell surface, two quantities, the binding field and the cross-linking field, are needed to characterize the cross-linking curve, i.e., the equilibrium concentration of cross-linked receptors plotted as a function of the total ligand site concentration. The *binding field* is the sum of all ligand site concentrations weighted by their respective binding affinities, and the *cross-linking field* is the sum of all ligand site concentrations weighted by the product of their respective binding and cross-linking affinity and the total receptor density. Assuming that the cross-linking affinity decreases if the binding affinity decreases, we find that the height of the cross-linking curve decreases, its width narrows, and its center shifts to higher ligand site concentrations as the affinities decrease. Moreover, when we consider cross-linking-induced proliferation, we find that there is a minimum cross-linking affinity that must be surpassed before a clone can expand. We also show that under many circumstances a polyclonal antiserum would be more likely than a monoclonal antibody to lead to cross-linking-induced proliferation.

INTRODUCTION

Frequently, cellular responses, such as antigen-induced proliferation of B cells or histamine release from basophils, exhibit dose-response relationships that are bell-shaped when the amplitude of the response is plotted against the logarithm of the antigen concentration (e.g., Celada, 1971; Dintzis et al., 1976; Metzger, 1992). It has been shown (DeLisi and Perelson, 1976; Dembo and Goldstein, 1978; Perelson and DeLisi, 1980) that such log bell-shaped dose-response functions result when the response depends upon the fraction of receptors cross-linked on the cell surface. (Other mechanisms can also give rise to phenomenological log bell-shaped dose-response functions (cf. Sulzer et al., 1993).) At low ligand concentration there is little binding and hence little cross-linking. At high ligand concentration almost every receptor site is occupied by a singly bound ligand, preventing the formation of cross-links. Therefore, cross-links are formed efficiently only at intermediate ligand concentrations. Clearly, the ligand has to be at least bivalent to be capable of forming cross-links.

Models of the B cell response induced by antigen or anti-immunoglobulin (anti-Ig) as a triggering signal have commonly employed log bell-shaped functions to describe the dose dependence of the B cell response (De Boer, 1988; Varela et al., 1988; Weisbuch et al., 1990; De Boer et al.,

1992; Sulzer et al., 1993). In all of these models, dose-response functions have depended upon a single variable, usually called the *field*. The field is defined as a linear superposition of the concentration of all ligands weighted by interaction constants that are often identified with the affinity of the corresponding ligand for its receptor.

Phenomenological dose-response relationships defined by these one-parameter functions describe correctly the overall shape of the dose dependence of the cellular response. However, they do not capture essential properties of the dose dependence of the concentration of cross-links, i.e., of *cross-linking curves*. For example, the cross-linking curves generated by ligand-receptor pairs with the same affinity but with different equilibrium constants for cross-linking have different widths and heights (Dembo and Goldstein, 1978; Perelson and DeLisi, 1980). However, the commonly used one-parameter dose-response functions, by design, always have the same width and height. Thus in situations where cross-linking underlies the biological response, it would be desirable to utilize dose-response functions that more accurately reflect the cross-linking process.

Recently, Faro and Velasco (1993, 1994) have modeled the formation of cross-links on B cells by multivalent ligands. Following Bell (1974), they assume that the formation of cross-links is an irreversible process, and they measure the rate of B cell activation by the characteristic time it takes to form infinite-size ligand-receptor aggregates (in a system of infinite size). The shorter the time to achieve infinite clusters the larger the rate of cell activation. They argue that a one-parameter response function does not describe correctly the rate of formation of cross-links. Below, we also argue that one-parameter dose-response functions are not appropriate. However, we do so for different reasons

Received for publication 17 April 1995 and in final form 21 November 1995.

Address reprint requests to Dr. Alan S. Perelson, Theoretical Biology and Biophysics, T-10, MS K710, Los Alamos National Laboratory, Los Alamos, NM 87545. Tel.: 505-667-6829; Fax: 505-665-3493; E-mail: asp@t10.lanl.gov.

© 1996 by the Biophysical Society

0006-3495/96/03/1154/15 \$2.00

because there is no evidence that infinite-size or even very large aggregates are needed to trigger a cell. For example, experiments by Dintzis et al. (1976) suggest that aggregates of about ten should suffice.

We consider bivalent ligands, such as anti-Ig or two dinitrophenyl (DNP) groups separated by a small spacer, for which cross-linking is well described as a reversible chemical process (cf. Dembo and Goldstein, 1978; Dembo et al., 1978; Dower et al., 1984). For many bivalent ligands, binding and receptor aggregation reactions are fast compared to the kinetics of the cellular response (Dembo and Goldstein, 1978; Goldstein, 1988). This suggests that for such ligands the rate of cellular response may be a function of the equilibrium distribution of cross-linked receptors. Whereas cross-linking of receptors by a single bivalent ligand has been thoroughly studied (DeLisi and Perelson, 1976; Dembo and Goldstein, 1978; Dembo et al., 1978; Reynolds, 1979; Perelson and DeLisi, 1980; Perelson et al., 1980; Perelson, 1984; Macken and Perelson, 1985; Wofsy and Goldstein, 1987; Posner et al., 1995), little has been done to analyze situations where collections of bivalent ligands compete for binding to receptors, even though this is surely the case when cells interact with polyclonal rather than monoclonal anti-receptor antibody.

Based on the binding model presented below we conclude that a polyclonal antibody serum may give rise to a significantly greater amount of cross-linking than a monoclonal antibody (cf. Fig. 1), although the average affinity of the serum and the affinity of the monoclonal antibody are the same. Prerequisite for this greater efficiency in forming cross-links is that the equilibrium constant for cross-linking, i.e., the *cross-linking affinity*, increases when the corresponding equilibrium constant for binding from solution, i.e., the *binding affinity*, increases. Fig. 1 shows that in this case the overwhelming majority of cross-links (more than 96%) is formed by the high-affinity component of the serum (black portion in P1). A significantly smaller fraction of cross-links (73%) are constituted by the high-affinity component when the cross-linking affinity is identical for all antibodies in the serum (black portion in P0). In this case, we find no increase in the amount of cross-linking over the corresponding monoclonal antibody (M and P0 have the same height in Fig. 1). We return to the comparison of polyclonal and monoclonal antibody preparations under Efficiency of Monoclonal and Polyclonal Stimuli. Because the equilibrium binding properties of collections of ligands depend on how the cross-linking affinity changes with the binding affinity (as illustrated by the example), we devote a major part of our paper to the exploration of the consequences of different relationships between binding affinity and cross-linking affinity.

In the next section we derive the equilibrium concentration of cross-links, the crosslinking curve, for collections of ligands and receptors with different affinities. As we discuss further under Excess Ligand Regime, two quantities, the *binding field* and the *cross-linking field*, are necessary and sufficient to characterize the cross-linking curve of bivalent

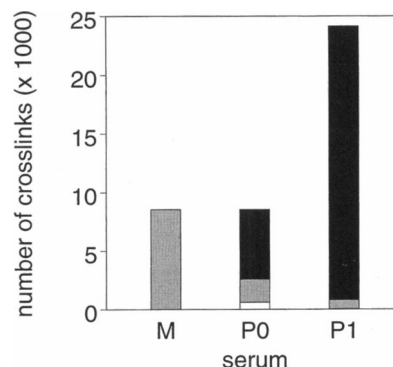


FIGURE 1 Cross-linking induced by three different kinds of serum. M denotes a monoclonal antibody of binding affinity $K = 10^6 \text{ M}^{-1}$ and nondimensional cross-linking affinity $K_{x,i}R_T = 1$ (see Ligands of Different Affinities Cross-linking Receptors on Different Cell Clones for notation). P0 and P1 are polyclonal sera, both composed of 70% low-affinity antibody Ab₁ ($K_1 = 10^5 \text{ M}^{-1}$), 23% medium-affinity antibody Ab₂ ($K_2 = 10^6 \text{ M}^{-1}$), and 7% high-affinity antibody Ab₃ ($K_3 = 10^7 \text{ M}^{-1}$). All antibodies in P0 have the same nondimensional cross-linking affinity $K_{x,i}R_T = 1$, $i = 1, 2, 3$. In serum P1 the nondimensional cross-linking affinity increases in proportion to the binding affinity, i.e., $K_{x,1}R_T = 0.1$, $K_{x,2}R_T = 1$, and $K_{x,3}R_T = 10$. Thus, all preparations have identical average binding affinity and average cross-linking affinity. Shading of the bars shows how many cross-links are formed by Ab₁ (white), Ab₂ (gray), and Ab₃ (black). Comparing P0 and P1, we observe a pronounced enhancement of the number of cross-links formed by the high-affinity antibody Ab₃ in the polyclonal serum P1, yielding a much greater total number of cross-links. Thus the polyclonal serum P1 is more effective in cross-linking than the monoclonal antibody M. Data are taken at a total ligand concentration of 10^{-6} M .

ligands. This means that even for collections of arbitrarily many bivalent ligands a function of only two variables suffices to capture all essential features of cross-linking curves. We study the situation in which the cellular response is a monotonically increasing function of the amount of receptor cross-linking under Cellular Response. We show that clones with low cross-linking affinity are essentially nonresponsive (Response Curves of Cells with Different Affinity), a feature that has been missed in models that used a one-parameter phenomenological dose-response function. Using our theory we also explain why polyclonal anti-receptor antiserum can be more effective in stimulating a biological response than monoclonal antiserum under Efficiency of Monoclonal and Polyclonal Stimuli.

LIGANDS OF DIFFERENT AFFINITIES CROSS-LINKING RECEPTORS ON DIFFERENT CELL CLONES

To introduce our model let us first consider the binding of one type of bivalent ligand to a single kind of cell surface receptor. Binding of free ligand, at concentration C , to a free receptor site, at concentration F , yields singly bound ligand, C_1 . Doubly bound ligand, C_2 , is formed when the second site on a singly bound ligand binds a free receptor site. We measure the concentration of free ligand in mol/liter $\equiv M$ and the cell surface concentrations, F , C_1 , and C_2 , in units

of $1/\text{cm}^2$. The following mass-action rate equations describe the kinetics of ligand binding and cross-linking receptors (see Fig. 2; compare DeLisi and Perelson, 1976; Perelson and DeLisi, 1980):

$$\dot{C}_1 = 2k^+FC - k^-C_1 + 2k_x^-C_2 - k_x^+FC_1 \quad (1)$$

$$\dot{C}_2 = k_x^+FC_1 - 2k_x^-C_2. \quad (2)$$

The initial binding of unbound ligand to a free receptor site is governed by the forward rate constant k^+ and the reverse rate constant k^- , which are measured per site. We define the corresponding binding affinity by $K \equiv k^+/k^-$. The second binding step on the cell surface leading to cross-links depends on the forward cross-linking rate constant k_x^+ and the reverse cross-linking rate constant k_x^- with the associated cross-linking affinity $K_x \equiv k_x^+/k_x^-$. We measure k^+ in M^{-1}/s , k_x^+ in cm^2/s , and the reverse rate constants k^- and k_x^- in $1/\text{s}$. Accordingly, the unit of the binding affinity K is M^{-1} and that of the cross-linking affinity K_x is cm^2 . There are two free sites on the ligand, and either can bind to a free receptor site; thus a factor of 2 appears in the first term in Eq. 1. Similarly, a doubly bound ligand can dissociate from either of two sites, and hence k_x^- is multiplied by 2.

Conservation of receptor sites and ligand requires

$$R_T = F + C_1 + 2C_2, \quad (3)$$

$$C_T = C + \alpha BC_1 + \alpha BC_2, \quad (4)$$

where C_T is the total ligand concentration and R_T the total receptor site concentration. In Eq. 4 the cell surface concentrations of bound ligand C_1 and C_2 have to be multiplied by the cell concentration B and a scale factor α to render them compatible with the volume concentrations C_T and C (cf. Sulzer and Perelson, manuscript submitted for publication). For the units of measurement specified above, α is the ratio of the mean cell surface area and Avogadro's number times 10^3 . Assuming that small B cells are spherical cells with a radius of $3.5 \mu\text{m}$ (Strand, 1978), i.e., a surface area of $1.5 \times 10^{-6} \text{ cm}^2$, we obtain $\alpha \approx 2.5 \times 10^{-27} \text{ cm}^2 \text{ mol}$. A typical value for the total receptor concentration is $R_T = 6.7 \times 10^{10} \text{ cm}^{-2}$, corresponding to 10^5 antibody sites per B cell (Paul, 1993).

Implicit in this formulation is the assumption that a cell carries one and only one specificity of receptor that binds the ligands at hand, and that if the receptor has multiple sites that they bind ligand independently and with the

same rate constants (this latter feature is called the equivalent site hypothesis). The assumption of a single receptor specificity is valid for the binding of antigens to immunoglobulin on B cells. (We use the term "specificity" here because B cells have receptors with different constant regions, e.g., IgD and IgM, but with the same variable region and hence the same binding specificity.) When a multivalent ligand binds to a bivalent or multivalent receptor, ligand-receptor aggregates can form that may contain rings (Perelson and DeLisi, 1980; Posner et al., 1995). Here we neglect ring formation. Thus the theory developed here is rigorous for monovalent receptors, such as Fc receptors, but is only an approximation for multivalent receptors for which ring formation can occur.

Before we generalize Eqs. 1 through 4 to capture the binding between collections of different ligands and cell clones, we switch to new variables, which prove to be more convenient for our purpose. For a bivalent ligand composed of two identical haptens separated by a spacer, concentrations can be expressed in terms of the hapten concentration or in terms of the ligand concentration. Because binding would typically be via the haptens, we develop our theory here in terms of hapten concentration or more generally ligand binding site concentrations. Thus, let

$$L \equiv 2C \quad (5)$$

be the concentration of binding sites on unbound ligand. Moreover, we express all cell surface concentrations in terms of the receptor sites involved. This choice seems appropriate, because the response of the cell depends on the state of its receptors. We denote the fraction of free receptor sites by R , the fraction of receptor sites occupied by singly bound ligand by S , and the fraction of receptor sites occupied by ligand that has both sites bound by X . Then, the relations

$$R \equiv F/R_T \quad S \equiv C_1/R_T \quad \text{and} \quad X \equiv 2C_2/R_T \quad (6)$$

define the transformation between the new, nondimensional variables and the cell surface concentrations used in Eqs. 1–4.

We describe the binding of a set of N different bivalent ligands to the receptors on a set of M different clones of cells by monitoring S_{ij} , the fraction of receptor sites of type i occupied by a singly bound ligand of type j , and X_{ij} , the fraction of receptor sites of type i occupied by a ligand of type j that has both sites bound. That is, X_{ij} is the fraction of

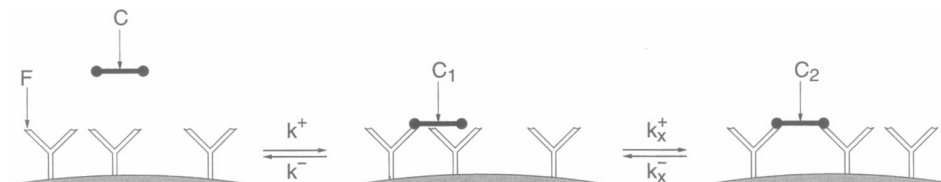


FIGURE 2 Binding and cross-linking of bivalent receptors by bivalent ligand. Unbound ligand, C , binds to free receptor sites, F , with rate constant k^+ , giving rise to singly bound ligand, C_1 . Cross-linking occurs when the free site of a singly bound ligand binds to a free receptor site with rate constant k_x^+ resulting in doubly bound ligand, C_2 . Cross-links dissociate with rate constant k_x^- , and singly bound ligand dissociates with rate constant k^- .

receptor sites i in crosslinks formed by ligand j . Using Eqs. 5 and 6 in Eqs. 1 and 2 we obtain

$$\dot{S}_{ij} = k_{ij}^+ R_i L_j - (k_{ij}^- + R_i R_{iT} k_{x,ij}^+) S_{ij} + k_{x,ij}^- X_{ij}, \quad (7)$$

$$i = 1, \dots, M; \quad j = 1, \dots, N,$$

$$\dot{X}_{ij} = 2(R_i R_{iT} k_{x,ij}^+ S_{ij} - k_{x,ij}^- X_{ij}), \quad (8)$$

$$i = 1, \dots, M; \quad j = 1, \dots, N.$$

The factor 2 in Eq. 8 is due to the fact that X_{ij} describes the number of receptor sites in cross-links; formation and dissociation of cross-links affect two receptor sites simultaneously. We assume that the total concentration of receptor sites R_{iT} is constant.

Conservation of receptor and ligand sites, Eqs. 3 and 4, now reads

$$1 = R_i + \sum_{j=1}^N (S_{ij} + X_{ij}), \quad i = 1, \dots, M, \quad (9)$$

$$L_{jT} = L_j + \alpha \sum_{i=1}^M B_i R_{iT} \left(S_{ij} + \frac{1}{2} X_{ij} \right), \quad j = 1, \dots, N, \quad (10)$$

where L_{jT} is the total concentration of ligand sites j .

At equilibrium, the fraction of receptor sites occupied by singly bound ligands and those of receptor sites in cross-links are given by

$$S_{ij} = R_i K_{ij} L_j, \quad i = 1, \dots, M; \quad j = 1, \dots, N, \quad (11)$$

$$X_{ij} = R_i R_{iT} K_{x,ij} S_{ij}, \quad i = 1, \dots, M; \quad j = 1, \dots, N. \quad (12)$$

Using Eqs. 11 and 12, the conservation equation for receptor sites at equilibrium becomes

$$1 = R_i^2 h_{x,i} + R_i (h_i + 1), \quad i = 1, \dots, M, \quad (13)$$

where we have defined the cross-linking field, $h_{x,i}$, and the binding field, h_i , by

$$h_{x,i} \equiv \sum_{j=1}^N R_{iT} K_{x,ij} K_{ij} L_j, \quad i = 1, \dots, M, \quad (14)$$

$$h_i \equiv \sum_{j=1}^N K_{ij} L_j, \quad i = 1, \dots, M. \quad (15)$$

We emphasize that these two ligand-dependent quantities are necessary to describe the cross-linking of receptors by a collection of bivalent ligands of different affinities and the ensuing cellular response properly. Models of the B cell response frequently refer to cross-linking as a possible explanation for the bell-shaped dose-response curve they assume (De Boer, 1988; Varela et al., 1988; Weisbuch et al., 1990; De Boer et al., 1992; Sulzer et al., 1993). However, they have not taken into account that two parameters are required and have, instead, used dose-response functions

that depend upon a single parameter that is equivalent to the binding field, h_i , in Eq. 15.

The equilibrium concentrations of free ligand sites, which are needed to specify the fields (Eqs. 14 and 15), are given by

$$L_j = \frac{L_{jT}}{1 + \alpha \sum_{k=1}^M B_k R_k R_{kT} K_{kj} (1 + \frac{1}{2} R_k R_{kT} K_{x,kj})}, \quad j = 1, \dots, N, \quad (16)$$

an expression derived by inserting Eqs. 11 and 12 into the conservation equation for ligands (Eq. 10).

Formally, the conservation equation for receptor sites, Eq. 13, looks similar to the conservation equation used in the case of binding of a single ligand type to a single receptor type (Perelson and DeLisi, 1980). In general, however, the conservation equations for different clones of cells expressing receptors with different affinities are coupled, because the free ligand site concentrations that occur in the fields (Eqs. 14 and 15) depend on all of the free receptor site concentrations. In the next section we show that the conservation equations for receptor sites (Eq. 13) decouple in the excess ligand approximation, where we assume $L_j = L_{jT}$, and we recover a generalized form of the results of Perelson and DeLisi (1980). The excess ligand approximation is valid when the ligand site concentration is much larger than the receptor site concentration.

EXCESS LIGAND REGIME

Biologically, we frequently encounter situations where the ligand site concentration is much larger than the corresponding receptor site concentration. Under such circumstances, ligand binding hardly affects the concentration of free ligand sites, and one can assume $L_j = L_{jT}$. This we call the excess ligand approximation. At the initiation of an immune response, for instance, the antigen concentration usually exceeds the concentration of membrane-bound immunoglobulin on the few antigen-specific B cells by orders of magnitude. Only late in the response, when the specific B cell populations have expanded considerably and antigen is almost eliminated, is the excess ligand approximation expected to fail. In vitro experiments have been set up so that the excess ligand approximation is valid (e.g., Dembo et al., 1978). Thus, it is worthwhile to examine the excess ligand regime in detail. The analysis in the remainder of this paper is performed under the assumption of the excess ligand regime.

We use the excess ligand approximation when the second, cell-concentration-dependent term in the denominator of the equilibrium concentration of free ligand sites (Eq. 16) is much less than 1, i.e.,

$$\alpha \sum_{k=1}^M B_k R_k R_{kT} K_{kj} \left(1 + \frac{1}{2} R_k R_{kT} K_{x,kj} \right) \ll 1. \quad (17)$$

Then the concentration of free ligand sites hardly differs from the total concentration of ligand sites, because the tiny

amount of binding to a few receptors hardly affects the vast pool of unbound ligand.

The excess ligand approximation is exact when the cell densities B_i vanish. Here, the free ligand site concentration becomes identical to the total ligand site concentration (cf. Eq. 16). The conservation equations for receptor sites (Eq. 13) decouple, because the cross-linking and the binding fields now depend on the constant total ligand site concentrations, i.e.,

$$h_{x,i} = \sum_{j=1}^N R_{iT} K_{x,ij} K_{ij} L_{jT}, \quad (18)$$

and

$$h_i = \sum_{j=1}^N K_{ij} L_{jT}, \quad (19)$$

and hence are independent of the number of free receptor sites.

The fraction of free receptor sites on cells of type i can easily be computed in the excess ligand regime from Eq. 13, with the fields given by Eqs. 18 and 19:

$$R_i = \frac{-(h_i + 1) + \sqrt{(h_i + 1)^2 + 4h_{x,i}}}{2h_{x,i}}, \quad (20)$$

$i = 1, \dots, M.$

We define (cf. Perelson and DeLisi, 1980)

$$\delta_i(h_i, h_{x,i}) \equiv \frac{h_{x,i}}{(h_i + 1)^2} \quad (21)$$

and obtain from Eqs. 11 and 12

$$X_i = \sum_{j=1}^N X_{ij} = R_i^2 h_{x,i} = \frac{1 + 2\delta_i - \sqrt{1 + 4\delta_i}}{2\delta_i}, \quad (22)$$

$i = 1, \dots, M,$

for the fraction of receptor sites in cross-links on cells of type i .

Equations 18 through 20 show that in the excess ligand regime the binding of ligands to a certain clone of cells does not affect and is not affected by the binding of ligands to other cell clones. Because of this mutual independence of the binding properties for cells carrying different receptors, we focus on a particular type of cell and drop the index i for the following considerations in which we derive some fundamental properties of the cross-linking curve.

The fraction of receptor sites in cross-links, X , when plotted against the ligand site concentration, is called a cross-linking curve. As noted by (Perelson and DeLisi, 1980) in the case of a single ligand, X is a monotonically increasing function of δ , i.e.,

$$\frac{dX(\delta)}{d\delta} = \frac{1 + 2\delta - \sqrt{1 + 4\delta}}{2\delta^2 \sqrt{1 + 4\delta}} > 0, \quad (23)$$

and, thus, the symmetries of $X(\delta)$ are identical to those of $\delta(h, h_x)$ itself. Perelson and DeLisi (1980) have shown that δ has a single maximum at $h = 1$ irrespective of the value of h_x , or that δ expressed as a function of the ligand site concentration L has a maximum at $L = K^{-1}$.

We now generalize these results to the multi-ligand case. Defining the ligand composition $\mathbf{f} \equiv \{f_j | L_{jT} = f_j L_T, j = 1, \dots, N\}$, where $L_T \equiv \sum_{j=1}^N L_{jT}$ is the total ligand site concentration (f_j is the fraction of ligand j in the set of ligands, $\{L_{jT}, j = 1, \dots, N\}$), we can rewrite the binding and cross-linking fields in the form

$$h = \sum_{j=1}^N K_{ij} f_j L_T \equiv \langle K \rangle L_T, \quad (24)$$

and

$$h_x = R_T \sum_{j=1}^N K_{xj} K_{ij} f_j L_T \equiv R_T \langle K_x K \rangle L_T. \quad (25)$$

Under the condition that the ligand composition is fixed, we can write δ as function of a single variable, the total ligand site concentration, L_T , i.e.,

$$\delta(L_T) = \frac{R_T \langle K_x K \rangle L_T}{(\langle K \rangle L_T + 1)^2}. \quad (26)$$

$\delta(L_T)$ attains its maximum where the total ligand concentration is equal to the inverse of the average binding affinity, $L_T = \langle K \rangle^{-1} \equiv L_{T,\max}$, since

$$\frac{d\delta}{dL_T} = \frac{R_T \langle K_x K \rangle (1 - \langle K \rangle L_T)}{(\langle K \rangle L_T + 1)^3}. \quad (27)$$

Moreover, $\delta(L_T)$ as a function of the total ligand site concentration for fixed ligand composition \mathbf{f} is symmetric on a logarithmic scale around its maximum at $L_{T,\max}$, i.e. (compare Dembo and Goldstein, 1978, as well as Perelson and DeLisi, 1980, Theorem 2),

$$\delta(L_{T,\max} \ell) = \frac{R_T \langle K_x K \rangle \langle K \rangle^{-1} \ell}{(\langle K \rangle \langle K \rangle^{-1} \ell + 1)^2} = \frac{R_T \langle K_x K \rangle \langle K \rangle^{-1} \ell^{-1}}{(\langle K \rangle \langle K \rangle^{-1} + \ell^{-1})^2} \quad (28)$$

$$= \frac{R_T \langle K_x K \rangle \langle K \rangle^{-1} \ell^{-1}}{(1 + \langle K \rangle \langle K \rangle^{-1} \ell^{-1})^2} = \delta(L_{T,\max} \ell^{-1}).$$

Equation 28 holds for any value of ℓ , a real number measuring the distance from $L_{T,\max}$. Only the height of the maximum depends upon the cross-linking affinity. It is given by (compare Perelson and DeLisi, 1980, Theorem 3)

$$X_{\max} \equiv X[\delta(h = 1, h_x)] \quad (29)$$

$$= 1 + \frac{2\langle K \rangle}{R_T \langle K_x K \rangle} \left(1 - \sqrt{1 + \frac{R_T \langle K_x K \rangle}{\langle K \rangle}} \right).$$

Summarizing, the bell-shaped nature of the cross-linking curve and the location of its maximum in the case of a collection of ligands remain the same as in the single

ligand-single cell case (Perelson and DeLisi, 1980), when we generalize the binding affinity to be the average binding affinity and the ligand site concentration to be the total ligand site concentration.

CELLULAR RESPONSE

Cross-linking of its receptors may activate a cell and induce a response such as cell division. In this section we discuss how to model the cellular response as a function of the average concentration of receptor sites in cross-links. Our approach will be phenomenological in that we do not attempt to capture the triggering of intracellular signaling cascades by cross-linked receptors, which eventually cause the cell to respond (cf. Cambier and Ransom, 1987; Cambier et al., 1994).

We consider the proliferative response of B cells to bivalent antigen. In De Boer et al. (1996) we apply the theory developed here to idiotypic networks, in which case the bivalent antigen is an anti-idiotypic antibody binding to B cell receptors. Ligand-receptor binding reaches equilibrium within a few minutes for most antibody-hapten combinations. Pecht and Lancet (1977), for instance, show that in a large sample of antibody-hapten combinations the reverse rate constant k_{ij}^- is on the order of 0.1 s^{-1} or larger except for a few high-affinity interactions. The inverse of the reverse rate constant gives a lower bound to the time scale on which equilibrium is attained. The same is true for bivalent ligands when we assume that the rate constant for the dissociation of crosslinks, $k_{x,ij}^-$, is comparable to k_{ij}^- (Perelson and DeLisi, 1980). In this case, the time to reach chemical equilibrium is small compared with typical time scales of the cellular response; a B cell divides at most once every 6 h (Zhang et al., 1988). Consequently, we assume that the equilibrium concentration of cross-links on a B cell is the relevant quantity determining its response.

When binding relaxes to equilibrium on a time scale comparable to the cellular events one has to consider how the cross-linking curve changes in time. Qualitatively, the height and width of the cross-linking curve increase, and it attains its maximum at progressively lower ligand concentration (Perelson and DeLisi, 1980; Perelson, 1980). The response rate may then depend on the number of cross-links that can be established before the occurrence of cellular events that prevent further receptor-mediated signaling. B cells, for instance, internalize their antibody receptors within an hour after binding has started (Puré and Tardelli, 1992).

More dynamical notions of cell activation are conceivable, too. Instead of the concentration of cross-linked receptors the rate of recruitment of new receptors into the cross-linked state may be the signaling event on the cell membrane. If cross-linking is an irreversible process the average time needed to form a sufficiently large ligand-receptor cluster may be a better candidate for the signaling event (Bell, 1974; DeLisi and Perelson, 1976; Faro and

Velasco, 1993, 1994); note that in the case of irreversible cross-linking all receptors are cross-linked at equilibrium. If the reverse binding constant is on the order of 10^{-3} s^{-1} or smaller the reaction will attain its equilibrium on the time scale of hours and a dynamical description of cell activation will be more appropriate than our equilibrium analysis. Dynamical notions of receptor ligation and cell activation are beyond the scope of this paper, however.

Even if receptor cross-linking reaches equilibrium rapidly compared with a cellular response, we are still confronted with the question: How does the rate of the cell's response depend upon the number of cross-links on its surface? In general, the response may not be exactly proportional to the fraction of receptor sites in cross-links and may differ depending on the particular response being modeled, e.g., proliferation, antibody secretion, etc. The amount of histamine released by basophils has been shown to depend monotonically upon the number of cross-links on the cell surface (Dembo et al., 1978). Goroff et al. (1991) show that cross-linking of their IgD receptors is necessary for the activation of B cells and subsequent antibody secretion. In the absence of a quantitative relationship between the degree of cross-linking and the rate of B cell responses we assume that, in analogy to basophils, the observed response is a monotonically increasing function of X , the fraction of receptor sites cross-linked. A class of functions possessing this property is the set of sigmoid response functions F_r of the form

$$F_r(X) \equiv \frac{X^q}{X^q + \vartheta^q}, \quad (30)$$

which we shall use for our analysis below. In this Hill function the signaling threshold ϑ determines the number of cross-links required for half-maximum response. The Hill coefficient q controls the steepness of the transition from no to full response. Different types of responses may be characterized by different signaling thresholds and/or Hill coefficients (e.g., Monroe and Cambier, 1983). This fact constitutes one of the tacit assumptions of the model of the B cell response introduced by Sulzer et al. (1993) where proliferation and terminal differentiation of B cells may require a different degree of cross-linking. Consistent with the notion of different signaling thresholds are the results of Mongini et al. (1991), which show that cross-linking-induced progression into different phases of the cell cycle has different affinity requirements. Implicit in this formulation is the assumption that T cell help is not limiting for the response under consideration.

Nonmonotonic relationships between the equilibrium concentration of cross-links and the cellular response are also observed. The histamine release of basophils, for instance, has been shown to decrease in some patients when the concentration of cross-links still increases (Becker et al., 1973; Magro and Alexander, 1974). In many cases, this nonmonotonic dependence can be explained as the net result of two antagonistic, elementary reactions with different

dose-response relationships (cf. Dembo and Goldstein, 1980; Sulzer et al., 1993). Perelson et al. (1982) discuss a kinetic method for determining whether the decline in histamine release at high ligand concentrations is due to a lack of cross-links (type I inhibition) or is an example of this nonmonotonic behavior, i.e., is due to too much cross-linking (type II inhibition).

For cross-linking-induced B cell responses we expect signaling thresholds ϑ between 10^{-4} and 10^{-1} because, on one hand, at least a few receptors must be cross-linked to distinguish spontaneous from induced responses (10 bound receptor sites on a cell with 10^5 receptor sites yields the lower estimate; here we assume that a B cell carries about 50,000 antibody receptors) and, on the other hand, aggregation of all receptors cannot be required or else the cell cannot be sensitive to a reasonable range of stimuli (10% receptor sites bound yields the upper estimate).

Let us assume that the B cell proliferative response obeys a dose-response relationship of the type defined by Eq. 30, where the threshold for proliferation is denoted ϑ_p and the proliferative response function is called F_p . We show the fraction of receptor sites in cross-links (*low dashed curve*) and one-parameter families of dose-response functions (*solid lines*) for proliferation in Fig. 3. The width of the proliferation function F_p increases as the signaling threshold, ϑ_p , decreases (*left panel*), because the cells require a smaller number of cross-links to start a response. As the Hill coefficient q increases (*right panel*) the transition from no proliferation to maximum proliferation becomes steeper. Dose-response functions for other kinds of cellular responses may be expected to depend in the same way upon the parameters ϑ and q .

Now we are ready to ask how the proliferative response of B cells depends on the affinities of the antibody-antigen

interaction. To address this question we employ a simple model for B cell proliferation defined by

$$\dot{B}_i = [pF_p(X_i) - d]B_i. \quad (31)$$

Different kinds of B cells, B_i , distinguished by the affinities of their antibody receptors for the antigen, are assumed to die at a constant rate d and to proliferate at maximum rate p modulated by the fraction of receptor sites in cross-links, X_i . For the dose-response function, F_p , we employ a Hill function, Eq. 30, with proliferation threshold, ϑ_p . We take the ligand site concentration to be an externally controlled parameter, and thus, the concentration of cross-links is also controlled externally. Clearly, a certain minimum number of cross-links is required to induce enough proliferation to overcome cell decay. The dotted line in Fig. 3 shows an example of the ratio d/p . All antigen concentrations, L , for which the proliferation function, F_p , is larger than the dotted line induce a net growth of the B cell clone characterized by F_p . This is the *stimulatory range* of antigen concentration. We call a cell B_i *responsive* when an antigen concentration exists such that its proliferation rate exceeds its death rate. Besides the parameters ϑ_p and q characterizing the response function, the responsiveness of a cell depends on the affinity of its antibody receptor for antigen. Not all B cells are responsive to a given antigen.

A cell is capable of responding in the sense defined above only if its proliferation at the optimally stimulatory ligand site concentration, i.e., at the maximum of the cross-linking curve, exceeds the decay rate. In the case of the binding of a single type of bivalent ligand to cell receptors, the situation in an immune response against an antigen, the height of the cross-linking curve is determined by $R_T K_x$ only (cf. Eq. 29; when we consider the binding of a single type of ligand, the averages in Eq. 29 reduce to the corresponding value for the single ligand-receptor interaction):

$$X_{\max} = 1 + \frac{2}{R_T K_x} (1 - \sqrt{1 + R_T K_x}). \quad (32)$$

The cell population can expand, if the maximum possible proliferation overcompensates the death rate, i.e.,

$$pF_p(X_{\max}) > d \quad (33)$$

$$\Leftrightarrow K_x > K_{x,\min} \equiv \frac{4(p/d - 1)^{1/q} \vartheta_p}{R_T [(p/d - 1)^{1/q} - \vartheta_p]^2} \approx \frac{4\vartheta_p}{R_T (p/d - 1)^{1/q}}.$$

The critical cross-linking affinity, $K_{x,\min}$, increases linearly with the signaling threshold ϑ_p unless $\vartheta_p \approx (p/d - 1)^{1/q}$, in which case the approximation in Eq. 33 does not hold.

B cell clones that have a cross-linking affinity slightly larger than $K_{x,\min}$ will expand for a small range of antigen concentrations centered at the optimal antigen concentration, $L_{T,\max}$, which yields maximum cross-linking. The larger $R_T K_x$ the wider the range of antigen concentrations for which the B cell clone grows. However, if the cross-linking affinity is smaller than $K_{x,\min}$ the clone will not grow at any concentration of the antigen. Thus, in a strict, math-

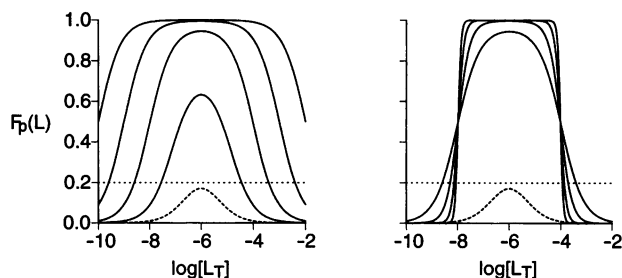


FIGURE 3 Proliferation functions for different response characteristics. We show the dose-response relationship for proliferation of B cells employing the phenomenological sigmoid response function, Eq. 30, varying the parameters ϑ_p and q of the response function. The low dashed curve in both panels displays the cross-linking curve for ligand-receptor interaction with binding affinity $K = 10^6 \text{ M}^{-1}$ and a nondimensional cross-linking parameter $R_T K_x = 1$. In the left panel, $\vartheta_p = 10^{-1}, 10^{-2}, 10^{-3}$, and 10^{-4} (inside to out) for $q = 1$. In the right panel, $q = 1, 2, 4$, and 8 (bottom to top at the maximum) for $\vartheta_p = 10^{-2}$. The dotted line shows an example of the ratio of the decay rate to the proliferation rate of B cells: $d/p = 0.2$. A ligand/antigen is stimulatory at concentrations where F_p is larger than d/p (see Eq. 31). We observe that the range of stimulatory ligand site concentrations increases as the signaling threshold ϑ_p decreases.

emational sense, the responsiveness of a B cell hinges on its cross-linking affinity. The binding affinity determines only the location of the maximum of the cross-linking curve, i.e., where the range of stimulatory antigen concentrations is located. However, as a practical matter, cross-linking affinity and binding affinity may be related (see below). And even if $K_x > K_{x,\min}$, the antigen concentration required to stimulate a cell with a low binding affinity may be higher than achievable under physiological conditions. In this sense, the binding affinity also plays a role in determining the observed responsiveness of cells.

The cross-linking affinity, which, according to Eq. 33, determines the responsiveness of a given cell type, is difficult to measure (Erickson et al., 1991) and therefore is generally not available. Instead, ligand-receptor interactions are usually characterized by the binding affinity, which, by itself, has only little influence on responsiveness. It is therefore desirable to establish a relationship between cross-linking and binding affinities that would allow us to then link a cell's responsiveness to its binding affinity for the ligand. In fact, we expect cross-linking and binding affinities to be related, because both are in part determined by local interactions (van der Waals forces, etc.) between the contact sites on the receptor and ligand. These local interactions should remain roughly the same irrespective of whether the ligand is in solution or already bound to the cell at one site. This issue has previously been discussed from a statistical mechanical perspective (Dembo and Goldstein, 1978; Dwyer and Bloomfield, 1981).

We expect that as the binding affinity of ligands increases, their cross-linking affinity will also increase. Goldstein and Wofsy (1994) argue that the difference between binding from solution and cross-linking on the cell surface basically is that the bound ligand sees an effective concentration of receptor sites such that

$$R_T K_x = C_e K, \quad (34)$$

where the effective receptor site concentration C_e depends upon the geometry and flexibility of the receptor and ligand, and the spatial distribution of receptors, but is independent of the chemistry between ligand and receptors. Equation 34 implies a proportionality between binding and cross-linking affinities. If this is the case, then the existence of a minimum cross-linking affinity implies that there is also a minimum binding affinity for the B cell response. Experiments by Klinman (1972), Teale and Klinman (1980), Riley and Klinman (1986), and Mongini et al. (1991) support the idea of a threshold affinity for triggering B cell events.

PARAMETERIZING THE MUTUAL DEPENDENCE OF BINDING AND CROSS-LINKING AFFINITIES

We introduce a parameterization for the relationship between binding and cross-linking affinities that will facilitate our ensuing analysis of the dependence of cross-linking curves upon the two types of affinities. Consider two ligand-

receptor interactions, with binding and cross-linking affinities ($K_i, K_{x,i}$) and ($K_j, K_{x,j}$), respectively. Denote the ratio of binding affinities by

$$\kappa_{ij} \equiv \frac{K_i}{K_j} \quad (35)$$

and the ratio of cross-linking affinities by

$$\kappa_{ij}^{\eta_{ij}} \equiv \frac{K_{x,i}}{K_{x,j}}. \quad (36)$$

This parameterization has the advantage that the case of constant cross-linking affinity can be characterized independently of the difference in affinity: $\eta_{ij} = 0$ means that the two cross-linking affinities are identical. Likewise, proportionality of binding and cross-linking affinity corresponds to $\eta_{ij} = 1$ (again irrespective of the value of κ_{ij}). Note, however, that this parameterization can describe any relation between cross-linking affinities only if $K_i \neq K_j$. Therefore, we exclude the case $K_i = K_j$ from the following arguments and comment on it at the end of the next section.

The parameter η_{ij} conveniently describes how changes in cross-linking affinity are related to changes in binding affinity. The cross-linking affinity increases linearly with the binding affinity, when $\eta_{ij} = 1$. It increases sublinearly when $\eta_{ij} < 1$ and superlinearly when $\eta_{ij} > 1$, and is constant when $\eta_{ij} = 0$. We allow also for cases with $\eta_{ij} < 0$, which implies that an increase in binding affinity is accompanied by a decrease in cross-linking affinity. Notice that the case of almost identical effective concentrations of receptor sites C_e implies $\eta_{ij} \approx 1$.

It is easy to compare the cross-linking curves of ligand-receptor interactions with identical $\eta_{ij} = \eta$ (for all i and j). Restricting our attention to this subset of all possible interactions among a given collection of ligands and receptors, it makes sense to talk about the affinity of an antibody-antigen interaction, because once we specify one affinity characterizing the interaction (e.g., K) the other affinity (e.g., K_x) is determined. All of the next section will be concerned with subsets of interactions that are homogeneous with respect to η . Different subsets of ligand and receptors, of course, can behave differently, depending on the appropriate value of η . The choice $\eta_{ij} = \eta = 0$ for the whole set of interactions deserves special mention, because in this case we recover the description of the B cell response previously used in models of the idiotypic network (De Boer, 1988; Varela and Stewart, 1990; Weisbuch et al., 1990). This special case is studied by De Boer et al. (1996).

RESPONSE CURVES OF CELLS WITH DIFFERENT AFFINITY

During an immune response a variety of clones with different affinities for the antigen become activated and grow. In fact, the spectrum of affinities of the responsive clones may change during the course of the response. In studies examining the response to 2,4-dinitrophenyl

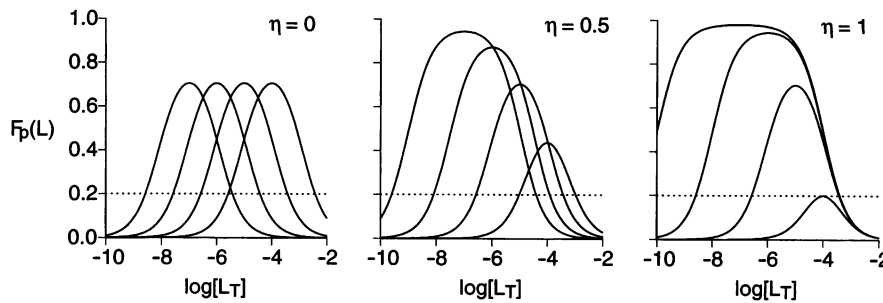


FIGURE 4 Proliferation functions for different values of η . In each panel the proliferation function F_p is plotted for four different binding affinities: $K = 10^7, 10^6, 10^5$, and 10^4 M^{-1} (left to right). The parameters of F_p are $\vartheta_p = 0.01$ and $q = 1$. The horizontal line denotes $d/p = 0.2$. (Left) $\eta = 0$. With $\eta = 0$, the cross-linking affinity is independent of the binding affinity and hence is the same for each interaction. Here $R_T K_x = 0.1$ for all four curves. The stimulatory range keeps its width and is shifted to the right for interactions of lower binding affinity. Low-affinity clones are stimulated better than high-affinity clones at high ligand site concentration. (Middle) $\eta = 0.5$. Here $R_T K_x = 1.0, 0.1 \sqrt{10}, 0.1$, and $0.01 \sqrt{10}$ (left to right). The width of the stimulatory range decreases with decreasing affinity. Still, low-affinity clones receive better stimulation at high ligand site concentration. (Right) $\eta = 1$. Here $R_T K_x = 10, 1.0, 0.1$, and 0.01 (left to right). The proliferation function of a low-affinity clone is embedded entirely within the proliferation function of the clones with higher affinity. Clones with high affinity are always stimulated more than those with low affinity.

(DNP) attached to a protein carrier, it was found that early in an immune response antibodies that bound DNP have relatively low binding affinity (10^5 to 10^6 M^{-1}), whereas later in the response antibodies with high binding affinity (10^7 to 10^8 M^{-1}) become prevalent (Eisen and Siskind, 1964). Here we shall not discuss how this increase in affinities for the antigen comes about. However, we explore the circumstances under which B cells with high binding affinity receive better stimulation than B cells with low binding affinity.

B cells carrying high-affinity immunoglobulin receptors are stimulated better than those carrying low-affinity receptors (irrespective of the ligand site concentration) when the cross-linking curve for any antibody-antigen interaction with given affinity is entirely embedded within the cross-linking curve for every interaction with higher affinity. Whether low-affinity cross-linking curves are actually embedded in high-affinity cross-linking curves depends crucially on how changes in binding affinity are related to changes in cross-linking affinity.

Fig. 4 shows how proliferation functions change as the affinities change, assuming homogeneous subsets of interactions with different relationships between binding and cross-linking affinities. We consider three cases: $\eta = 0$, $\eta = 0.5$, and $\eta = 1$. If the cross-linking affinity is the same for all antibody-antigen interactions ($\eta = 0$), the stimulatory range has identical width for all clones irrespective of the corresponding binding affinity. The cross-linking curves and proliferation functions are simply shifted to the right with decreasing affinity. Lower binding affinity can always be compensated by higher ligand site concentration. For any given antigen concentration, a particular clone is stimulated best. Clones with lower as well as higher affinity receive less stimulation, and the reduction in stimulation increases with the distance from the optimally stimulated clone. Because with $\eta = 0$ the height of the cross-linking curve remains the same for different values of the binding affinity, either all clones

or no clones respond to antigen, i.e., have a maximum proliferation rate that is greater than their death rate. However, if cells respond then the ligand concentration will be important in determining the degree of response.

If the cross-linking affinity increases sublinearly with the binding affinity ($\eta = 0.5$), the stimulatory range increases in width with increasing affinities. At high ligand site concentration low-affinity clones can still be stimulated better than high-affinity clones. However, the range of antigen concentrations where this happens is considerably narrower than in the case $\eta = 0$.

If the cross-linking affinity increases linearly (or super-linearly) with the binding affinity ($\eta \geq 1$), low-affinity clones are stimulated less than high-affinity clones, irrespective of the antigen concentration. The proliferation function of a clone of any affinity is nested within the proliferation functions of clones having higher affinity for the antigen. We conclude this section by formally proving this fact.

For the following arguments we choose the binding affinity, K , and the cross-linking affinity, K_x , of an arbitrary, but fixed, ligand-receptor interaction in the subset of ligand-receptor pairs being studied as a point of reference and measure all binding and cross-linking affinities with respect to K and K_x , respectively. Then any ligand-receptor interaction can then be characterized by

$$K_i = \kappa_i K \quad \text{and} \quad K_{x,i} = \kappa_i^\eta K_x. \quad (37)$$

To determine whether response functions for interactions of different affinities intersect, we consider the derivative of the response function F_r (or in the case of proliferation F_p) with respect to κ_i for the response to a single ligand at total ligand site concentration L_T . A given response function is completely embedded within those for interactions with higher affinities when this derivative is positive irrespective of the ligand site concentration. Under these conditions, an increase in affinity will lead to higher response at all ligand site concentrations.

Because of the monotonic dependence of the response function F_r on the concentration of cross-links X , and of X upon δ (cf. Eq. 23), it suffices to consider the derivative of δ as a function of κ_i . Dropping the subscript i for convenience, from Eqs. 18, 19, 21, and 37,

$$\delta(\kappa) = \frac{\kappa^{1+\eta} R_T K_x K L_T}{(1 + \kappa K L_T)^2}, \quad (38)$$

and hence

$$\frac{\partial \delta}{\partial \kappa} = \frac{\kappa^\eta R_T K_x K L_T}{(1 + \kappa K L_T)^3} [(\eta + 1) + (\eta - 1) \kappa K L_T]. \quad (39)$$

For $\eta \geq 1$, the derivative $\partial \delta / \partial \kappa$ is strictly positive for all values of L_T . Thus an increase in affinity will lead to a higher δ and hence a higher response at all ligand site concentrations. This shows that the response functions are nested within one another.

We can obtain further useful information from Eq. 39. When $\eta < 1$, there exist values of L_T for which $\partial \delta / \partial \kappa > 0$ is no longer true and thus the response curves will cross. This is seen in Fig. 4. For $\eta = 1$,

$$\frac{\partial \delta}{\partial \kappa} = \frac{2 \kappa R_T K_x K L_T}{(1 + \kappa K L_T)^3}, \quad (40)$$

which approaches 0 for $L_T \rightarrow \infty$. Thus, as indicated in Fig. 4, the response curves will converge at high ligand site concentrations.

In summary, cross-linking curves are embedded within one another when cross-linking affinities increase at least as fast as the corresponding binding affinities, i.e., $\eta \geq 1$. Under these conditions a high-affinity clone is always stimulated more than a low-affinity clone. If cross-linking affinities increase sublinearly with the binding affinities, there is a range of ligand site concentrations where low-affinity clones are stimulated more than high-affinity clones. Let us compare the response of two clones, clones 1 and 2, having high and low affinity for an antigen, respectively. We choose to measure affinities with respect to $(K_1, K_{x,1})$, which implies $\kappa_1 = 1$ and $\kappa_2 < 1$. The low-affinity clone 2 is stimulated more than the high-affinity clone 1 when $\partial \delta / \partial \kappa < 0$, i.e., for

$$L_T > \frac{1 + \eta}{1 - \eta} \frac{1}{\kappa_2 K} > \frac{1}{K}, \quad (41)$$

when $0 < \eta < 1$. This range is located at ligand site concentrations larger than the optimally stimulatory one ($K^{-1} = K_1^{-1}$) for the high-affinity clone.

Finally, if for a subset of interactions the binding affinities are the same but the cross-linking affinities are different, cross-linking curves of interactions with low cross-linking affinity are embedded within those of interactions with higher cross-linking affinity. The cross-linking affinity only modulates the height of the cross-linking curve but leaves the location of the maximum unchanged.

The graphs shown in the right panel of Fig. 4 bear a striking resemblance to curves published by Dembo and Goldstein (1978) for the inhibition of cross-linking by monovalent haptens. This turns out not to be an accident but a result of the mathematics of cross-linking. Cross-linking curves at different hapten concentrations H_T are nested within each other exactly like the cross-linking curves for different affinities when the cross-linking affinity increases linearly with the binding affinity. This equivalence is easy to understand. In the presence of monovalent haptens at concentration H_T binding with affinity K_h to the same receptor as the ligand L_T , the concentration of cross-links X is determined by Eq. 22 with δ now modified to take into account hapten binding. According to Dembo and Goldstein (1978),

$$\delta = \frac{R_T K_x K L_T}{(1 + K L_T + K_h H_T)^2} = \frac{R_T K'_x K' L_T}{(1 + K' L_T)^2}, \quad (42)$$

where $K' = K/(1 + K_h H_T)$ and $K'_x = K_x/(1 + K_h H_T)$. Thus, both the binding and the cross-linking affinity of the bivalent ligand are reduced by a factor $1/(1 + K_h H_T)$. Hence, the effective binding and cross-linking affinities decrease proportionally to each other as the hapten concentration increases. In other words, the effective cross-linking affinity changes linearly with the effective binding affinity. The case of a linear change of a true cross-linking affinity as function of the true binding affinity is thus formally equivalent to the change of cross-linking curves by inhibitory monovalent haptens.

EFFICIENCY OF MONOCLONAL AND POLYCLONAL STIMULI

Frequently, antibodies against cell surface markers and receptors are used to stimulate, tolerize, or even kill cells in the immune system (e.g., Braun and Unanue, 1980; Jonsson and Eichmann, 1990; Gascan et al., 1991; Finkelman et al., 1995; Morel et al., 1992; Shizuru et al., 1992). Polyclonal antibody sera frequently prove to be more potent immune modulators than monoclonal antibodies (J. Uhr, personal communication). One possible explanation for this difference, in systems that respond to cross-linking, is that rare high-affinity antibodies present in the polyclonal serum at low concentrations may be disproportionately effective at cross-linking receptors. We show this by the following example.

For illustrative purposes, consider the simple polyclonal serum composed of a collection of three different antibodies (70% low-affinity (10^5 M^{-1}), 23% medium-affinity (10^6 M^{-1}), and 7% high-affinity (10^7 M^{-1})) that we have introduced in the first section. Assume $\eta = 1$, so that the cross-linking constants for these three antibodies also vary by factors of 10 (serum P1 in Fig. 1). This serum has an average binding affinity of $\langle K \rangle = 10^6 \text{ M}^{-1}$ and an average nondimensional cross-linking affinity of $R_T \langle K_x \rangle = 1$. In Fig. 5 we compare cross-linking induced by the serum P1 with

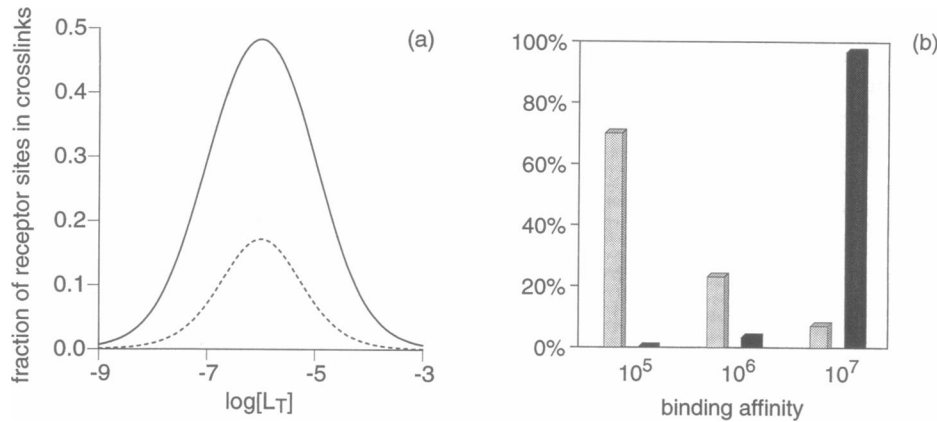


FIGURE 5 Cross-linking induced by polyclonal versus monoclonal sera. (a) compares the crosslinking curve for polyclonal (*solid*) and for monoclonal (*dashed*) sera, both of which have the same average binding affinity (10^6 M^{-1}). We observe that the polyclonal serum yields about three times as many cross-links as the monoclonal antibody over the entire range of ligand concentrations. For the polyclonal serum, (b) shows the percentages of antibodies of different affinities in the total antibody population (*light gray bars*) and in the population of antibodies forming cross-links (*dark gray bars*). The high-affinity antibodies are drastically enriched in cross-links. Binding affinities are paired with cross-linking affinities such that $(K, R_T K_x) = (10^5, 0.1)$, $(10^6, 1)$, and $(10^7, 10)$, i.e., $\eta = 1$.

that induced by a monoclonal antibody with the average binding affinity and the average cross-linking affinity of the serum. As illustrated in Fig. 5 *a*, the polyclonal serum induces about three times as many cross-links as the monoclonal antibody. If cell response is proportional to the degree of receptor cross-linking, the polyclonal serum will be a more potent stimulator of the cellular response than the equivalent monoclonal antibody.

Receptors that require cross-linking to induce a signal are very efficient in selecting the high-affinity fraction of ligand collections. Fig. 5 *b* shows that in this example more than 96% of the cross-links are formed by the high-affinity antibodies, although they constitute only 7% of the total antibody concentration in the polyclonal serum. Conversely, the low-affinity antibodies, which at 70% represent the majority of the total antibody population, can be found in less than 0.1% of the cross-links. The fact that cross-linking involves two sequential binding events implies that improvements in affinity are sensed twice by the receptor. The ratio of cross-links formed by antibody *k* and antibody *l*, with cross-linking and binding affinities $K_{x,k}, K_k$ and $K_{x,l}, K_l$, respectively, is (cf. Eqs. 11 and 12)

$$\frac{X_k}{X_l} = \frac{K_{x,k} K_k L_{CT}}{K_{x,l} K_l L_{CT}} \quad (43)$$

Thus, the partial concentration of cross-links formed by a particular ligand increases linearly with the product of binding and cross-linking affinity. The same effect is not seen with regard to singly bound antibodies because the corresponding ratio of singly bound antibodies of different types is

$$\frac{S_k}{S_l} = \frac{K_k L_{CT}}{K_l L_{CT}} \quad (44)$$

If $\eta = 1$, the case considered in Fig. 5, then a 10-fold difference in binding affinity between the two antibodies, e.g., $K_k = 10K_l$, implies

$$\frac{X_k}{X_l} = \frac{100L_{CT}}{L_{CT}} \quad \text{and} \quad \frac{S_k}{S_l} = \frac{10L_{CT}}{L_{CT}} \quad (45)$$

Our prediction can be verified by mixing three monoclonal antibodies binding to the same site on the receptor such that the average binding affinity of the mixture is identical to the binding affinity of the medium-affinity antibody. Assuming that the affinities are ordered such that $K_1 < K_2 < K_3$, then the composition of the mixture must satisfy

$$\frac{1 - f_2}{f_3} = \frac{K_3 - K_1}{K_2 - K_1} \quad (46)$$

$$f_1 = 1 - f_2 - f_3 \quad (47)$$

with $0 < f_i < 1$, for the average binding affinity of the mixture to be identical to K_2 . Our theory predicts that if $\eta > 0$ the mixture will be more effective than the monoclonal antibody of average affinity.

This example is a fairly crude caricature of polyclonal sera in that it contains only three antibodies with affinities differing by a factor of 10. We obtain the same qualitative results for large collections of antibodies with an almost continuous distribution of affinities (unpublished results).

CONCLUSIONS

We have modeled the cross-linking of cell surface receptors by a set of bivalent ligands having different affinities for the receptor such as might be found in a polyclonal anti-receptor serum. We have assumed that the equilibrium concentration of receptor cross-links on the cell surface determines

the extent of ensuing cellular responses such as proliferation or differentiation. Then, the concentration of cross-links expressed as a function of the ligand site concentration, i.e., the cross-linking curve, and the dose-response relationship of a cell are closely related to each other.

On a logarithmic scale the cross-linking curve is a bell-shaped function of the total ligand site concentration under the condition that the relative concentrations of all ligands are constant, i.e., the ligand composition is fixed. Thus, the bell-shaped form of the cross-linking curve, which has been previously established for the case of the binding of a single bivalent ligand (Dembo and Goldstein, 1978; Perelson and DeLisi, 1980), is preserved in the case of a set of many different ligands.

Binding and cross-linking affinity

A complete description of the cross-linking curve requires the definition of two quantities that both depend upon the concentration of each ligand individually. The first, the binding field, is the sum of all ligand site concentrations weighted by their respective binding affinities. (We call the equilibrium constant for the binding of a ligand from solution to a site of a cell-surface receptor the *binding affinity* (this is conventionally called the affinity of ligand for the receptor) and the equilibrium constant for the binding to a receptor site of the second site on a ligand already bound at one site the *cross-linking affinity*, because the latter process cross-links receptors.) The second, the *cross-linking field*, is the sum of all ligand site concentrations weighted by the product of their respective binding and cross-linking affinities and multiplied by the receptor site density. Both fields are necessary to capture the essential features of the cross-linking curve. In general, neither field can be reduced to a function of the other. For a fixed ligand composition (see Excess Ligand Regime, above, for the definition), the binding field determines the location of the maximum of the cross-linking curve whereas the cross-linking field affects the curve's width and height. Both the width and height of the cross-linking curve increase as the cross-linking field increases.

Ligand-receptor interactions are usually characterized by their binding affinity only, because the value of the cross-linking affinity is difficult to measure (Erickson et al., 1991). On the other hand, as we have just argued, knowledge of the cross-linking affinity and receptor site density is essential for predicting the concentration of cross-links. Can we infer the value of the cross-linking affinity knowing the value of the binding affinity? Goldstein and Wofsy (1994) suggest that cross-linking affinity is proportional to binding affinity. Generalizing this, we have suggested that for a class of ligands and receptors, and in particular for a single bivalent ligand binding to a set of receptors that have similar physical structure, e.g., immunoglobulins of a given subclass, there may exist a linear relationship between binding and cross-linking affinity. In general, however, one has to

consider a variety of different relationships between binding and cross-linking affinities.

How binding and cross-linking affinity depend upon each other determines the mutual relationship of the different cross-linking curves resulting from the binding of a pure sample of a ligand to cells carrying different receptors. Linear dependence separates two qualitatively different kinds of relationship. When the cross-linking affinities increase at least linearly with the binding affinities, the cross-linking curve for any particular ligand-receptor interaction is entirely nested within those of higher-affinity interactions (Fig. 4, *right*). Here we assume a homogeneous relationship between binding and cross-linking affinity, i.e., $\eta_{ij} = \eta$, for all i and j . In this case, specifying one affinity for a ligand-receptor interaction determines the value of the other affinity (see Parameterizing the Mutual Dependence of Binding and Cross-linking Affinities). Then talking about the affinity of an interaction is meaningful. When, on the other hand, the cross-linking affinities increase sublinearly with the binding affinities, low-affinity interactions give rise to a larger number of cross-links than high-affinity interactions at high ligand site concentrations. This qualitative difference has important consequences for the behavior of idiotypically interacting B cell clones (De Boer et al., 1996).

Is the equilibrium number of cross-links relevant for the cellular response?

The theory presented here depends on two key assumptions: 1) the number of receptor sites on a cell surface is constant, and 2) receptor-ligand binding and cross-linking come to equilibrium rapidly compared with the time scale for a cellular response. The receptors on a B cell are internalized within an hour after the start of binding (Puré and Tardelli, 1992). Thus, for our theory to be valid equilibrium needs to be reached on a time scale of less than an hour. The time scale on which binding from solution relaxes to its equilibrium, assuming excess ligand, is $1/(k^+ L_T + k^-)$. If this time is on the order of 10^3 s or greater our approach is no longer valid.

Faro and Velasco (1993, 1994) developed a kinetic criterion for B cell activation based on the idea that a very large receptor cluster needs to be formed within some critical time to activate the cell. Their model assumes that the formation of cross-links is an irreversible process. For binding and cross-linking reactions that are slow to equilibrate, we agree with Faro and Velasco (1993, 1994) in that a dynamical criterion is needed to describe the relationship between receptor binding and cell activation. However, we feel it would be desirable to develop dynamical criteria using models with slow but reversible cross-linking. In this case, Perelson (1980) shows that, as binding relaxes to its equilibrium, the cross-linking curves are bell-shaped, attain their maximum at lower ligand concentrations, and become higher and wider as time progresses.

Multivalent ligands

We have rigorously analyzed cross-linking curves and response functions for the binding of bivalent ligand to receptor sites. We remark, however, that the qualitative features of cross-linking curves for multivalent ligands are the same as those of the cross-linking curves for bivalent ligands. In particular, the width and the height of cross-linking curves increase with the cross-linking affinities, and the position of its maximum moves to lower concentrations (Perelson, 1981). Consequently, high-affinity interactions are again favored compared with low-affinity interactions. The range of stimulatory ligand site concentrations is larger and the stimulation is higher anywhere provided the cross-linking affinities grow at least linearly with the binding affinities.

Describing the cross-linking curve for an n -valent ligand accurately requires n fields. However, it is not possible to obtain an explicit expression for the concentration of cross-links when the valence of the ligand is larger than 4. (Because the order of the polynomial describing the fraction of free receptor sites (cf. Eq. 13) becomes larger than 4, one can no longer obtain analytical solutions.) Qualitatively, the main effect of increasing the valence is that the formation of multiple cross-links on a single ligand allows for a larger number of cross-links at low ligand site concentrations. The number of cross-links hardly differs for ligands of different valence at ligand site concentrations larger than the inverse of the binding affinity—the location of the maximum of the cross-linking curve of bivalent ligand. Consequently, cross-linking curves become asymmetric around their maximum and the asymmetry increases with valence and cross-linking affinity (Perelson, 1981; Sulzer and Perelson, 1996).

Cellular response

Knowing just the number of cross-links on a cell does not tell us whether the cell will become activated and perform any kind of response. Here we have adopted the view that the rate at which a cell responds increases strictly monotonically with the equilibrium number of cross-links on the cell. A phenomenological response function relates the number of cross-links to the observed response rate. In general, different types of response will be characterized by different response functions. Moreover, responses typically depend on signals other than cross-linking signals. Thus, in T cell-dependent B cell responses, other cell surface receptors need to be engaged to generate a response, and the degree of their engagement may modulate the response to cross-linking (cf. De Boer and Hogeweg, 1989; Carter and Fearon, 1992; Neumann and Perelson, manuscript in preparation). This means that responses may differ in their requirement for cross-linking, a phenomenon seen in histamine release from human basophils (cf. MacGlashan et al., 1986; MacGlashan, 1983).

Being a function of the concentration of cross-links, dose-response relationships depend upon the binding as

well as the cross-linking field. Phenomenological dose-response functions frequently used in models of B cell response (De Boer, 1988; Varela et al., 1988; Weisbuch et al., 1990; De Boer et al., 1992; Sulzer et al., 1993) depend only on one parameter, an analog of the binding field. Because these functions depend only on a binding affinity-like parameter they miss an important aspect of the underlying cross-linking function.

An improvement over one-parameter response functions concerns the relative stimulation of clones with different affinity for the antigen at the same antigen concentration. When the cellular response is modeled by a one-parameter function, low affinity can always be compensated for by a large ligand concentration. However, when the cellular response depends on both binding affinity and cross-linking affinity this is no longer the case. As discussed under Response Curves of Cells with Different Affinity, high-affinity clones are stimulated more than low-affinity clones at any antigen concentration provided the cross-linking affinities increase linearly or superlinearly with the corresponding binding affinities. Even if the cross-linking affinities grow sublinearly, the range of antigen concentrations where the stimulus of low-affinity clones exceeds that of high-affinity clones is much narrower than for one-parameter response functions. These results are consistent with *in vitro* data of Rudich et al. (1988), which show that the proliferation of human B cells increases as the affinity of the stimulating monoclonal antibody increases.

We have examined the proliferative response of B cells due to cross-linking of their membrane immunoglobulin receptors under Cellular Response, above. Because B cells are subjected to a natural decay process, cross-linking-induced proliferation must overcompensate cell decay before a B cell clone can expand. A minimum cross-linking affinity is required for sufficient cross-linking and, in turn, for proliferation to outpace death. Assuming that binding and cross-linking affinities are covariant, we conclude that B cell clones having too low an affinity for the antigen do not expand and are apparently nonresponsive. We consider the lack of expansion of low-affinity clones at any antigen concentration a very realistic feature of our phenomenological model of the B cell response (Klinman, 1972; Teale and Klinman, 1980; Riley and Klinman, 1986; Mongini et al., 1991). This feature was absent from models where only the binding field determined the dose dependence of the proliferation rate.

Finally, we have proposed an explanation for the observation that polyclonal sera are frequently more effective stimuli than monoclonal antibodies under circumstances where signaling requires cross-linking. When exposed to a polyclonal serum, cell-bound receptors preferably bind the high-affinity component of the serum. Thus the cells preferentially accumulate on their surface the high-affinity fractions of the serum, provided both binding and cross-linking affinity are large (cf. Fig. 1). These results show that comparison of the binding properties of receptor-ligand interac-

tions depends critically on both the binding affinity and the cross-linking affinity.

We thank Byron Goldstein and Carla Wofsy for helpful suggestions.

Portions of this work were done under the auspices of the U.S. Department of Energy and supported by NIH grants RR06555 and AI28433 (ASP) and the Santa Fe Institute through the Joseph P. and Jeanne M. Sullivan Foundation Program in Theoretical Immunology.

REFERENCES

- Becker, K. E., T. Ishizaka, H. Metzger, K. Ishizaka, and P. M. Grimley. 1973. Surface IgE on human basophils during histamine release. *J. Exp. Med.* 138:394–409.
- Bell, G. I. 1974. Model for the binding of multivalent antigen to cells. *Nature*. 248:430–431.
- Braun, J., and E. R. Unanue. 1980. B lymphocyte biology studied with anti-Ig antibodies. *Immunol. Rev.* 52:3–28.
- Cambier, J. C., C. M. Pleiman, and M. R. Clark. 1994. Signal transduction by the B cell antigen receptor and its coreceptors. *Annu. Rev. Immunol.* 12:457–486.
- Cambier, J. C., and J. T. Ransom. 1987. Molecular mechanisms of transmembrane signaling in B lymphocytes. *Annu. Rev. Immunol.* 5:175–197.
- Carter, R. H., and D. T. Fearon. 1992. CD19: lowering the threshold for antigen-receptor stimulation of B lymphocytes. *Science*. 256:105–107.
- Celada, F. 1971. The cellular basis of immunologic memory. *Prog. Allergy*. 14:223–267.
- De Boer, R. J. 1988. Symmetric idiotypic network: connectance and switching, stability, and suppression. In *Theoretical Immunology, Part Two. Vol. III of SFI Studies in the Science of Complexity*. A. S. Perelson, editor. Addison-Wesley, Redwood City, CA. 265–289.
- De Boer, R. J., M. C. Boerlijst, B. Sulzer, and A. S. Perelson. 1996. A new bell-shaped function for idiotypic interactions based upon crosslinking. *Bull. Math. Biol.* In press.
- De Boer, R. J., and P. Hogeweg. 1989. Idiotypic network models incorporating T-B cell cooperation: the conditions for percolation. *J. Theor. Biol.* 139:17–38.
- De Boer, R. J., A. U. Neumann, A. S. Perelson, L. A. Segel, and G. Weisbuch. 1992. Recent approaches to immune networks. In *Proceedings of the First European Biomathematics Conference*. V. Capasso and P. Demongeot, editors. Springer, Berlin. 243–261.
- DeLisi, C., and A. S. Perelson. 1976. The kinetics of aggregation phenomena. I. Minimal models for patch formation on lymphocyte membranes. *J. Theor. Biol.* 62:159–210.
- Dembo, M., and B. Goldstein. 1978. Theory of equilibrium binding of symmetric bivalent haptens to cell surface antibody: application to histamine release from basophils. *J. Immunol.* 121:345–353.
- Dembo, M., and B. Goldstein. 1980. A model of cell activation and desensitization by surface immunoglobulin: the case of histamine release from human basophils. *Cell*. 22:59–67.
- Dembo, M., B. Goldstein, A. K. Sobotka, and L. M. Lichtenstein. 1978. Histamine release due to bivalent penicilloyl haptens: control by the number of cross-linked IgE antibodies on the basophil plasma membrane. *J. Immunol.* 121:354–358.
- Dintzis, H. M., R. Z. Dintzis, and B. Vogelstein. 1976. Molecular determinants of immunogenicity: the immunon model of immune response. *Proc. Natl. Acad. Sci. USA*. 73:3671–3675.
- Dower, S. K., J. A. Titus, and D. M. Segal. 1984. The binding of multivalent ligands to cell surface receptors. In *Cell Surface Dynamics: Concepts and Models*. A. S. Perelson, C. DeLisi, and F. W. Wiegel, editors. Marcel Dekker, New York. 277–328.
- Dwyer, J. D., and V. A. Bloomfield. 1981. Binding of multivalent ligands to mobile receptors in membranes. *Biopolymers*. 20:2323–2336.
- Eisen, H., and G. W. Siskind. 1964. Variation in affinities of antibodies during the immune response. *Biochemistry*. 3:996–1008.
- Erickson, J., R. Posner, B. Goldstein, D. Holowka, and B. Baird. 1991. Analysis of ligand binding and cross-linking of receptors in solution and on cell surfaces. Immunoglobulin E as a model receptor. In *Biophysical and Biochemical Aspects of Fluorescence Spectroscopy*. F. G. Dewey, editor. Plenum, New York. 169–195.
- Faro, J., and S. Velasco. 1993. Crosslinking of membrane immunoglobulins and B-cell activation: a simple model based on percolation theory. *Proc. R. Soc. Lond. B*. 254:139–145.
- Faro, J., and S. Velasco. 1994. Numerical analysis of a model of ligand-induced B-cell antigen-receptor clustering. Implications for simple models of B-cell activation in an immune network. *J. Theor. Biol.* 167:45–53.
- Finkelman, F. D., J. M. Holmes, O. I. Dukhanian, and S. C. Morris. 1995. Cross-linking of membrane immunoglobulin D, in the absence of T cell help, kills mature B cells in vivo. *J. Exp. Med.* 181:515–525.
- Gascan, H., J. F. Gauchat, G. Aversa, P. van Vlasselaer, and J. E. de Vries. 1991. Anti-CD40 monoclonal antibodies or CD4⁺ T cell clones and IL-4 induce IgG4 and IgE switching in purified human B cells via different signaling pathways. *J. Immunol.* 147:8–13.
- Goldstein, B. 1988. Desensitization, histamine release and the aggregation of IgE on human basophiles. In *Theoretical Immunology, Part Two. Vol. III of SFI Studies in the Science of Complexity*. A. S. Perelson, editor. Addison-Wesley, Redwood City, CA. 3–40.
- Goldstein, B., and C. Wofsy. 1994. Aggregation of cell surface receptors. *Lectures Math. Life Sci.* 24:109–135.
- Goroff, D. K., J. M. Holmes, H. Bazin, F. Nisol, and F. D. Finkelman. 1991. Polyclonal activation of the murine immune system by an antibody to IgD. XI. Contribution of membrane IgD cross-linking to the generation of an in vivo polyclonal antibody response. *J. Immunol.* 146:18–25.
- Jonsson, J. I., and K. Eichmann. 1990. Anti-CD4 modifies the kinetics but not the magnitude of anti-CD3-induced interleukin 2 production in peripheral T cells. *Eur. J. Immunol.* 20:1611–1614.
- Klinman, N. R. 1972. The mechanism of antigenic stimulation of primary and secondary clonal precursor cells. *J. Exp. Med.* 136:241–260.
- MacGlashan, D. W. J. 1983. Qualitative differences between dimeric and trimeric stimulation of human basophils. *J. Immunol.* 130:4–6.
- MacGlashan, D. W. J., S. P. Peters, J. Warner, and L. M. Lichtenstein. 1986. Characteristics of human basophils sulfidopeptide leukotriene release: releasability defined as the ability of the basophil to respond to dimeric cross-links. *J. Immunol.* 136:2231–2239.
- Macken, C. A., and A. S. Perelson. 1985. Branching Processes Applied to Cell Surface Aggregation Phenomena. Vol. 58 of *Lecture Notes in Biomathematics*. Springer Verlag, New York.
- Magro, A. M., and A. Alexander. 1974. Histamine release: in vitro studies of the inhibitory region of the dose-response curve. *J. Immunol.* 112:1762–1765.
- Metzger, H. 1992. Transmembrane signaling: the joy of aggregation. *J. Immunol.* 149:1477–1487.
- Mongini, P. K. A., C. A. Blessinger, and J. P. Dalton. 1991. Affinity requirements for induction of sequential phases of human B cell activation by membrane IgM-cross-linking ligands. *J. Immunol.* 146:1791–1800.
- Monroe, J. G., and J. Cambier. 1983. B cell activation. I. Anti-immunoglobulin-induced receptor cross-linking results in a decrease in the plasma membrane potential of murine B lymphocytes. *J. Exp. Med.* 157:2073–2086.
- Morel, P., J. P. Revillard, J. F. Nicolas, J. Wijdenes, H. Rizova, and J. Thivolet. 1992. Anti-CD4 monoclonal antibody therapy in severe psoriasis. *J. Autoimmun.* 5:465–477.
- Paul, W. E., editor. 1993. *Fundamental Immunology*, 3rd ed. Raven Press, New York.
- Pecht, I., and D. Lancet. 1977. Kinetics of antibody-hapten interactions. In *Chemical Relaxation in Molecular Biology*. I. Pecht and R. Rigler, editors. Springer, New York. 306–338.
- Perelson, A. S. 1980. Receptor clustering on a cell surface. II. Theory of receptor cross-linking by ligands bearing two chemically distinct functional groups. *Math. Biosci.* 49:87–110.
- Perelson, A. S. 1981. Receptor clustering on a cell surface. III. Theory of receptor cross-linking by multivalent ligands: description by ligand states. *Math. Biosci.* 53:1–39.
- Perelson, A. S. 1984. Some mathematical models of receptor clustering by multivalent ligands. In *Cell Surface Dynamics: Concepts and Models*. A. S. Perelson, C. DeLisi, and F. W. Wiegel, editors. Marcel Dekker, New York. 223–275.

- Perelson, A. S., and C. DeLisi. 1980. Receptor clustering on a cell surface. I. Theory of receptor cross-linking by ligands bearing two chemically identical functional groups. *Math. Biosci.* 48:71–110.
- Perelson, A. S., C. DeLisi, and R. Siraganian. 1982. A method for determining whether the descending limb of a biphasic histamine release curve reflects insufficient crosslinking. *Mol. Immunol.* 19:13–20.
- Perelson, A. S., B. Goldstein, and S. Rocklin. 1980. Optimal strategies in immunology. Part III. The IgM-IgG switch. *J. Math. Biol.* 10:209–256.
- Posner, R. G., C. Wofsy, and B. Goldstein. 1995. The kinetics of bivalent ligand-bivalent receptor aggregation: ring formation and the breakdown of the equivalent site approximation. *Math. Biosci.* 126:171–190.
- Puré, E., and L. Tardelli. 1992. Tyrosine phosphorylation is required for ligand-induced internalization of the antigen receptor on B lymphocytes. *Proc. Natl. Acad. Sci. USA.* 89:114–117.
- Reynolds, J. A. 1979. Interaction of divalent antibody with cell surface antigens. *Biochemistry.* 18:264–269.
- Riley, R. L., and N. R. Klinman. 1986. The affinity threshold for antigenic triggering differs for tolerance-susceptible immature precursors vs mature primary B cells. *J. Immunol.* 136:3147–3154.
- Rudich, S. M., K. H. Roux, R. J. Winchester, and P. K. A. Mongini. 1988. Anti-IgM-mediated B cell signaling. Molecular analysis of ligand binding requisites for human B cell clonal expansion and tolerance. *J. Exp. Med.* 168:247–266.
- Shizuru, J. A., S. E. Alters, and C. G. Fathman. 1992. Anti-CD4 monoclonal antibodies in therapy: creation of nonclassical tolerance in the adult. *Immunol. Rev.* 129:105–130.
- Strand, F. L. 1978. Physiology. New York.
- Sulzer, B., A. U. Neumann, J. L. van Hemmen, and U. Behn. 1993. Memory in idiotypic networks due to competition between proliferation and differentiation. *Bull. Math. Biol.* 55:1133–1182.
- Sulzer, B., and A. S. Perelson. 1996. Binding of multivalent ligands to cells: effects of cell and receptor density. *Math. Biosci.* In press.
- Teale, J. M., and N. R. Klinman. 1980. Tolerance as an active process. *Nature.* 288:385–387.
- Varela, F., A. Coutinho, B. Dupire, and N. M. Vaz. 1988. Cognitive networks: immune, neural and otherwise. In *Theoretical Immunology, Part Two. Vol. III of SFI Studies in the Science of Complexity*. A. S. Perelson, editor. Addison-Wesley, Redwood City, CA. 359–374.
- Varela, F. J., and J. Stewart. 1990. Dynamics of a class of immune networks: global stability of idio-type interactions. *J. Theor. Biol.* 144:93–101.
- Weisbuch, G., R. J. De Boer, and A. S. Perelson. 1990. Localized memory in idiotypic networks. *J. Theor. Biol.* 144:483–499.
- Wofsy, C., and B. Goldstein. 1987. The effect of co-operativity on the equilibrium binding of symmetric bivalent ligands to antibodies: theoretical results with application to histamine release from basophils. *Mol. Immunol.* 24:151–161.
- Zhang, J., I. MacLennan, Y.-J. Liu, and P. Lane. 1988. Is rapid proliferation in B centroblasts linked to somatic mutation in memory B cell clones? *Immunol. Lett.* 18:297–300.