Results from modeling of B-Cell receptors binding to antigen

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Abstract

In the late 80's, Dintzis et al. conducted an experiment which showed that T-Cell independent activation of B-cells needs high-valence antigen and happens only in a narrow range of antigen concentration. These experiments were believed to be explained by the “immunon” theory that requires that a minimum number of receptors need to be cross-linked to activate a cell. However, the immunon theory does not take into account receptor dynamics and cannot explain the lack of immune response at high antigen concentration or low antigen valence. We propose instead a simple, new mechanism for the T-Cell independent activation of B-Cell, which includes receptor endocytosis. Our model focuses on the fact that for the majority of antigens where the B-Cell is activated with T-Cell help, the kinetic parameters for binding, unbinding and endocytosis must be tuned so that there is an equilibrium between the number of receptors bound on the surface of the B-Cell and the number of antigen-bound receptors endocytosed. This equilibrium mechanism is probably generic and will also occur even when the B-Cell is activated by antigen without T-Cell help. By computer modeling, we show that if we accept this hypothesis of the requirement for equilibrium between the two mechanisms of binding and endocytosis, then we can explain both the valence cutoff and the low and high zone tolerance seen in the Dintzis experiment.

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1. Introduction

This paper discusses some computer modeling experiments in immunology done in collaboration with Yoram Louzoun of Bar Ilan University and Martin Weigert of Princeton University (Louzoun et al., 2003).

Section 2 defines the problem which relates to some puzzling aspects of experiments done by Dintzis et al. (Dintzis et al., 1989; Reim et al., 1996) on the immune response...
of mice treated with antigens of various sizes, which were able to activate B-Cells without T-Cell help. Models previously suggested do not explain the Dintzis experiments. Section 3 is a brief introduction to the immune system, with a focus on the issues relevant to what follows. Section 4 describes our computer experiment, our results, and the role of endocytosis in the modeling and in the activation of B-Cells. Finally, Section 5 contains a summary.

2. The Dintzis experiment and the paradox

Dintzis and collaborators (Dintzis et al., 1989; Reim et al., 1996) looked at the immune response of mice treated with five different fluoresceinated (FL) polymers of different masses and hapten valence (valence = number of binding sites on the antigen). These FL polymers were synthetically prepared by size fractionation and consisted of strands of one natural polymer (dextran), one modified natural polymer (carbomethyl cellulose) and three synthetic polymers (Ficoll, polyvinyl alcohol and polyacrylamide). The polymers were haptenated with FL and size fractionated to create a number of molecules with different hapten valence, molecular mass and hapten density. Different concentrations of these molecules were then injected into mice and their anti-FL IgM response was measured. An in vitro measurement was also done in culture using unfractionated spleen cells from naïve mice.

The Dintzis experiment showed that these antigens activated the B-Cells without T-Cell help and the following conditions were necessary for activation (i.e. for anti-FL antibodies to be found in the serum or in the culture):

1. The polymers had to exceed a threshold value (~20) of hapten valence.
2. The polymer concentration (measured in number of haptens per unit volume) had to be in a finite range (~0.1–100 ng/ml).

No immune response was measured outside these parameter ranges.

This immediately raises two questions. Why is the immune response so specific for these types of antigens? How do the B-Cells achieve this specificity of response? Dintzis and colleagues believed that the explanation of their experiment was in the “immunon” model for immune response (Vogelstein et al., 1982). This model claimed that the immune response, at its most elementary level, is quantized. A specific number of antigen receptors must be connected together in a spatially connected cluster (called an immunon), before an immune signal is delivered to the responding cell. But when the immunon model was studied on the computer (Sulzer and Perelson, 1996), it could only explain the general features of the curve of receptor binding as a function of hapten concentration. It was not able to explain either the finite activation concentration range or the valence threshold. A simple argument is sufficient to understand why a model that merely requires a bound cluster of receptors to activate the cell is insufficient to explain the valence threshold. This is because, even for valence = 2, clusters of arbitrary size can form by cross-binding of the two arms of the B-Cell receptor.
3. Introduction to the immune system

The immune system has evolved as a method of maintaining cell and organ function in the face of pathogenic assault (Janeway et al., 2001). The response is of two distinct types. The first is an innate response, which turns on immediately, is identical in all individuals of a species, and which ate many toxins, parasites, fungi and worms. The innate response involves phagocytes, signaling cascades and complex repair mechanisms primed by evolution to identify and eliminate specific toxins and pathogens encountered multiple times in the past by evolutionary ancestors of the species.

The second response, of interest to this paper, is the adaptive immune response. It takes 3–10 days to go into effect and is mediated by specialized cells called lymphocytes (B-Cells and T-Cells) with the support of macrophages, cytokines and organs such as the spleen, the lymph nodes, the thymus and the bone marrow. The actions of this response depend on the activating antigen in a dynamic way. It evolves during an individual’s lifetime through a memory mechanism where information about past infections is stored for future use. The mechanism of the adaptive response has evolved to deal with infection by novel bacteria, viruses, toxins and chemicals not encountered by the species in the past. It is necessary because pathogens themselves adapt and evolve on a time scale that is much shorter than the time scale over which the innate immune response is established.

The main components of the adaptive immune system are T-Cells and B-Cells. Both these originate in the bone marrow from stem cells and differentiate into one of killer T-Cells (also called CD8 T-Cells) or helper T-Cells (CD4 T-Cells) or B-Cells. Every cell of the body expresses pieces of its cellular proteins on the surface in specialized organelles called MHC molecules. The purpose of this presentation is to allow the immune system to distinguish self from non-self. Naïve T-Cells mature in the thymus where they are presented with the body’s own peptides in MHC molecules. T-Cells that do not recognize the MHC molecules or strongly react to the body’s own peptides are killed. The T-Cells that pass these tests are released into the body’s lymphatic and circulatory systems.

T-Cells have receptors on their surface with a single binding arm. These cells identify unusual processes in the interior of cells by attaching to MHC-peptide complexes on cells. When CD8 T-Cells identify infected cells, they trigger a cascade in the cells which results in programmed cell death. On the other hand, CD4 T-Cells, on identifying an infection, will proliferate and migrate to specialized organs called germinal centers (located in the spleen and lymph nodes) where they provide a second signal necessary for the activation and proliferation of B-Cells.

B-Cells mature in the bone marrow in a way similar to T-Cells. They are presented with the body’s peptides and are killed if they react sufficiently strongly to them. B-Cells have bivalent receptors on their surfaces with which they bind to antigen. When B-Cells get an appropriate signal from bound antigen and are co-stimulated by activated CD4 T-Cells, they proliferate and produce antibodies specific to the antigen that triggered them.

A small subclass of antigen can activate B-Cells without T-Cell help. The antigens used in the Dintzis experiment belong to this class. The process by which T-Cell activated B-Cells get a second signal from T-Cells is through endocytosis of antigen/receptor complex, followed by processing and presentation on MHC complexes for the second signal. We expect that this
mechanism of endocytosis must also exist in B-Cell that is activated without T-Cell help and we will assume for the rest of this paper that this is true.

It has been observed that unbound B-Cells recycle their receptors (endocytose and degrade them) at the rate of about once per 30 min. However, we will show that for the Dintzis experiment to agree with the computer model of B-Cell activation that we developed from experimental data, the endocytosis rate of B-Cell receptors bound to antigen has to be much higher. We will show that the rate of endocytosis plays a crucial role even in the activation of B-Cells without T-Cell help. By making the rate of endocytosis competitive with the time of activation of B-Cells (approximately 0.1–100 s), we are able to explain all the features of the Dintzis data.

4. The computer experiment

We simulated the surface dynamics of receptors on a square lattice. Each lattice point represents a region of $3 \times 3$ nm$^2$ and can contain a single bivalent receptor, and a single hapten. The cell surface was chosen to have periodic boundary conditions as this simplifies the geometry of the modeling without affecting the results. Since the size of our cell surface is equivalent to 20% of a real non-activated B-Cell, there are 50,000 receptors on the surface of a B-Cell, we used 10,000 receptors in our modeling. These were initially placed on random sites of the lattice. To model B-Cell surface dynamics properly, the size of the modeled surface must be bigger than the biggest relevant length scale and the time steps used must be smaller than the smallest dynamical time scale. The size of a B-Cell receptor is about 3 nm. The size of a typical antigen (ligand) in our simulation was 5–40 nm. The surface diffusion rate of receptors is of the order of $D \approx 2.0 \times 10^{-14}$ m$^2$/s (Saxton and Jacobson, 1997) and the time scale for activation of a cell is about a minute ($\tau \approx 100$ s). Hence the minimum linear size $L$ necessary in our modeling satisfies 

$$L^2 > D\tau \sim 1(\mu$m$)^2$.

We choose a single lattice spacing to represent a receptor. Each lattice spacing represents a distance of $dL = 3$ nm. The linear size of our surface was chosen to be 1000 lattice units which represents a physical length of 3 µm. Hence, the maximum modeling time allowed is about 900 s ($\tau < L^2/D$). Only after this time will the torus nature of the lattice become visible to the receptors due to their diffusion on the cell surface.

The receptors dynamics was simulated using a synchronous simulation, with a time step of $\delta t = 0.05$ ms. At each time step, the position of every receptor was updated by moving it to a neighboring site (if the site was empty) with a probability $P = D\delta t/\delta L^2 \sim 0.05–0.1$. Receptors bound to ligands were not allowed to move. At any given time step, a site was considered to be empty if there was no receptor on the site.

As mentioned before, B-Cell receptors are bivalent. In our simulation, they were assumed to be able to bind to antigens located either at the same or at neighboring sites. The interaction between receptors and soluble antigens was through receptor–hapten binding. The simulated antigens were linear and multivalent. The average distance between the hapten on the ligand was fixed at 9 nm or three lattice units. The ligand was free to bend, as long as the distance between neighboring haptnets remained constant. For simplicity we defined distance as the “Manhattan” distance or the number of hops along the lattice. Once the antigen was bound we assumed it could no longer bend.
The ranges of affinity which can activate B-Cells is measured to be $10^6$–$10^{10}$ M$^{-1}$ (Batista and Neuberger, 1998, 2000). The off rate of these antigens is

$$T_{1/2} = 0.1 \text{ s} - 200 \text{ min} \rightarrow k_{\text{off}} = (1.0 \text{ e} - 4 \text{ to } 10) \text{ s}^{-1}. \quad (1)$$

Hence, the dimensionless probability for a bound hapten to unbind (dimensionless off rate) is

$$P_{\text{off}} \approx k_{\text{off}}[05 \text{ ms}] = 5.0 \text{ e} - 4 \text{ to } 5.0 \text{ e} - 8. \quad (2)$$

Also, we can estimate

$$k_{\text{on}} \approx 10^6 - 10^7 \text{ M}^{-1} \text{ s}^{-1}. \quad (3)$$

From this one can compute the dimensionless binding probability to be (see Louzoun et al., 2003 for details)

$$P_{\text{on}} = [5.0 \text{ e} - 2 \text{ to } 5.0 \text{ e} - 5]. \quad (4)$$

At every time step, each receptor with at least one free arm could bind to haptens at neighboring grid points, and each bound receptor arm could unbind from its receptor. Once a ligand became unbound from all receptors, it was released. Once every time step, the receptors were presented with new ligands at a constant rate proportional to the total ligand concentration. The ligand concentration was a free parameter in our simulation and was varied over a large range of values.

In addition to modeling no endocytosis at all (i.e. ignoring endocytosis as relevant in T-Cell independent activation), we modeled endocytosis in two different ways. In the first model, endocytosis was hapten concentration (density) independent, which meant that the probability to endocytose a bound receptor was independent of whether it was singly or multiply bound. When a receptor was endocytosed in this model, it took with it the hapten bound to it but it left the remainder of the antigen intact. Thus at any time step, each bound receptor had a fixed probability to endocytose the haptens that were bound to it. In the second model, endocytosis was hapten concentration dependent. This meant that the endocytosis rate dropped abruptly (by a factor of 5) if more than one hapten was bound within a ligand. We tested both binding arms of each receptor. A receptor arm was called singly bound if the ligand carrying the hapten bound to it was only singly bound (i.e. no other hapten within the ligand was bound to other receptors). If one arm of the receptor was singly bound and the other arm was unbound or if the two arms were singly bound, the receptor had a high rate of endocytosis. Otherwise (i.e. if at least one arm was bound to a multiply bound ligand), the endocytosis rate was decreased by a factor of five. Unbound receptors were not endocytosed.

The surface of a B-Cell in the computer was initialized by populating it with receptors at random locations. The receptors were then allowed to diffuse on the surface. Multivalent antigens were presented to the virtual surface at random positions, and the position of each hapten was compared to the position of the BCRs. If a BCR and a hapten were close enough, they were allowed to bind and unbind with a constant probability. We tracked the total number of bound receptors as a function of time.

We first measured (Fig. 1) the number of bound receptors as a function of antigen valence after a fixed time interval (5 s) after antigen presentation began at fixed antigen concentration. Fixed antigen concentration meant that the total number of haptens presented per unit time to a receptor was kept constant. Thus, at the same concentration, an antigen with valence two was
presented at 10 times the rate at which an antigen with valence 20 was, because the latter has 10 times as many binding sites for the receptors. On the basis of entropy alone, one expects the number of antigen–receptor complexes to increase as a function of valence at fixed concentration. This is because, once an antigen binds, it is fixed in place for a finite amount of time. During this time more receptors can bind. The multiply bound antigen will then unbind with a probability that decreases exponentially with the number of haptens bound per antigen. In other words, the entropic cost of bringing the antigen to the surface and aligning it to the receptors is entirely covered by the first binding event. The cost of sequential binding events is only the entropy required for the lateral alignment of the receptor and antigen. Thus the avidity for high-valence antigens is much higher than for low-valence antigens. This explains why, in Fig. 1, the number of bound receptors is an increasing function of the number of haptens per antigen.

The high number of bound receptors for high-valence antigen leads to a higher probability of an immunogenic signal. One might argue that this result explains Dintzis’ experiments. However, this is incorrect. If this was all there was to it, the total number of bound receptors would keep rising as a function of antigen concentration. Hence, at high concentration, low-valence antigens would be able to activate the cell, contrary to the Dintzis experiment. Moreover, high-valence antigens would be immunogenic at high concentrations, also contrary to experimental

Fig. 1. The total number of bound receptors to antigens of varying valence. We varied the number of haptens in each antigen, but kept the total number of haptens presented in a given time constant (this is equivalent to a constant hapten concentration). The rise in the total number of hapten–receptor complexes is only due to cooperative effects of haptens on the same antigen. We measured the number of receptors bound to at least one hapten after 5s of antigen presentation, for different antigen valences. Each simulation started with no bound receptors. The on and off rates were 0.0005 and 0.0002 for the low affinity (thin full line, left scale) and 0.0005, 0.0002 for the higher affinity, respectively (thick dashed line, right scale), and the diffusion rate of the receptors was $10^{-14}\text{m}^2/\text{s}$. For antigens containing 20 haptens, we presented 0.1 antigens/ms, while for antigens containing a single hapten we presented 2 antigens/ms.
observations where high zone tolerance is clearly observed (i.e. at high antigen concentration, even high-valence antigen did not activate the cells). Thus, we conclude that the prevalent model of B-Cell binding and activation does not explain the Dintzis experiment.

We will now discuss the first of the enlarged models where we include density-independent endocytosis. In order to resolve the discrepancy between the model presented so far and the experimental results, we added the effect of antigen endocytosis. B-Cells activated with T-Cell help must balance the rate of endocytosis and the amount of antigen binding in a delicate way to ensure that these mechanisms operate simultaneously. This balancing will most likely also be operative for B-Cells that are activated without T-Cell help (this is a fundamental assumption in this paper).

Endocytosis of antigens is independent of the cell activation (Weiser et al., 1994) and hence, it must precede activation. Let us first assume that the probability to endocytose a receptor also does not depend on the mass of the aggregate surrounding it. If this was included in our modeling with the probability of endocytosing a bound receptor kept fixed at $10^{-2}$ per activated hapten per second, we were able to reproduce the high zone tolerance seen experimentally. At high enough antigen densities, we found that most receptors were bound and rapidly endocytosed. We would like to see that, in agreement with the Dintzis et al. experiments, B-Cells should be activated over three orders of magnitude of antigen concentration only for high-valence antigen and not at all for low-valence antigen. Fig. 2 shows the results of our modeling which is that

![Fig. 2. Number of bound (full line) and endocytosed receptors (dashed line) as a function of antigen concentration for high- and low-valence antigens. The current model includes receptor diffusion, antigen binding and unbinding and endocytosis. One can observe three regions. A low-concentration region where the number of bound receptors stays close to 0, an activation region where the number of bound receptors is high, and a high-concentration tolerance region where the extremely high level of endocytosis lowers the number of bound receptors. The number of bound receptors at a given antigen concentration is higher for the high valence than for the low-valence antigens. However, both types of antigens have a concentration zone, where they are immunogenic.](image)
high-valence antigens are indeed immunogenic over three orders of antigen concentration. However, low-valence antigens are also immunogenic over three orders of magnitude of antigen concentration, albeit at higher concentration than for high-valence antigen.

At equal concentrations, the number of bound receptors is a few orders lower for low than for high valence, but the maximum number of bound receptors is still the same order of magnitude for high- and low-valence antigens. Thus if one wants to completely eliminate activation by low-valence antigens, one is forced to add a new mechanism into the analysis.

This is achieved via a slightly enlarged model where endocytosis is density dependent. Endocytosis can occur either before or after cell activation. Late endocytosis is mediated through the assembly of large antigen–receptor complexes into coated pits (Salisbury et al., 1980). However, local endocytosis can occur before cell activation (Lanzavecchia, 1987, 1990). Such endocytosis is limited in the types of receptor–antigen aggregates that can be endocytosed. We simplify this type of endocytosis, assuming that only singly bound antigens can be endocytosed. Simulation shows that this assumption indeed produces a different maximum number of bound antigens across valences, and this is shown in Fig. 3.

In order to understand the significance of limited endocytosis on activation, one has to understand the interplay between receptor diffusion, binding and endocytosis. The fraction of the

![Graph showing number of bound receptors vs antigen density](image)

**Fig. 3.** Results of model with aggregate-size dependent endocytosis. We show the number of bound receptors as a function of antigen concentration for high- (full line) and low (dashed line) valence antigens. The current model is similar to the one presented in Fig. 2, but the endocytosis rate is higher by a factor of five when the ligand is bound to a single receptor (either on one or the two arms of the receptor). High-valence antigens have a similar activation pattern to the one observed in Fig. 2, while low-valence antigens bind a low number of receptors for every antigen concentration. This model explains all the experimentally observed results.
B-Cell surface available for binding (covered by receptors) is about 1%. First consider the case when the receptors are fixed (no diffusion). Since the receptors cover 1% of the surface, the probability of an antigen with \( n \) haptens to bind once is \([1 - 0.99^n \approx 0.01n]\). An antigen with 10 or fewer haptens will have less than a 10% probability to bind once, and less than a 1% probability to bind twice, even if its affinity is very high (i.e. even if the antigen always binds, and never unbinds). If one now adds a low constant rate of endocytosis for singly bound antigens, then, at some time, all the antigens bound to this small number of receptors will be endocytosed. In the presence of receptor diffusion, the number of receptors that bind to an antigen grows with time. The total number of bound haptens is determined by the endocytosis rate, and the rate at which an antigen can bind a new receptor. An antigen with a high valence will, on initial encounter, bind with multiple high-affinity receptors in a few time steps, and have a low endocytosis probability. If the total number of bound antigens is very high, they individually compete for access to free receptors. Consider the number of bound receptors for high-valence antigen. At low concentration, every antigen that is bound is eventually endocytosed. As antigen concentration is raised, some of the antigens will be endocytosed, while some other will bind rapidly to a large number of receptors, and stay on the surface. Finally, when too many antigens are presented they start to compete for access to receptors. In this case, even though the initial number of bound receptors is high, their number will eventually decrease because of the high endocytosis rate, and the competition over access to receptors. Thus, the enlarged model explains the tolerance to both high and low antigen concentrations. It also sets an upper limit on the total number of receptors that can be bound for a given valence. This number is significantly lower for low valence than for high-valence antigens.

5. Summary

Normally, B-Cell activation requires two signals, one directly from the antigen, and one from a T-Cell. These two signals imply a dual role for the B-Cell. One role is to present processed antigen on MHC-II molecules to receive a co-signal from T-cells. The other is to produce antibodies upon activation by a sufficient level of high-affinity antigen. The first role requires significant endocytosis even for low affinity and low concentration of antigens. The second role requires the presence of receptor–antigen complexes on the surface of the cell. Endocytosis, which is necessary for presentation, decreases the number of receptor–hapten complexes on the cell surface and reduces the chances of activation.

In the Dintzis experiment, the antigens are such that the B-Cell is activated without T-Cell help. In spite of this, it is very likely that the mechanism of endocytosis is operative, even though it is not necessary for activation. In this paper we assume that this is true and propose that the need for equilibrium between endocytosis and binding mechanism can explain the results of the Dintzis experiment. As a byproduct of our modeling, we note that cross-linkage of BCR emerges as a part of the activation process, as a result of the tradeoff between endocytosis of BCR. A critical test of our proposal is the rate of endocytosis of bound B-Cell receptors, which we claim will be significantly higher than that of unbound receptors. An experimental measurement of this quantity should be possible.
References


