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TOLERANCE, DANGER, AND THE EXTENDED FAMILY*

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Abstract

For many years immunologists have been well served by the viewpoint that the immune system's primary goal is to discriminate between self and non-self. I believe that it is time to change viewpoints and, in this essay, I discuss the possibility that the immune system does not care about self and non-self, that its primary driving force is the need to detect and protect against danger, and that it does not do the job alone, but receives positive and negative communications from an extended network of other bodily tissues.

INTRODUCTION

Among the fundamental questions in immunology, there are three that lie at the heart of the regulation of immunity. They are: 1) How is self-tolerance induced and maintained? 2) How is memory induced and maintained? and 3) How is the class of response determined? This essay is about the first one, tolerance (actually T cell tolerance), but it is also about something deeper, something that affects the way we think about every aspect of immunity. It is about the belief that the immune system's primary driving force is the need to discriminate between self and non-self. I have abandoned this belief.

Over the years that I have been trying to understand immunological tolerance, I have been intrigued, mystified, and dissatisfied by a range of

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phenomena that didn't fit with the view that the immune system reacts against anything foreign and is tolerant of anything that is self. For example, so difficult to accept was the evidence that normal individuals contain natural antibodies to self-antigens like DNA and keratin that I came up with all sorts of excuses for them: for example, that these antibodies are not really anti-self, they only cross-react when tested on a carpet of denatured antigen in ELISA assays, or they are of too low affinity to make any difference in vivo, or they are usually tested in vitro at 4°C and may not react at body temperature. I had similar problems with the evidence that most of us do not get demyelinating diseases, yet normal T cells can be immunized to myelin basic protein. Then there was the problem of tissue-restricted antigens. What mechanism can induce tolerance to tissue-specific antigens found only on skin, kidney, or liver cells, and yet allow responses to tissue-specific viruses? And finally there was the sheer *number* of things to which we must be tolerant—all the potential peptides made by 55,000 different bodily proteins, plus more than 10^{12} potentially different B and T cell idiotypes—while maintaining the ability to respond to foreign antigens. These, as well as other sorts of phenomena that were worrisome when approached from the basis of self–non-self discrimination, suddenly fell into place when approached from a different direction: the perspective that the immune system is far more concerned with danger and potential destruction than with the distinction between self and non-self. This is not a new theory but rather a different way of looking,[1] a different perspective from which to try to make sense of the information. As such, it does not immediately suggest new experiments nor is it refutable. Yet I have found it to be a valuable way of looking at immunity.

This essay is a description of T cell tolerance based on the view that the driving force for the immune system is the need to recognize danger and prevent destruction. It covers such things as why it is normal to find B and T cells specific for some self-antigens, why virgin and memory T cells should have different activation requirements, why B cells should not activate virgin T cells and how they might be involved in high- and low-zone tolerance, why neonates are easy to tolerize, why veto cells veto, how T killer cells get help, why foreign MHC antigens elicit strong responses while silicone, well-boiled bone fragments, or solitary haptens elicit none, as well as some more recent topics such as anergy and ignorance. The

[1] The concept was foreseen by Ehrlich (152), who worried about "horror autotoxicus" not "horror autoreactus," and Coutinho has often argued that the immune system is not afraid to recognize some aspects of self. However, it was Ephraim Fuchs who convinced me, after two years of discussion (153). As writers so often say in the introduction to their books, any credit for the idea goes to Ephraim, whereas any fault in my ability to delineate its usefulness should be ascribed strictly to me.

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model is not perfect. In its infancy, it leaves several questions unanswered. However, it does serve up explanations for some long-standing observations, it generates a few new questions, and it works reasonably well as a framework for immunological tolerance. Thus, in spite of its imperfections, I hope to show that the perspective from which it originated may be a useful one.

PART I: THE PERSPECTIVE

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The assertion that the immune system does not discriminate between self and non-self is a strong one. It is in absolute opposition to the fundamental belief, held by most immunologists, that the immune system is designed to attack anything foreign while remaining tolerant of self. Yet, radical as it may seem at first glance, I do not think that this is actually as different from the generally accepted view as it first appears. Let me explain.

Whenever immunologists convene to discuss self–non-self discrimination, one of the first questions asked is "What is self?" The answers have varied extensively, and none has ever satisfied everyone (1). Some scientists define "self" as everything encoded by the genome. Others include everything under the skin, including structures encoded by commensal genomes. Others exclude the "privileged" sites such as brain, cornea, and testes, defining self as any tissue accessable to lymphocytes. For T cells, a slightly more modern definition of self is, the set of peptides found complexed with MHC molecules. Waldmann suggested that a restricted subset of peptides would win the competition for MHC slots, thus reducing the number of "self" antigens to which tolerance must be induced (2), and Zinkernagel et al reduced it further, arguing that "self" consists only of APCs and thymic epithelium and that all other tissues are ignored (3). For B cells, Cohn defined "self" as cell surface and soluble molecules, proposing that antibodies against intracellular components might serve a housekeeping function to help clear cellular debris (4). Mitchison defined "self" as the set of bodily proteins that exist at a concentration above a certain threshold (5), and Jerne used the same reasoning to exclude antibody idiotypes from the definition of self because their individual concentrations are too low (6). In contrast, Coutinho and his colleagues define self in terms of an idiotype–anti-idiotype network, which they call a "positive definition of self" (7).

The definition of non-self can be equally problematical. Beginning with "everything outside the skin," we quickly find plenty of non-self structures to which the immune system does not mount an attack, e.g. silicone, bone fragments, solitary haptens, many peptides (depending on the responder's MHC type), and food (at least some of it). In a step toward the view that

self–non-self discrimination is less important than recognition of danger, Janeway pointed out that very few antigens are particularly immunogenic; he made the case that the immune system focuses on certain subsets of foreign antigens that carry "markers" of foreign-ness, like those of bacterial cell walls that act as adjuvants (8).

These definitions show how some of the most creative thinking in immunology has evolved in the search for practical definitions of self and non-self. Ultimately they all boil down to variations of the view that the immune system makes its own definitions. It regards a certain subset of the body as self and a particular fraction of the rest of the universe as foreign. In short, it doesn't really discriminate self from non-self, but *some* self from *some* non-self.

It only takes a small sidestep to go from the established view to the new one. If we begin with the notion that the immune system recognizes only a subset of bodily antigens as "self" and only a fraction of all possible foreign structures as "non-self," then we must also accept the existence of structures that fit into neither category, structures to which the immune system is neither tolerant nor reactive. This is not a homogeneous group because it includes those bodily structures to which the immune system is not tolerant but against which it does not normally respond, as well as the set of "foreign" structures to which the immune system simply doesn't react. So there are actually at least four classes of structures distinguished by the immune system: (i) "visible self," structures toward which the immune system is tolerant, (ii) "visible non-self," structures to which the immune system normally responds, (iii) "invisible self," bodily structures, like acetylcholine receptor or myelin basic protein, to which the immune system is not tolerant but to which it does not normally respond, and (iv) "non-immunogenic" structures, like isolated haptens or silicone, that the immune system simply ignores. If we accept that these categories exist, the logical next step is the question "by what criteria does the immune system define these subsets?" What features of a bodily antigen make it recognizably "self" (and an object of tolerance), and what characteristics distinguish a nonbodily structure as "non-self" (and an object to attack)? When put this way, there is no immediately obvious answer to the question; perhaps we could learn something by rephrasing it. For example, we could ask "what criteria does the immune system use when deciding to attack, to ignore, or to be tolerant of a particular structure?" I would suggest that the criteria have to do with what is dangerous rather than with what is "self."

At this stage many immunologists would perhaps agree, in a slightly bemused way, that distinguishing dangerous from harmless structures would be more efficient and would make more evolutionary sense than

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discriminating between subsets of "self" and "non-self." On the flip side, if the immune system were self-tolerant only to the minimal level needed to prevent self destruction, it would retain a greater flexibility with which to fight dangerous foreign antigens. But what distinguishes dangerous from harmless foreign structures? There is no consistent structural difference between them. Perhaps self–non-self discrimination is the best the immune system can do?

Here is the sidestep. If we move away from the idea that self–non-self discrimination is the immune system's primary goal, and consider instead that it might simply be the best mechanism that the immune system could find to distinguish dangerous from nondangerous structures, we open a window to a different perspective. We could now go two ways. We could try again to redefine self and find the exact type of self–non-self discrimination that would be needed, or we could shift and try to come up with another way to recognize danger. The shift, when it came for me, was away from an emphasis on recognition, away from an emphasis on the specificity of individual lymphocytes, and toward the interactions between them. In effect it was a shift from signal one to signal two.

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Definitions

In the discussion of T cell tolerance that follows, I call "self" any part of the body, and "non-self" any part of the rest of the universe. I also assume that the immune system doesn't really care about these categories, but that its primary goal is to recognize danger. Therefore, when I mention "self," I am not necessarily talking about a structure to which the immune system ought to be tolerant. I simply mean some structure that is a normal part of the individual's body.

There are also a couple of other definitions to get out of the way:

1. MAPs: Each tissue should have its own particular set of diverse MHC/peptide complexes (9–12), which Bonomo and I call a MAP (for MHC Antigen Profile) (11). Although the MAPs of different tissues overlap a great deal, they should also differ depending on each tissue's function and protein content. For example, though B cell and skin cell MAPs are similar because they both express such housekeeping molecules as cytochrome c or fatty acid synthetases, the B cell MAP also contains peptides of proteins expressed only by B cells, such as antibody or B220, while the skin cell MAP should instead contain peptides from keratin and the skin-specific antigen Sk (13).

2. Cells: A *virgin* cell is a mature T cell that has not yet met antigen, whereas an *experienced* cell has responded at least once. I use the term experienced because the more commonly used "memory" cell has collected too many connotations during its history, for example, regarding life span

and cell surface phenotype. An *effector* cell is an activated experienced cell, ready to kill targets, help B cells or macrophages, or secrete antibody. It will die in a few days or revert to a resting state. Antigen presenting cells (APCs) fall into two groups. Several types of APC may be able to stimulate experienced T cells, but the virgin T cell needs special signals. Lassila, Vainio, and I coined the term *professional* APC (14) for any cell that can activate virgin T cells. In the following model, I argue that B cells do not function as professional APCs. Though able to reactivate experienced T cells, they cannot stimulate virgin cells. I'm not entirely sure about macrophages. Although they can stimulate experienced cells, it is not clear to me yet that they can also activate truly virgin T cells. I do not yet exclude them from the category of professional APCs because, in contrast to B cells, there is no a priori reason to exclude them. However, the interdigitating dendritic cells, in their various tissue guises, are my (and others') (8, 15, 16) favorite candidates for the true professional APCs. Though there may be other cell types that can turn on virgin T cells, I use the terms dendritic cell and professional APC interchangeably for the time being.

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PART II: ASSUMPTIONS

The concept of a second signal was first proposed 23 years ago by Peter Bretscher and Mel Cohn (17) as a mechanism to maintain self-tolerance in the face of B cell hypermutation. In the mid-1970s, Lafferty and Cunningham proposed a two signal model to explain alloreactivity (18). Although the two models are often confused with each other, they are actually very different, and because they are the foundation for what I describe below, I use a bit of space here to point out some of the differences.

Both models define signal one as that which occurs when a lymphocyte's antigen-specific receptor (Ab or TCR) contacts the appropriate antigen, i.e. native structures for B cells and peptide/MHC complexes for T cells. Both models also suggest that this is not enough for activation, that a second signal is required. That is the extent of their similarities. Bretscher and Cohn's signal one was an OFF command that, if not countered by a second signal, shuts the reacting cell down, while Lafferty and Cunningham's signal one was simply not enough to activate a T cell. The other main difference between the models is in the source of signal two. Bretscher and Cohn's second signal is supplied by an antigen responsive cell (the T helper) and is called "help," whereas Lafferty and Cunningham's second signal comes from an antigen presenting cell and has been named co-stimulation. In an immune system based on self–non-self discrimination, the presence or absence of co-stimulation cannot be the critical component

determining tolerance because, as Mel Cohn points out, the APC does not make a distinction between self and non-self. It presents captured foreign and self-antigens as well as its own intrinsic cellular antigens and cannot discern whether it is presenting to an autoreactive T cell or one specific for a foreign antigen. In fact, Lafferty and Cunningham did not invoke co-stimulation as a mechanism to induce tolerance but instead to explain MHC restriction and alloreactivity. In contrast, Bretscher and Cohn's model can function to maintain tolerance because signal two comes from an antigen *specific* cell (a lymphocyte), not an antigen *presenting* cell. For example, in a tolerant individual, a newly mutated autoreactive B cell would be paralyzed by the self-antigen (signal one) because there should be no T helper cells able to offer help (signal two). In the same vein, a self-reactive killer T cell would become tolerant because of a lack of T helper cells responsive to the same antigen. This reasoning, however, has led Cohn into a chicken-egg dilemma with the T helpers themselves. If tolerance versus activation depends on the presence or absence of activated T help, who helps the first helpers?[2]

The upshot is that the presence or absence of co-stimulation cannot account for self-tolerance because the APC does not distinguish self from non-self. Nor can the presence or absence of T cell help, because it does not solve the problem of tolerance in the T helpers themselves. This is the state of affairs from the perspective of self–non-self discrimination. However, if we step sideways and look again from the perspective that the immune system is primarily concerned with danger, we can generate a model of tolerance by putting the two models together.

The Laws of Lymphotics

The model is based on the guiding principle that the presence or absence of second signals determines responsiveness or tolerance. This principle generates two laws for the behavior of resting lymphocytes:

The First Law of Lymphotics is: DIE IF YOU RECEIVE SIGNAL ONE IN THE ABSENCE OF SIGNAL TWO.

Signal one is *always* OFF, regardless of the antigen recognized or whether the responder is a virgin or experienced B or T cell. Cells that receive signal one can be rescued by the addition of an appropriate second signal, and this combination of signals leads to activation. Putting together the original two signal models, I would suggest that T helper cells are driven by the Lafferty-Cunningham type of co-stimulation whereas B cells need the Bretscher-Cohn type derived from helper T cells. Although it is not a strict requirement, much of killer T cell behavior is more easily

[2] For his solution to this problem, see Ref. (4).

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explained if resting killers, like helpers, also need co-stimulatory signals from APCs.

The Second Law of Lymphotics is: ACCEPT SIGNAL TWO ONLY FROM APCS.

Although a TCR may bind to many different cell types to trigger signal one, only APCs can co-stimulate. It follows from the first law that a T cell recognizing a non-APC will receive signal one in the absence of signal two and be tolerized. There will be some subtle twists to this principle when we come to discuss B cells as APCs, but in the main this guideline will hold for virgin as well as experienced T cells whether they be killers or helpers.

Activated effector cells must also be controlled. They must die or revert to a resting state from which they can be reactivated only in the presence of signal two. This concept is critical to any model of the immune system and has been laid out in depth by Mel Cohn (4). At first glance, an immune system, once attacked, would seem to be best prepared for the next invasion by keeping its primed effector cells ready. However, though efficient, this is simply too dangerous to allow. Autoreactive effector CTLs could destroy tissues, effector T helpers could secrete large amounts of lymphokines, and each plasma cell can secrete up to 2000 antibodies per second. Resting cells, by contrast, are completely innocuous (until activated). The immune system is therefore stingy with its effectors and does not allow them unlimited life spans. Plasma cells are thought to die after a few days, and killer and helper effectors either die or revert to the resting state, from which they must be reactivated to once again become effectors. The transition from resting cell to effector is thus a critical and carefully controlled step that occurs only in the presence of signal two, i.e. help for B cells and co-stimulation for T cells.

Before leaving this topic, I want to point out the difference between activation and "triggering." "Activation" is what happens to a resting cell. It is the set of events that galvanizes resting T cells to initiate the transcriptional and translational processes leading to cell division, lymphokine production, granule formation, etc, and turns them into effectors. "Triggering" is what induces effector CTLs (the loaded gun) to release their granules and kill their targets, or induces helpers to send activation signals to B cells and macrophages. Though activation and triggering differ in several ways, two are significant here. First, activation induces a change of state (from resting cell to effector) whereas triggering does not. Second, activation requires co-stimulation while triggering does not. Though both triggering and activation are consequences of the same external event, recognition of antigen, and though some of the early intracellular signals are the same, the signaling pathways of resting vs effector cells must differ.

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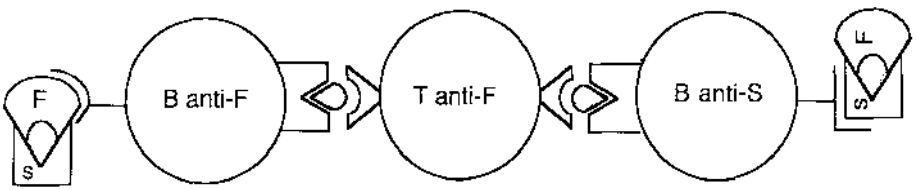
Killers, for example, are quite capable of killing targets that cannot deliver co-stimulation, and they do it equally well with or without help (19). This is why effectors must either die or revert. The return to the resting state guarantees that any dangerously autoreactive cell can be tolerized by signal one in the absence of signal two.

The Limits of Tolerance

BOTH T AND B CELLS MUST BE TOLERIZABLE Although there is no disagreement about the need to tolerize T cells, it is often said that B cells need not be self-tolerant since, in the absence of T helper cells specific for self-antigens, autoreactive B cells cannot be activated. This assertion is clearly not so.

Foreign antigens are not uniquely foreign. They are complicated structures that often share features with self-antigens. Since T and B cells must recognize linked but not necessarily identical determinants, helpers specific for a foreign part of an antigen, "F," would be able to help B cells specific for F as well as B cells specific for a shared self-determinant "S."



Both B cells could capture the antigen, process it, and present the various processed peptides to activated T helpers, which would not be able to distinguish between them. The T helpers would recognize the F peptide on the surface of both types of B cells and offer help. Both B cells would be activated, would multiply and secrete antibody. For example, take the antibody response to MHC molecules. Although MHC alleles are variable, large portions of the molecule are constant, yet the antibody response is specific for foreign determinants. If B cells were not tolerant to self-MHC molecules, they would receive help from helpers specific for the foreign parts, make anti-self as well as anti-foreign antibody, and there would be no allele specific sera. Three quarters of a century of serology has given us evidence for the existence of B cell tolerance.

There are, of course, limits to both B and T cell tolerance. They fall into three classes:

TOLERANCE CREATES VALLEYS IN THE REPERTOIRE, NOT HOLES The picture of a "hole in the repertoire" comes from the concept of a clean edged deletion that removes every cell that could possibly react to a particular

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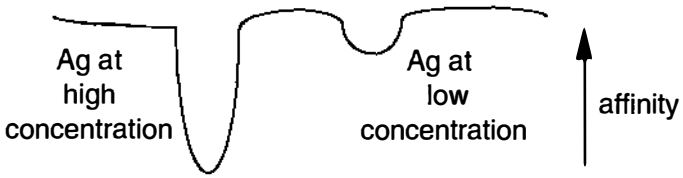
determinant. But there are two things wrong with this picture. First, the repertoire is not a smooth set of affinities; It is knobby and unpredictable, a set with boundaries more like a blob of rough concrete. Second, and more important, since deletion has an affinity cutoff, its effect on the repertoire will be to make a dent rather than a hole.



Highly concentrated antigens will induce deletion of a greater number of cells than dilute antigens, but none will delete every potentially reactive cell, only those with a certain threshold avidity. Cells below the threshold will survive.

Consequently there will always be cells able to respond to self-antigens if presented at high enough levels by professional APC. I am no longer surprised by reports showing that lymphocytes can respond to self-proteins when immunized with massive doses in adjuvant or when presented at high levels on APCs (such as autologous B cells that have increased their MHC expression due to infection with EBV).

IT IS NOT NECESSARY TO BE TOLERANT OF EVERY SELF STRUCTURE B cells need not be particularly tolerant of intracellular antigens, because they are behind the plasma membrane, and such antibodies may even be useful to help clear cellular debris. We come to this again when discussing autoimmunity.

NEW T AND B CELLS ARISE EVERY DAY Each day approximately 2 million new T cells (20) and 20 million new B cells (21) are generated. Thus, in any immune response to foreign antigens, there should be a small component of self-reactivity caused by newly emerging, not yet tolerant lymphocytes. I discuss this in the sections on experienced cells and on Anergy.

PART III: THE RULES OF THE GAME

The rules are designed to generate an immune system whose default reaction is OFF, in which a response is difficult to initiate and needs constant attention from the appropriate cells in order to continue. The basic scheme is that T cells should be totally unresponsive to the normal surface MAPs of APCs (including intrinsic APC antigens and any antigens that APCs

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normally capture) and that APCs should be the only cells capable of activating T cells. In this case there should only be T cells capable of being activated by an APC if the APC picks up a new antigen.

I. Rules for lymphocytes
   A. Rules for thymocytes
      1. Die if you receive signal one in the absence of signal two.
      2. Be unable to receive signal two from any source.
   B. Rules for virgin T cells
      1. Die if you receive signal one in the absence of signal two.
      2. Respond only to second signals offered by professional APCs (i.e. dendritic cells).
      3. Circulate from lymph node to lymph node or spleen.
   C. Rules for experienced T cells
      1. Die if you receive signal one in the absence of signal two.
      2. Be able to receive second signals from B cells and macrophages as well as dendritic cells.
      3. Circulate through tissues as well as from lymph node to lymph node.
   D. Rules for B cells
      1. Die if you receive signal one in the absence of signal two.
      2. Receive signal two only from experienced/effector T cells.
   E. Rules for effector T and B cells
      1. Ignore signal two. Perform your function when you receive signal one regardless of the presence or absence of signal two.
      2. Die or revert to a resting state after a reasonably short period of time.
II. Rules for APCs
   A. Rules for professional APCs
      1. Become activated in the presence of tissue destruction.
      2. Express co-stimulatory signals that can be received by both virgin and experienced T helper cells.
      3. Upregulate co-stimulatory signals for CTL precursors upon receiving the proper signals from T helper cells.
      4. Capture antigens randomly from your environment and present them to T cells.
   B. Rules for B cells
      1. Express co-stimulatory signals that can be received by experienced but not by virgin T helper cells.
      2. Capture antigens nonrandomly from your environment by binding them with your surface antibody, concentrate them and present them to T cells.

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Of course these rules are somewhat oversimplified. Nevertheless, we can use them to describe an immune system that mounts effective responses while preventing destructive auto-reactions, and to paint a picture that accounts for much of the experimental evidence.

PART IV: THE RULES IN PRACTICE

Thymocytes

This part of the model begins like that of Lederberg, who first suggested that lymphocytes might pass through a developmental stage in which antigen encounter resulted in death rather than activation (22). The evidence for such a stage during T cell development is now very strong. Starting with the findings that thymocytes expressing Vβ17 or Vβ6 are deleted in animals expressing IE or MLs[a] respectively (23, 24), and moving to the T cell receptor (TCR) transgenic mice, where the development of a T cell can be followed (25), we see that T cells are almost always deleted by thymic encounter with antigen. There are four questions one can ask about the induction of death and deletion in the thymus

1. What antigens should thymocytes be deleted to?
2. At what affinity threshold should they be cut off?
3. At what stage of their development should they be tested?
4. How is it done?

Question 1: *What antigens?* This is one of the main differences from models based on self–non-self discrimination. Here the only antigens to which T cells must be deleted are those normally found on APCs. There is no need (and no opportunity) to delete to skin antigens, for example, because skin cells cannot activate T cells.[3] Deletion should cover T cells specific for antigens intrinsic to APCs as well as for antigens captured from the environment such as hemoglobin, serum proteins, hormones, etc.[4] In this way, T cells that leave the thymus will be tolerant of the normal surface MAP of a professional APC. The evidence is that thymocytes do indeed delete when they recognize intrinsic cellular antigens (26) or captured circulating antigens (27–30) on dendritic cells. The available evidence, in fact, indicates that thymocytes are deleted when they see an antigen on any cell. We come to this again in the section on mechanism of deletion.

[3] One might ask what happens to a skin specific T cell, activated by a professional APC to a cross-reactive foreign antigen. We cover this later.

[4] A side effect will be deletion to any current infective agent. However, deletion would be complete only if the infection occurs very early in life and persists, as with neonatal infection by LCMV, since peripheral T cells would otherwise clear it.

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Question 2: *What affinity cutoff?* Mel Cohn has pointed out that every inducible cell must be tolerizable (4). Thus, the threshold affinity for deletion should never be higher than the peripheral activation threshold, lest autoreactive cells escape the thymus and become activated in the periphery. Can it be lower? T cells with too low an affinity to a self-antigen to be activated by normal levels of that antigen are not dangerous and need not be tolerized. Thus, in theory the affinity cutoff should be the same for deletion and activation. Janeway argued that the immune system might build in an extra margin of safety and set the deletion threshold lower. The margin could be set to take into account affinity increases due to changes in adhesion molecules in activated effector cells (31, 32). This is a matter of taste. The immune system could afford to lose a few cells in order to play it safe, but the deletion threshold shouldn't be too far below the activation threshold, or large numbers of potentially useful cells will be needlessly deleted. Some have suggested that it may be 10- to 100-fold lower, though these figures are tentative because of difficulties in equalizing experimental conditions for the two modes of reaction (33).

Question 3: *What stage?* The stage at which a thymocyte is deleted will depend on where and when it reacts strongly enough to its antigen to reach the necessary affinity threshold. The data on this point are contradictory, so let's examine the theoretical aspects first.

The exact point at which a thymocyte functionally encounters its antigen is influenced by the location and concentration of the antigen, the surface level of TCR, and the affinity of the TCR for the antigen. Since the level of TCR changes as thymocytes develop,[5] and since thymic tissue MAPs differ in different locations, opportunities for antigen encounter will also change. Under normal circumstances double negative thymocytes cannot be deleted because they express no TCR. Nor can they be deleted when they begin to express the TCR β chain for, at this stage, whether the β is found on the surface as a homo or hetero dimer (34), they do not yet have their specificity. As they begin to show surface TCR $\alpha\beta$ dimers, they may pass into a tolerizable stage. Here affinity begins to matter. Since the early $CD4^+CD8^+$ blasts do not normally express large amounts of TCR, to be deleted by a self-antigen they must have a very high affinity and/or the antigen concentration must be high in the thymic cortex. Any deletion at this stage will knock off only a very small percentage of cells. As the

[5] I am assuming for this discussion that thymocytes progress from double negative to $CD8^+TCR^-$ to double positive (where they express alpha chains) to single CD4 or CD8 cells. I am not sure about this pathway, but since this is a discussion on tolerance and not the details of development, I will follow it for the moment. If it turns out to be different, the details of tolerance induction may change but the concepts remain the same.

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cells increase their TCR levels, efficient deletion is possible. These double positives have been suggested to be the main candidates for deletion.

I prefer the single positive stage. First, like Goverman and Hood (35), I have argued elsewhere (36) that the TCR itself is a broadly reactive molecule and that the class of MHC to which a cell is restricted is a function of its CD4 or CD8 accessory molecule. Consequently, though a CD8 T cell whose TCR binds to a self-peptide with MHC class I is dangerous and should be deleted, the same TCR is harmless when coupled with CD4 because the CD4 molecule cannot contribute to its activation by class I. CD8 cells with TCRs to self-MHC class II complexes are also harmless. Therefore deleting cells at the double positive stage would be wasteful. Most of the deletion should occur when the cells have decided to be CD4 or CD8 cells and have increased the level of TCR to that of mature cells. So goes the theory. What about the data?

The data, which come from normal, knockout, and transgenic mice, do not make an entirely clear picture. With normal mice, three different sorts of experiments were done. First, when measured functionally, the induction of tolerance was found to be MHC restricted (37–39), meaning that death generally follows the same rules as activation: the TCR and the accessory molecules must bind to the same MHC molecule. Second, when measured visually, using antibodies to follow a particular TCR Vβ in the response to a superantigen, both CD4 and CD8 single positive cells were deleted while the double positive cells were fairly normal (23, 24). Because the superantigens (IE and MLs[a]) were MHC class II related, the deletion was thought to have occurred at the $CD4^+8^+$ double positive stage, where an interaction with CD4 could affect the development of CD8 cells, perhaps, since the double positives appeared normal, at the transition from double to single. This conclusion was solidified by the evidence that anti-CD4 antibodies could block superantigen-induced deletion of CD8 cells (40, 41); however, it has now been considerably weakened by a series of findings in normal, transgenic, and knockout mice.

First, superantigens seem to be exceptions to the rule that T cell activation is MHC restricted. In the periphery, where CD4 can have no effect on CD8 cells, superantigens induce activation and deletion of both CD4 and CD8 cells carrying the suitable Vβ chains, indicating that superantigens that bind class II molecules can nevertheless affect CD8 cells (42–44). Second, in CD4 knockout mice, an environment completely lacking in CD4, both thymic and peripheral CD8 T cells are deleted by superantigens (45). Thus the superantigens delete CD8 T cells directly, undermining the conclusion that superantigen-induced deletion must occur at the double positive stage of the thymus.

In transgenic mice, deletion can also occur at the single positive stage.

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For example, in a TCR transgenic mouse specific for LCMV and MLs[a] (3), the CD8-associated TCR deletes as a mature single positive CD8 cell when it encounters MLs[a] and as an immature CD8, just before becoming double positive, if it encounters LCMV. Thus this mouse shows us that when a TCR is expressed precociously, as in all TCR transgenics, deletion can happen very early in T cell development or very late. Other examples are the mice carrying the anti H-Y TCR (46) or the anti-H-2K^b TCR (47), both of which can delete as immature single CD8 cells. These TCRs would be expected to delete later if their antigens were not expressed in the thymic cortex, and the anti H-2K^b TCR does indeed delete late if the K^b molecule is expressed only by the medulla (47). This mouse also illustrates the importance of the accessory molecule. Although its transgenic TCR is expressed at high levels by both CD4 and CD8 single positive cells,[6] only the CD8s arc deleted by the prcsence of K^b. The same receptor, when coupled with CD4 does not recognize the K^b antigen and is not deleted (47).

Thus, in transgenic mice, deletion can happen at any stage of development in which the TCR and accessory molecules are sufficiently expressed to make functional interactions. However, normal mice do not express high levels of TCR at the early stages.[7] Therefore, though these examples show us that deletion *can* happen at any stage, we do not yet know when the bulk of deletion actually does happen in normal mice. I am laying bets on the single positive stage.

Question 4: *What mechanism causes deletion in the thymus: murder, suicide, or starvation?* Miller suggested murder by veto cells, a specialized killer/APC cell type found in normal thymus and in the spleens of nude mice (48). However, it appears that tolerance can be induced by antigens presented by early thymocytes, by mature CD8 T cells, by B cells, islets, thymic epithelium, and dendritic cells (reviewed in 36). Thus, it appears that any cell can do it. Tolerance in the thymus is not a function of the APC but of the T cell itself or its environment.

Malkovsky and Medawar focused on starvation by the environment. Thymocytes responding to antigen, they said, should act like peripheral T cells and upregulate their IL-2 receptor. But, since IL-2 is scarce in the thymus, the responding thymocytes would starve while the rest, remaining quiescent, would not need IL-2 and would finish development. The advantage of this elegant little model is that the requirements for activation and

[6] A puzzle for positive selection models? See Ref. (36).

[7] They do however express Vβ on the surface, either as homodimers or perhaps dimerised with an alpha chain substitute (34). Could the premature expression of a complete TCR interfere with the normal selection processes?

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deletion are the same. They involve the same antigens, the same APCs, the same adhesion molecules, and the same intracellular events; the only difference is the environment. Unfortunately the theory did not pan out. The addition of IL-2 has no effect on tolerance induction (reviewed in 49). In retrospect, starvation may not after all be the best way to induce tolerance as it would be too easy to circumvent. For example, what would happen during a strong antiviral response? Enough IL-2 might be secreted to allow an army of autoreactive cells to pass through the thymus!

In order to eliminate thymocytes specific for professional APCs, Sylvie Guerder and I proposed that the decision between death and activation should be an intrinsic property of the thymocyte itself, such that a thymocyte dies even when it encounters antigen on a fully functional dendritic cell. We tested this in thymic organ cultures and found that spleen dendritic cells, which are the best activators of mature T cells, are also the best tolerizers of thymocytes. Cell for cell they induced tolerance 100-fold more efficiently than spleen cells and 1000-fold more efficiently than thymocytes (26). We found that the dendritic cells were able to activate T cell clones in these thymuses, showing that the thymic environment is not intrinsically lethal, and that neither CsA nor various combinations of lymphokines had any effect on tolerance induction (unpublished results). From these experiments we concluded that the decision between deletion and activation is not imposed from external sources, but is made by the thymocytes themselves. They simply commit suicide; but how?

One practical way to eliminate thymocytes is to make them unable to receive signal two from any source. By the first law of lymphotics, they should then die after any encounter with antigen. They could, for example, lack external receptors for co-stimulation or have defects in the associated intracellular pathways. Alternatively, they could lack receptors for growth factors such as IL-2, or perhaps have their TCRs hooked up to irreversible apoptotic pathways. Any of these mechanisms would ensure the elimination of any thymocytes that were specific for antigens found on dendritic cells, including antigens common to all cells, antigens specific to dendritic cells, and some peripheral antigens that dendritic cells routinely capture. As a side effect it would also eliminate any thymocytes able to react to T cells, macrophages, thymic epithelium, perhaps $CD5^+$ B cells, and any other cell found in the thymus.

The tolerogenicity of thymic epithelium has been a controversial issue. After all, the tissue that positively selects thymocytes should not turn around and delete them. Using several approaches and a variety of antigens, many groups studied the tolerizing capacity of thymic epithelium (reviewed in 11). Some reported that it was not tolerogenic, and others found that it was. Among the latter, some found that it induced complete

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or partial deletion, while others found anergy or a form of split tolerance in which the T cells reacted in vitro but not in vivo. Among the hypotheses put forward, it was suggested that the thymic epithelium might delete high but not low affinity T cells, that it might induce a form of partial anergy, that it is composed of two sorts of tissue expressing different antigens, one that positively selects and one that deletes, or that it establishes an immunological network that maintains tolerance in vivo but is disrupted in vitro.

To deal with these conflicts, Adriana Bonomo and I started with the idea that the surface MAP of peptide/MHC complexes on thymic epithelium might not be precisely the same as the MAP of any other tissue and that in vitro or in vivo reactivity to spleen cells or skin grafts was therefore not an appropriate test of the tolerogenic capacity of thymic epithelium. Testing this hypothesis (11), we found that BALB/c (MHC^d) nude mice grafted with B6 (MHC^b) thymic epithelium were indeed tolerant of B6 thymic epithelium but not of other B6 tissues. They generated MLR and CTL responses to B6 stimulator cells and rejected B6 skin, spleen grafts, and the bone marrow components of a second B6 thymus graft, but they accepted the B6 epithelium and proceeded to repopulate it with new BALB/c stem cells that then developed into T cells in a perfectly normal fashion. Using anti H-Y TCR transgenic mice, we showed that male thymic epithelium induced deletion of the H-Y specific cells. Looking back through the literature, we found that every case in which thymic epithelium appeared not to induce tolerance could be accounted for by the concept of MAPs. In these cases, tolerance was assessed against the complex surface MAPs of tissues other than thymic epithelium. We concluded that thymic epithelium, like all other tissues tested, induces deletion in the thymus[8] and that thymocytes must therefore be inherently suicidal. After passing through this stage of tolerizability, the remaining untolerized cells will finish their maturation and leave for the periphery.

Virgin T Cells

Virgin T cells leaving the thymus are tolerant of thymic tissues but have not yet encountered many other cell types. They haven't yet learned the difference between peripheral "self" peptides and foreign ones. The solution in most self–non-self discrimination models is to find ways to complete the job of tolerizing the virgin T cell population. The solution in a danger discrimination model is to ignore the question of what is self and simply

[8] As I have discussed elsewhere (36), this leaves open only two models of positive selection; the affinity based models (154, 155) and the "error peptides" model of Claverie and Kourilsky (156).

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activate only T cells that can bind to professional APCs and only B cells that can receive help. Since the virgin T cell population is tolerant of professional APC MAPs and, since other cell types cannot deliver signal two, the only antigens that should activate virgin T cells are those foreign antigens that become captured by the professional APCs. Surprisingly, this simple view works quite well to generate an effective response without endangering self tissues.

For the discussion that follows, I group the peripheral T cell repertoire into three classes: those that see tissue-restricted peptides in the MAPs of peripheral tissues, those that recognize foreign peptides, and those that have a dual specificity for foreign as well as self peptides.

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Virgin T Helper Cells and Their APCs

A T cell encountering a self or foreign antigen may do one of three things: (i) ignore the encounter, (ii) become activated, or (iii) be turned off. Thymocytes have only the first and third options. Mature virgin peripheral cells can be activated, though the conditions are tightly controlled through the availability of second signals. In this aspect, virgin and experienced cells differ. Although both can be activated by antigens presented by professional APCs such as dendritic cells, experienced T cells can also be activated by B cells, whereas virgin T cells cannot. Why should the activation requirements change during a T cell's life?

There are two theoretical reasons why B cells should not be APCs for virgin T cells. The first concerns antibody idiotypes. As Jerne pointed out (6), the immune system cannot be tolerant of every single idiotype, because there would be no cells left to see foreign antigens. Nor can the immune system respond to idiotypes, or it would be paralyzed by such introspective polyclonal activation. Jerne's solution to the problem led him to the network hypothesis (6). He proposed that the circulating amount of any one idiotype was too low to either immunize or tolerize and that it increased to immunizing concentrations only when a B cell responded to a foreign antigen. However, what Jerne could not know then is that the concentration of any particular idiotype, though low in the circulation and therefore infrequently presented by professional APCs, is high enough on the B cell making it to be presented to T cells (50, 51). If B cells were able to deliver costimulatory signals to naive T cells, there would often be anti-idiotype responses even in unimmunized animals.

The second theoretical reason that B cells should be unable to activate virgin T cells is that B cells, using their surface Igs, can capture antigens 10- to 10^4-fold more efficiently than other APCs (52), making them superb APCs at the tail end of primary immune responses or at the beginning of secondaries, when the antigen concentration is very low. However, if we

put the efficiency of antigen capture together with B cell hypermutation, we have a potential recipe for disaster. For example, consider a B cell that mutates and becomes specific for a common autologous serum or cell surface protein. Although T cells are tolerant of such proteins, that tolerance has an affinity cutoff set by the normal concentration with which the self-proteins are presented by normal APCs. The autoreactive B cell, however, can concentrate the protein to far greater densities than those to which the T cells are tolerant. If B cells could activate virgin T cells, they would initiate unstoppable autoimmune responses. The simplest way to prevent this type of autoaggression would be to require that virgin T cells be activated first by a professional APC before they can interact effectively with a B cell. In that case, because T cells are tolerant of the normal concentrations of self-proteins presented by the APCs, no T helper cells can be activated, and autoaggressive B cells should die for lack of help.

For these reasons, Olli, Olli, and I suggested that only professional APCs, and not B cells, should be able to activate virgin T cells (14). A typical immune response would then begin with an interaction between a T helper and an antigen presenting dendritic cell. Since T cells are tolerant of the normal MAPs of professional APCs, neither autoreactive nor idiotype specific virgin T cells would be activated, only those specific for the new antigens captured by the dendritic cells. The activated T cells then would expand, multiply, help turn on macrophages and B cells displaying the antigen, and revert to a resting state. As the response continued, the antigen specific B cells could present the antigen to *restimulate* these resting newly experienced T cells, maintaining the response till the foreign antigen was completely dealt with; however they would be unable to activate new virgin T cells. In fact, by the laws of lymphotics, B cells should induce tolerance of virgin T cells, because they offer signal one in the absence of signal two.

The data on this subject have been mixed. When Olli, Olli, and I tested the theory, using allogeneic chicken chimeras with MHC^a bodies and MHC^b B cells (14), we found that the B cells were able to accept T cell help, but they could not initiate the processes that led to the activation of the help they needed. The virgin T cells in the MHC^a chimeras needed first to be primed by professional MHC^b APCs. In addition we found that the prediction from the first law of lymphotics was also supported, as the chimeras were tolerant of the B cell MHC type. There have been a few other studies in which B cells were found to be inactive or tolerogenic as APCs for T cells, but there have also been many in which B cells, especially activated B cells, were perfectly functional APCs.

Janeway offered an explanation for the contradictions (8). He proposed that B cells act as an early warning system to distinguish noxious pathogens

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from harmless foreign substances by being especially susceptible to activation by such materials as bacterial lipopolysaccharides (LPS). If activated B cells expressed co-stimulatory signals for T cells, but resting B cells did not, then with one fell swoop, this generated a method by which the immune system could respond specifically to bacteria, and it also might explain much of the contradictory data on B cells as APCs. However, the theory created a dire problem for tolerance. If B cell presentation of self-proteins and of their own idiotypes creates a risk of autoaggression, then presentation by activated B cells is as much of a problem as presentation by resting B cells. Janeway realized this and attempted to show that this is precisely how autoimmunity can arise (53). But presentation of self components by activated B cells should happen constantly, and yet autoimmunity is rare.

Therefore, Fuchs and I offered another explanation for the contradictions. We said that it was the state of the T cell that mattered, not the state of the B cell. We argued that both resting and activated B cells should tolerize virgin T cells and reactivate experienced T cells (54). If we look carefully at the published works from this viewpoint, we find that the contradictions clear up. With three apparent exceptions, B cells are indeed stimulatory for primed T cells but not for naive T cells. The first exception is the case of T cells responding to MHC or MLs antigens, where B cells have been reported to activate, not activate, or tolerize (55–59); it illustrates the importance of the antigen one uses. If virgin T cells might behave differently from experienced T cells, then it is crucial to choose an antigen to which the immune system under test is not primed. Neither MHC nor MLs are such antigens. Foreign MHC is a complicated MAP containing many different peptides (9, 60, 61). Because of this complexity, and because TCRs are broadly cross-reactive, many T cells specific for environmental antigens also cross-react on foreign MHC. Estimates of the extent of these cross-reactions range from 1% to 10% when antigen specific T cells are tested against single foreign MHC types, but the numbers go up spectacularly when they are tested on a larger panel. An extensive analysis of T helper clones from B10.A mice specific for cytochrome c, insulin, or GAT showed that 38/63 (60%) also reacted to one or more of nine different MHC haplotypes (62). Since there are well over one hundred different MHC alleles, it appears that nearly every T cell can respond to at least one foreign MHC antigen. Thus the response to MHC is not purely a virgin response. The responders are a mixed population of virgin cells and experienced cells specific for cross-reactive environmental antigens. Responders to MLs and other superantigens are also unlikely to be purely virgin, since each superantigen can bind to large numbers of T cells through several different Vβ chains, without regard to whether the T cells are virgin

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or experienced. Because the T cells responding to MHC or MLs are likely to be such mixtures of naive and experienced cells (63, 64), it follows that presentation of MHC or MLs antigens by B cells should initiate two opposing reactions: tolerance of the naive T cells and reactivation of the cross-primed memory cells. The end result, for any antigen, will thus depend on the relative proportions of naive and memory T cells that exist.

The second exception is the finding by Liu and Janeway that B cells activated by LPS or poly IC can act as costimulators for the anti-CD3 mediated activation of naive T cells (65). In this in vitro study, the authors used the CD45RB cell surface marker to separate $CD4^+$ T cells into "naive" (RB^+) and "memory" (RB^-) populations. However, since RB^- cells can convert to the RB^+ phenotype (33, 66), it is becoming clear that these surface markers, once thought to label memory cells, may actually be markers of fairly recent activation rather than previous experience.

It was for these reasons that Fuchs and I chose the male specific antigen H-Y for our study. Virgin female mice seem to be unprimed to H-Y because, in contrast to MHC or MLs, they do not make primary in vitro responses to H-Y. Furthermore there are probably not many environmental H-Y-like antigens, since H-Y primed T cells lose their memory when transferred into unprimed recipients (67). The choice of H-Y allowed us to look at the in vivo effects of B cells without confusing virgin and primed T cells and without the need to do in vitro studies with arbitrarily separated populations. When we injected resting or activated B cells into virgin or previously immunized female mice, the outcome was as we predicted. Virgin T cells were tolerized by both resting and activated B cells and experienced T cells were boosted (54).

The third exception came from Eynon and Parker, who used the outer portion of rabbit Ig, also an antigen likely to stimulate mostly virgin cells in a mouse. Although they found that injections of monovalent Fab fragments of rabbit anti mouse IgD (which targets to B cells) tolerized naive mice to rabbit Ig, they also found that injections of divalent $F(ab)'_2$ fragments did not (68). Because $F(ab)'_2$ fragments can activate B cells by cross-linking surface Ig, the authors proposed, like Janeway, that activated, but not resting B cells, could serve as APCs. However there is an alternative explanation. Although IgD is primarily a surface molecule, it also circulates at low levels. Thus the responses obtained with $F(ab)'_2$ fragments may not have resulted from priming by activated B cells but rather by professional APCs that had captured complexes of circulating IgD with the injected anti-IgD $F(ab)'_2$, complexes that could not be made by injected Fab fragments.

Although the data are not all in yet, it appears that the distinction between virgin and experienced T cells generally holds. Virgin cells do not

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accept second signals from B cells whereas experienced T cells do. The inadvertant tolerance that occurs when B cells present antigen to T cells offers explanations for several experimental observations.

EXPERIMENTAL NEONATAL TOLERANCE Ever since the classic experiments of Medawar and his colleagues, we have known that prenatal and neonatal immune systems are particularly susceptible to the induction of tolerance. This is generally assumed to reflect the uniquely tolerizable state of the lymphocytes in the young immune system, but the tolerizing capacity of B cells offers an alternative explanation.

What if the neonatal T cell pool is *not* uniquely tolerizable? Suppose that it simply consists of a small number of truly virgin T cells. Such an immune system would be extremely susceptible to tolerance induction by B cells. An injection of allogeneic bone marrow or spleen, in which B cells far outnumber dendritic cells, would therefore tolerize the vast majority of neonatal T cells. As the animal grows older and the numbers of peripheral T cells increase, the B cells in the tolerizing inoculum would no longer be sufficient to induce complete tolerance. Some T cells would thus remain, become activated by encounter with the professional APCs, and reject the inoculum.

There are actually two populations of cells involved in experimental neonatal tolerance, one that establishes tolerance and the second that maintains it for the life of the recipient. I would suggest that B cells induce the original state of tolerance, allowing engraftment of stem cells which then maintain it. Without the original tolerizing effect of the B cells, the stem cells might be rejected by the host. Thus, bone marrow may induce neonatal tolerance more easily than spleen, because it contains not a higher proportion of stem cells, but a higher proportion of B cells. This could easily be tested by removing B cells from the inoculum. Incidentally, T cells in the injected population should also induce tolerance, at least to MHC class I, because they too offer signal one without signal two. A prediction from this view is that a neonatal mouse injected with a population of spleen or bone marrow, depleted of B and T cells, should respond rather than be tolerized.

HIGH AND LOW ZONE TOLERANCE Mitchison's experiments on tolerance to soluble BSA showed that naive but not primed mice were tolerized by either very low or very high doses of antigen (69). High zone tolerance was achieved by a single mega-dose injection of antigen while the induction of low-zone tolerance required repeated injections of minute amounts. This is exactly what one would expect if presentation by B cells were tolerogenic for virgin T cells.

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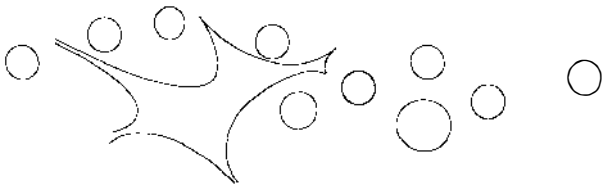
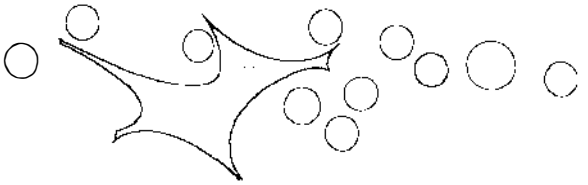
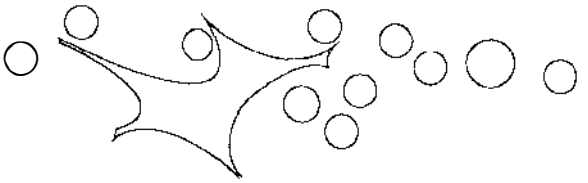


Because B cells, through their antigen-specific surface Igs, can be thousands of times better at capturing antigen than are other APCs (52), antigens at very low concentrations will be presented mainly by antigen specific B cells and will tolerize responding virgin T cells.



At medium doses the antigen will be captured by professional APCs and, since these APCs (about 1–3% of spleen) far outnumber the antigen specific B cells (about $1/10^6$–$1/10^5$), they will compete effectively to immunize the virgin T cells.



At very high concentrations the antigen would be captured nonspecifically by all B cells, and because the total number of B cells (about 60% of spleen) is greater than the number of professional APCs, the result would again be tolerance.

Since antigen-specific B cells are rare, complete tolerance of the virgin T cell pool would require repeated injections and, since B cells reactivate rather than tolerize experienced T cells, neither high nor low zone tolerance can be induced in previously primed mice.

TOLERANCE INDUCTION BY SUPERANTIGENS After injections of high doses of bacterial superantigens or of cells bearing endogenous viral superantigens, specific T cells expand and then die, the few remaining cells being unable to respond to the superantigen (70, 71). In a kinetic study of the response to SEB, D'Adamio et al (72) saw that there were three phases of

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response: an early death beginning hours after injection, a rapid expansion of the remaining T cells, and the subsequent demise of the expanded population. B cell presentation may account for the early death. Since SEB can bind directly to MHC class II molecules, it will mostly be presented by B cells and will tolerize most of the virgin T cell population. Experienced cells, and any virgin T cells primed by a dendritic cell, would expand, only to perish later by mechanisms not yet understood but which may well have to do with maintaining a well-balanced immune system rather than with maintaining tolerance. Because many of the endogenous superantigens are expressed by B cells but not by professional APCs, tolerogenic presentation by B cells would also explain why $V\beta 11^+$ T cells are not deleted in μ suppressed mice and why they delete when the B cells are allowed to return (58).

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THE BENEFICIAL EFFECT OF DONOR-SPECIFIC BLOOD TRANSFUSIONS

Transplants given to human recipients of blood transfusions (73, 74) or rodent recipients of APC depleted spleen cells (57, 75) are accepted for prolonged periods, though seldom completely. Since the population of T cells specific for allogeneic MHC is a mix of virgin and experienced cells, and since there will be a few professional APCs in the injections, the naive cells of the recipient should mostly be tolerized whereas the experienced cells will be boosted. Thus, variable depths of partial tolerance would be the expected result. There is also the matter of tissue specific MAPs. Since MHC is actually a plethora of MHC-peptide complexes, and since B cells can only tolerize T cells specific for B cell MAPs, there will be some remaining T cells that recognize kidney-specific or skin-specific MAPs. Thus, even if the recipient's immune system were totally virgin with respect to the antigens on these tissues, B cell induced tolerance (without stem cell backup) would not cover them completely.

Virgin T Killer Cells and Their APCs

The data on second signals for cytotoxic T cells (CTL) make two clear yet completely different stories. On the one hand it appears that CTL responses to many viruses are relatively independent of help (listed in 45). Although depletion of CD4 helper cells usually does have some effect on the magnitude of the response, there is often a residual response that seems to be helper independent. On the other hand, many CTL responses to viral and nonviral antigens are helper dependent. The CTL response to H-Y, for example, only occurs in mice carrying MHC class II molecules able to present H-Y to CD4 T cells, and depletion of CD4s abrogates the killer response (76–78). The response to Qa-1 is similar. In B6 mice, the class II

molecules do not seem to present Qa-1 to CD4 cells, and the killers do not respond, unless additional helper determinants are added (79). Both H-Y and the BALB strain minor histocompatibility antigens ("minors" for short) can activate T help for the Qa-1 killers. Thus, as in T-B collaboration, the antigen seen by the killer need not be the same as that seen by the helper; the only requirement is that they must both be presented by the same APC, a cellular form of hapten-carrier linkage.

Sylvie Guerder and I tested these helper dependent killers for their adherence to the first law of lymphotics (die if you receive signal one in the absence of signal two). Using Qa-1 and H-Y as antigens, we found that the killers did indeed become unresponsive to any further injections of their antigen if they encountered it first in the absence of help (76). This was true in mice that were depleted of CD4 cells and then injected with cells bearing Qa-1 and/or H-Y, and in intact mice injected with Qa-1$^+$ APCs lacking a helper determinant. Thus the absence of CD4 help, whether it was lacking because the helpers were physically removed or because they were simply not activated, set up a situation in which the killers were rendered unresponsive by signal one. Singer's group found that the tolerogenicity of Qa-1 without help extends to graft rejection (80), and Waldmann's group have found that both killer responses and graft rejection to multiple minor differences are stopped in animals injected with non-depleting anti-CD4 antibodies (81). Taken together these findings support the idea that T killer cells against these cellular antigens need help from CD4 helpers and are tolerized when they encounter their antigens in its absence.

Why should the activation requirements of CTL specific for viral antigens differ from those for CTL against cellular antigens? I believe that these differences are more apparent than real. Like B cells, CTL are potentially much too dangerous to be allowed to respond to signal one alone. They *must* need help. While contemplating the question of how help is delivered to those killer cells that do seem to need it, Sylvie Guerder and I came up with a model that potentially reconciles these differences (76).

Because CD4 T cells usually recognize peptides complexed with MHC class II and because resting CTL are class II negative, T helpers cannot bind to CTL as they do to B cells. For this reason it has long been thought that help for killers is mediated simply by the delivery of soluble IL-2. When Keene and Forman showed that help is only effective if both helper and killer recognize the same APC, the interpretation was that this enhanced the efficiency of help (79, 82); in effect, the helper cell needed to be close to the CTL precursor to prevent the secreted IL-2 from dissipating before it reached the killer, and this proximity would be ensured if both cells interacted with the same APC at the same time.

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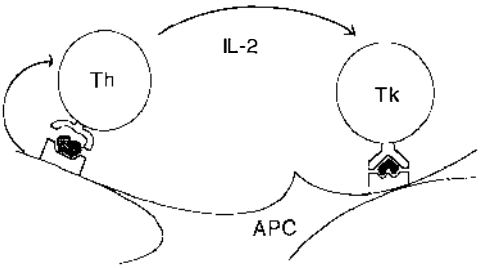
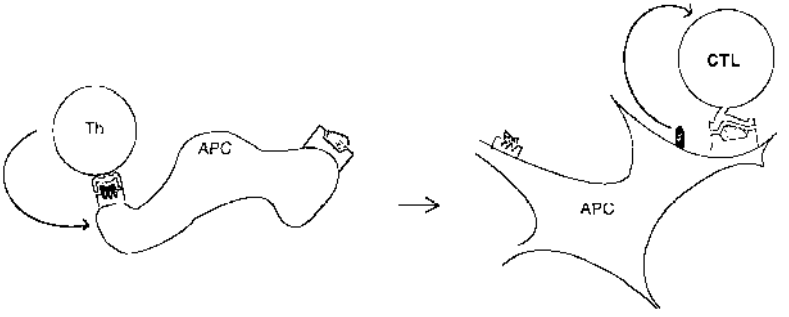


This view is not wholly satisfying. First, the few rare antigen specific helpers and killers must find the same APC and then stay there for the entire time that it takes the helper to produce IL-2 and then secrete it. The T helper and B cell have the same problem, but theirs is a two cell interaction, and each can continue to search until it finds its partner. In the case of helper and killer, neither can detect the presence of the other, and each could attach to separate APCs and wait in vain. Second, the helper must secrete IL-2 without any clue as to whether a killer in need of the lymphokine is in the neighborhood, an incredibly wasteful and inefficient process. Third, since properly stimulated killers make some IL-2 (83), why should they need help? Fourth, once the killer has received signal one, it will die without signal two. How long can it wait before it dies?

To get around these problems, we suggested the alternative view that help for CTL is routed through the APC rather than supplied directly. Based on the evidence that T helper cells can activate APCs (84, 85), we started with the idea that APCs do not normally express co-stimulatory signals for naive CTL but can be induced to do so by T helper cells. In effect, the T helper signals the APC, which then becomes activated and expresses distinct, CTL specific, co-stimulatory signals. The activated APC can now turn on CTL precursors in the absence of the original helper. In this way, the Bretscher-Cohn type of antigen specific helper signal is translated into a Lafferty-Cunningham type of co-stimulation.



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Delegating the help function to activated APCs solves many of the problems of T help for killers. First, rare helpers and killers need not meet. Second, helpers need not uselessly secrete IL-2 into their surroundings. Third, killers do not need to wait for signal two after receiving signal one. Since both signals come from APCs, they can be delivered simultaneously, as they are to helpers. Fourth, the activity of a few helpers can be amplified, since a single T helper cell can "arm" a number of APCs which can then, in their turn, activate a multitude of killers.

This model also provides an explanation for the contradictions about the helper dependence of CTL. To immunize mice to antigens such as Qa-1, H-Y, and other minor antigens, one usually uses normal spleen cells or grafts of normal skin, both of which contain normal resting dendritic cells or Langerhans cells which should not express co-stimulatory signals for CTL. Thus these responses need help. Viruses, however, are a different story. First, virus-infected tissues may produce a multitude of stimulatory molecules that activate the APCs directly, bypassing the need for T help. Second, the frequency of helpers may be far higher to the panoply of peptides presented by a virus than to a single, Ir gene–controlled antigen like H-Y, and it may be more difficult to completely deplete the responding T helpers. If the activity of a few remaining helpers can be amplified by their ability to activate several APCs each, then CD4-depleted mice would retain some responses to viruses, though they would inevitably respond less well than undepleted mice. This scenario fits with the observation that CD4-depleted mice often have weaker responses to viruses than do intact mice, a finding that is not explained if the CTL are truly helper independent.

In summary, both virgin T helpers and virgin T killers follow the first and second laws of lymphotics. They can be stimulated by APCs expressing antigen, but only if they simultaneously receive a second signal and they are tolerized in its absence. In the case of virgin helpers, the signal can only be supplied by professional APCs. In the case of virgin killers, the signal comes from APCs that have been activated by an interaction with a helper cell or, perhaps, by a stimulatory virus.

Experienced T Cells

By the two laws of lymphotics, an interaction between a T cell and a tissue that is not an APC leads to death for the T cell. This is the mechanism that allows the immune system to concentrate on detecting danger rather than defining self. It is a critical aspect of the model and, if the interactions I describe here are proven to be incorrect, the entire model comes crashing down.

One important difference in the rules for virgin and experienced T cells

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is that experienced cells can accept signal two from both professional and "amateur" APCs. Thus, for example, B cells tolerize virgin T helper cells but stimulate experienced ones. *Everything else stays the same.* Tissues that are not APCs cannot deliver second signals to either virgin or resting experienced cells and will thus be tolerogenic for any T cell that recognizes them. Since the long-term consequences of the two laws will depend on a cell's specificity, let's take the three different types in turn.

T CELLS SPECIFIC FOR FOREIGN ANTIGENS These cells were activated by a professional APC. They became effector cells, during which time they were oblivious to second signals, and then reverted to a resting state. If the infection is still going on, they will be reactivated, either by another antigen presenting dendritic cell or, since they are no longer virgin cells, by a B cell or macrophage. This cycle of activation, differentiation to effector, and reversion to resting cell should continue until the infection is finished. Because antigen specific B cells are incredibly efficient at capturing, concentrating, and presenting antigens, the response can be maintained until there is simply no trace of the foreign invader left. The experienced cells, or at least a selected few of them, will now rest down and circulate, perhaps being restimulated periodically from the store of antigens held by follicular dendritic cells (86), and wait for the next encounter.

AUTOREACTIVE T CELLS SPECIFIC FOR SELF PEPTIDES THAT ARE FOUND ON PERIPHERAL TISSUES[9] So far the scenario is that thymocytes become completely tolerant of dendritic cells and, when they migrate to the periphery, they can only be activated by dendritic cells. If the system were perfect, the only immunogenic molecules would therefore be foreign antigens captured by dendritic cells, but a problem with this simple scene arises when we consider that dendritic cells in peripheral micro-environments are certain to capture different sets of tissue restricted self-antigens. Any common antigens may be taken to the thymus often enough to induce deletion, but there are bound to be some local antigens which virgin T cells have never encountered and to which they are not tolerant.

To get around this problem, some self–non-self discrimination models have proposed that T cells leave the thymus in a tolerizable state and circulate through the body for a certain period of time so that autoreactive cells may meet their antigen and be tolerized. Theoretically, this is Russian roulette. Since T cells circulate from blood to lymph and back about once

[9]Dealing with these cells is an important part of any model. Therefore this section is detailed and long.

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every 24 hours (87), and since virgin T cells may not circulate to tissues at all (88), the tolerizable period would have to last *weeks* to give autoreactive cells even a minimal chance to be tolerized by their antigen. Even then, if the antigen were expressed on an MHC class II$^-$ tissue, no CD4 cell could be tolerized. In practice, there is no evidence for this view. Studies with adult, neonatal, and organ cultured thymocytes as well as with recent thymic migrants have shown that they make proliferation, lymphokine secretion, and killing responses (26, 89–91). Thus, T cells do not seem to leave the thymus in a uniquely tolerizable state. How then do they refrain from damaging peripheral tissues?

It takes only a small sidestep to look at this problem from the view of a danger discrimination model. In this case we simply say that mature T cells are *always* tolerizable and always activatable; we apply the laws of lymphotics.

Consider the virgin autoreactive T cell that encounters a professional APC presenting a tissue-restricted self-antigen along with a foreign one. The autoreactive T cell becomes an effector for a while and then reverts to a resting state, ready to be restimulated. Up to this point the picture looks the same as the one describing the T cell specific for a foreign antigen, but now things change. Because the self-antigen is always present on non-APCs as well as (temporarily) on APCs, the resting autoreactive cell will not necessarily see its antigen on an APC. It may well see it on a normal cell, in which case instead of being reactivated, it will be tolerized by the lack of signal two. Of course, this could also happen to T cells specific for foreign antigens, but the balance between tolerance and activation should be heavily loaded in favor of tolerance for autoreactive cells and reactivation for foreign antigen specific cells because of the ratio of APC to non-APC cells displaying the self-antigens.

For example, take a T killer cell specific for the skin antigen, Sk. In general it should pose no problem. Because of the relative inefficiency with which captured antigens are presented with MHC class I (92, 93), very few tissue-specific killer cells should ever be activated. However, the segregation between class I and class II pathways is not perfect (77, 94, 95), and the experiments on cross-priming tell us that there are in vivo mechanisms by which antigens can be reprocessed and presented with class I (95, 96), and therefore that a few killers specific for tissue-restricted antigens could become activated. Even a small number could be catastrophically destructive if not stopped.

They can be stopped, however. Consider an Sk-specific and a virus-specific cell that are activated by professional APCs in a lymph node draining from a virally infected skin lesion. Like the virus-specific CTL,

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the Sk-specific killer will multiply, leave the node in search of its targets, kill a few skin cells—not necessarily the infected ones, not even necessarily in the same location—and then revert to a resting cell, ready to be restimulated. The Sk-specific CTL is now in a critically different situation from the virus specific killer. Since Sk is everywhere on normal skin, the sk specific killer will not necessarily return to the site of infection. Circulating from blood to tissues and back, it will meet Sk on skin cells far more often than on APCs and will be tolerized by the absence of signal two. In contrast, the virus specific killer will accumulate at the site of infection and return to the resting state in the draining lymph node where it is more likely to meet a virus-presenting APC than an infected non-APC. After clearing the infection, the virus-specific CTL will have no opportunity to encounter their antigen in a tolerogenic way while, as time goes on, the Sk specific population will be completely tolerized by normal skin cells.

Thus, although the laws of lymphotics apply to both autoreactive and virus specific CTL; the difference in antigen source makes the difference between tolerance and activation. The antigen for an autoreactive cell is continuously expressed by normal cells whose sheer numbers and persistence ensure that an autoreactive cell should meet its antigen on a non-APC far more often than on an activated APC. Therefore, should a rare tissue-specific CTL be activated, it will cause only a small amount of destruction before it is tolerized. A prediction from this scenario is that self-reactive killers should be found during the early phases of most responses to foreign antigens, and they should disappear with time. A second prediction is that, if it becomes possible to unambiguously separate virgin from experienced cells using surface markers, the frequency of autoreactive CTL in the virgin population should be higher than that among experienced cells.

Autoreactive T helpers can be a mixed blessing. Should a significant number of virgin helpers be activated to tissue-restricted peptides, their experienced daughters will later have a reasonable probability of running into macrophages and/or B cells that have captured the same antigens from damaged or otherwise shocked tissues. At times this might be useful in that the helpers may activate macrophages at the sites of damage. At others it could be harmless. For example, helpers activated to tissue-restricted *intracellular* antigens could help CTL and B cells specific for those antigens. The CTL will eventually be tolerized by the tissues themselves and, at worst, the antibodies could do no harm. The plasma membrane stands between them and their antigens, and they could even be useful to help clear debris from damaged cells (97). Since 90% of the proteins in our bodies are intracellular, most autoreactive helpers can only be beneficial or harmless. The majority of the remaining proteins are

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secreted serum proteins to which tolerance should be easily achieved in the thymus by dendritic cells that capture them from plasma (27–30).

The problem comes with T helpers specific for tissue-restricted cell *surface* antigens. These are far more difficult to deal with than killers. The helper that has been activated by a professional APC displaying a peptide from a tissue-restricted surface antigen cannot see the antigen on the native tissue, because most tissues do not express MHC class II, and cannot become tolerized in the same manner as killers. By themselves, such helpers are harmless but should one meet a B cell specific for the same antigen, the ensuing auto-antibodies could create damage. Though B cells should generally be tolerant of cell surface auto-antigens, hypermutation during an immune response can generate an autoreactive B cell at any time. Though the chances are slim that a particular response will generate both a newly mutated B cell and an autoreactive T helper specific for the same tissue-restricted surface molecule, it is not impossible. The evidence that it probably happens, if only occasionally, is that the body has gone to some lengths to protect itself from it, with such safety valves as decay accelerating factor—the species-specific molecule that prevents complement from drilling holes in the membranes of normal cells (98), or the Ly49 molecule which shuts off natural killer cells when it binds to a normal MHC molecule (99). Nevertheless, the safety measures are not perfect, and dealing with these surface antigen-specific autoreactive T helpers is one of the most difficult, or rather incomplete, areas of this model. There are several possibilities.

One could begin by acknowledging that the frequency of such occurrences is exceedingly rare and that the immune system might live with it. Dawkins (100) pointed out several different sorts of evolutionary constraints on attaining perfection. His list includes time lags, historical constraints, lack of available genetic variation, constraints of costs and materials, imperfection at one level due to selection at another, mistakes due to environmental unpredictability, and the rare enemy effect, all of which can support the notion that the immune system lives with its imperfections. For example, when considering the role of cost constraints, the rule of thumb is that every improvement costs something. Consider gene rearrangement. The immune system improved its ability to recognize antigenic shapes by creating combinatorial associations of V, D, and J gene segments. The cost was a reduction in efficiency of B and T cell production because of loss due to out-of-frame rearrangements. Another improvement was affinity maturation, resulting from hypermutation and selection of high affinity B cells. The cost was death of large numbers of B cells and a need to introduce mechanisms to deal with newly mutated autoreactive cells. This is probably the most significant reason for the existence of T

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helper cells (33). After all, why should B cells need help? They would be much more efficient if they could respond by themselves, without needing to find a rare T helper specific for the same antigen. Without hypermutation, self-tolerant B cells would maintain their specificity, and there would be no need for the controllers that have been (mis)named helper cells (17, 33).[10] An improvement in the immune system's ability to recognize viruses and intracellular parasites came with the introduction of MHC molecules, which literally turn cells inside out by displaying internal peptides. The costs of this evolutionary maneuver have not yet been established, but they will include such things as the uselessness of T cells that are not able to bind to MHC (the driving force behind positive selection models), the construction of (or subversion of pre-existing) transporters to get the peptides to the MHC (101), the potential devastation on the immunological repertoire due to the need to be tolerant of all internal and external proteins, and all their peptides, rather than the small set of surface proteins in their native form. I could go on indefinitely. The point is that each improvement has compensatory costs, and we can never consider one without paying some attention to the other.

The principle behind the rare enemy effect is that it may cost more to be prepared for a rare enemy than it's worth. Are dangerous autoreactive helpers frequent enough to be worth dealing with? The thymic epithelium is composed of both endoderm and ectoderm (102), the bone marrow is mesodermally derived, and the dendritic cells present captured serum components as well as their own antigens. Thus, thymocytes specific for most self-antigens should be deleted. Very few autoreactive helpers specific for a tissue-restricted surface molecule will remain. In addition, since B cells specific for a cell surface molecule will also be exceedingly rare and short lived, the chances of an effective collaboration between the two might be vanishingly small.

Is it cost effective to deal with these rare events? The expense of adding control systems is not trivial. For example, the cost of needing T helper cell control over hypermutating B cells goes beyond the simple material costs of producing the helpers and such accessories as the MHC class II molecules necessary for effective T-B collaboration. It also imposes a sharp reduction in response efficiency, since each rare antigen-specific B cell must now find an equally rare T cell before it can respond. We cannot even begin to calculate the price of creating the circulation patterns, adhesion molecules, (perhaps the lymph nodes themselves?), required to ensure that

[10] A prediction from this reasoning is: Should we find a primitive immune system that has not evolved a mechanism for hypermutation, we may find that its B cells have no need for helper cells.

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the antigen specific B and T cells actually do meet each other. Because of potentially similar expenses, the immune system may have decided to put up with the rare helpers specific for tissue-restricted surface antigens, allowing a few individuals to become ill of autoimmune diseases rather than burdening every individual with yet another back up mechanism to ensure perfect tolerance.

Alternatively, the immune system may have chosen to evolve some protection, regardless of the expense. I can think of at least two ways in which this could be done. Both depend on the first law of lymphotics, but they differ in the source of signal one. The first supposes that peripheral tissues act as their own back-up tolerizers to inactivate T helpers, as they do for killers. They cannot do this constantly because most do not normally express MHC class II molecules. However, many peripheral cells can be induced to do so by lymphokines, and the induction of class II is not usually accompanied by a concomitant induction of co-stimulatory ability. Keratinocytes, for example, express class II molecules upon culture with γIFN, but they cannot stimulate T cells and, in fact, are tolerogenic (103, 104). Thus peripheral tissues that have been induced to express class II molecules could serve as a source of tolerogenic cells for circulating autoreactive T helpers.

The second source of tolerizers might be the professional APCs themselves. To my knowledge, it is not yet known whether dendritic or Langerhans cells sitting in tissue microenvironments can act as APCs before moving to the draining lymph nodes. If not, and if they nevertheless process their environment's antigens, *and* if the living cells around them slough their antigens continuously rather than only at times of stress, then these not-quite-yet APCs will offer signal one without signal two and act as tolerogenic cells for tissue specific T helpers of all kinds, including those specific for cell-surface antigens.

In either case, whether tolerance is induced by fledgling dendritic cells or by the tissues themselves, one might wonder what happens during an infection. Wouldn't infected non-APCs tolerize T cells specific for the foreign antigen? Again, as for killer cells, differences in the amount and persistence of antigen govern the result. The self-antigen is continuously present and, though each T cell makes only about one circuit per day, the autoreactive population will become tolerant over time. The foreign antigen, however, is transient. Thus, although a virus-infected or otherwise parasitized tissue may tolerize a few helpers specific for the foreign antigen, it is very unlikely to tolerize a significant proportion before local dendritic cells travel to the node to begin the process of initiating the response. For both killers and helpers, the rate at which tolerance to a particular tissue-specific antigen is established will depend on the size of the tolerizing tissue,

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the rate at which the cells flow through, the frequency of responders, and the level of MHC/peptide expression.

If the first and second laws of lymphotics hold, then any non-APC capable of expressing adequate levels of signal one (antigen) should be tolerogenic. This may explain the tolerogenic behavior of four different types of tissues.

1. *Transplants of normal tissues.* There are several examples of tissues that are accepted by allogeneic recipients once their passenger APCs are removed. For example, Lafferty and his colleagues (105) showed that thyroids or pancreases were not rejected if the grafts had been depleted of passenger leukocytes. If fresh donor type APCs were injected at the time of transplantation or soon after, the grafts were rapidly rejected, showing that they could serve as targets though not as immunogens. Over time, mice holding APC depleted grafts became tolerant and were unable to reject their grafts even when immunized with fresh APCs. Such tolerogenicity is not limited to endocrine tissues. Batchelor found that APC depleted kidneys were not rejected by fully competent allogeneic rats (106), and Kamada et al (107) showed that orthotopic transplants of intact rat liver, instead of being rejected, induce tolerance.

The case of liver seems rather extraordinary, since the transplants were *not* treated to deplete them of APCs, but though this is difficult to explain from a self–non-self discrimination viewpoint, it follows nicely from the first and second laws of lymphotics. Why should fully allogeneic liver transplants, replete with APCs, be tolerogenic when skin or heart grafts are rejected? The reason is that livers are big, and they are also able to recover from damage by regenerating new cells; consequently they are a large and stable source of signal one. The response to a liver graft is exactly what the laws would predict. APCs in the liver initiate a huge allogeneic response, and it becomes filled with lymphocytes precisely as in a rejection. However, after a few weeks the response wanes and eventually dies out altogether, leaving the recipients tolerant (107). My interpretation of this sequence is that the allo-MHC specific T cells are activated in the draining node by APCs from the graft; they migrate to the organ and begin to destroy it. The liver, of course, regenerates while its bone-marrow derived APCs do not. Therefore, after a certain period of time, the liver itself is the only remaining tissue that expresses the foreign MHC molecules to which the T cells respond. It continues to offer signal one without signal two and, rather quickly because of its size, tolerizes all the relevant T cells.

2. *Transgenic mice with peripherally expressed antigens.* The data are astonishingly varied, with some mice that are solidly tolerant of their peripherally expressed antigens, some that appear tolerant but respond in

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tests done in vitro, and some that appear tolerant but reject their transgene-carrying tissues if immunized properly.

Since this is not a review on transgenic mice, I am not going to go through these one by one. Interested readers can refer to *Immunological Reviews* No. 122 (25) as well as the wealth of literature since then. However the first and second laws of lymphotics, when combined with the concept of tissue specific MAPs and the knowledge that the TCR α chains do not show complete allelic exclusion in the TCR transgenics, create a framework within which much of the data seem understandable.[11] There are basically three groups.

(a) *Mice that are solidly tolerant.* These show that T cells can be tolerant of an antigen expressed exclusively in the periphery. Although there have been cases in which a supposed peripheral antigen was later found also to be expressed in the thymus, there are enough cases in which complete tolerance exists to a peripheral antigen to conclude that peripheral tissues may indeed induce T cell tolerance.

(b) *Mice that are solidly tolerant of the original transgenic tissue but react in vitro.* In vitro responsiveness is usually measured by MLR or CTL tests, which are directed against the antigens expressed by irradiated spleen cell stimulators (probably mostly dendritic cells) and/or tumor or mitogen activated T or B blast cell targets. These do *not* express the same MHC/peptide MAPs as islets of Langerhans, liver cells, thyroid cells, thymic epithelium, etc. Therefore, until it is shown otherwise, I would class these mice in the category of those that are solidly tolerant. Their reactivity in vitro (or in vivo, if tested against a different tissue, like skin) is a red herring.

(c) *Mice that accept their transgenic tissues until immunized.* The transgenic mice expressing viral proteins give three different patterns. Mice carrying SV40 large T antigen expressed early or influenza hemaglutinin (HA) on islets are solidly tolerant, and this tolerance cannot be broken by any sort of immunization (108). Slightly more reactive are mice in which MHC K^b is expressed by the liver (110). These mice are unreactive to their livers, even as double transgenics containing an anti-K^b TCR. However, when injected with T cells hyperimmunized to K^b, they behave like the liver transplanted mice discussed above. They begin with a massive liver infiltration and, within a few weeks, resolve the attack and become tolerant

[11] I exclude here any experiment in which the specificity of a cell depends on both the α and the β chains, but only the β is followed. Given that the endogenous α chains are not well excluded and that their expression on the peripheral cells is unpredictable, these experiments are inconclusive.

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again. The most reactive are mice in which an LCMV glycoprotein (GP) is expressed by the pancreatic islets. Both the single GP transgenic mice and double transgenic mice, carrying GP as well as an anti-GP TCR, are unreactive until immunized with whole LCMV, whereupon they quickly destroy their islets and become hyperglycemic (3).

There are three aspects of transgenic mice that must be kept in mind when interpreting the results of these experiments and others designed to test the tolerogenicity of peripheral tissues. First, the thymuses in TCR transgenic mice put out far larger numbers of T cells specific for any self antigen than could ever be normally expected. It may not be surprising that the rate of normal peripheral tolerance induction is sometimes too slow to deal with such overwhelming attack. Second, many of the T cells in TCR transgenic mice express two α chains, and this lack of allelic exclusion can affect the induction of tolerance as well as the specificity of activation. Consider a mouse transgenic for a peripherally expressed antigen (say X on the thyroid). These mice should be unreactive to X because thyroid is not an APC. If they were immunized with X given in adjuvant (and thus presented by APCs) the activated T cells responding to the inoculated X would also cause temporary destruction of the X-expressing thyroid. The response would soon stop, however, because the injected antigen X would be cleared and no longer available to APCs. Eventually, X-specific T cells that revert from effectors to resting cells would be tolerized when they encounter X presented by the thyroid itself.

Now consider the double transgenic mouse carrying X as well as a TCR specific for X (anti-X). Though T cells that express only the transgenic α and β anti-X TCRs should be deleted, those with a lower level of the transgenic α chain may not. These cells, carrying both endogenous and transgenic α chains will respond to environmental antigens (109) and, once activated, the cells carrying two α chains should be able to attack both the environmental antigen and the thyroid-X. Thus the thyroid would be destroyed even in the absence of overt immunization with X, but this destruction would be due to the dual specificity of the T cells, not because self tolerance has been broken. This is an example of how the need to maintain self tolerance may be the evolutionary driving force for allelic exclusion (97).

Third, tissues differ in their size and sensitivity. It seems that the liver, which is large and not barricaded from circulating lymphocytes (111), can deal with massive attacks while the islets of Langerhans cannot. Both size and the rate of T cell circulation may affect the ability of an organ to induce tolerance to itself. The tiny islets are simply not able to keep up. They are too small and the traffic rate through them is too low for tolerance to be established quickly. In addition, they seem to be extremely sensitive

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to the expression of some exogenous genes, dying in the absence of any immune attack (110), and this unusual death may well trigger immune responses that would not normally occur.

Overall, the transgenic mice that express an antigen on a peripheral tissue follow the first and second laws of lymphotics and do not reject their tissues. However, the tolerizing capacity can sometimes be overcome by a combination of constant stimulation through transgenic or endogenous TCRs in cases where the tolerizing organ is small and the number of responding autoreactive cells is large. Though these cases have little to do with reality, they do point to a possible explanation for why many autoimmune diseases involve small organs.

3. *Veto cells*. Veto cells, though currently thought to have special tolerogenic properties, may simply be another example of tolerance induction by tissues that are not professional APCs. Veto cells come in several forms. The original veto cells, found in normal thymus and nude spleen (48), seem to be NK cells. Their characteristic feature is that they turn off any CTLs which recognize them, whether the recognition is to intrinsic cellular antigens like MHC or to an antigen picked up and displayed by the VETO cell, like DNP. Activated T killer clones also have veto properties and have been added to the list of VETO cells, as have whole spleen cells, which can transiently shut off responses to themselves when injected into naive mice (112). Ephraim Fuchs and I could, of course, add B cells to the list, because they also have tolerogenic properties in naive mice (54), and the same could be done for keratinocytes, which can turn off the response of T cell clones (103, 104). We prefer, however, to think that these tolerizing tissues are simply cells that offer signal one in the absence of signal two. By the first and second laws of lymphotics, *any* cell that is not an APC should turn off responses to itself, as long as it expresses a sufficient level of signal one.

If veto phenomena are actually due to lack of signal two, then one would predict that the addition of second signals or of helper antigens able to elicit second signals would abrogate the veto effect. There are two systems in which this has been seen. In the first, spleen cells expressing K^b were found to be tolerogenic to H-$2K^{bm1}$ mice (which differ only at this single MHC locus), and this property was ascribed to the veto properties of the T cells in the K^b spleen cell inoculum (112). In the second, spleen cells expressing $Qa1^b$ were found to be tolerogenic to $Qa1^a$ mice, and the induction of tolerance was ascribed to the lack of help (76, 79). In both cases, the addition of helper antigens able to elicit delivery of second signals turned the tolerogenic inoculum into an immunogenic one. In the case of Qa1, the helper antigens were H-Y or BALB minors, whereas for K^b they were MHC class II alleles. Thus tolerance induction thought to

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be due to veto cells clearly mimics tolerance induction due to the presentation of signal one without signal two. My guess is that veto cells are peripheral tissues that do not express co-stimulation rather than a special class of regulatory cells.

4. *Tumors.* Tumors, per se, are not immunogenic. Whether they express new antigens or not, they are not APCs and should not activate T cells. Therefore, until they become necrotic or if they shed their antigens, they should grow happily without any intervention. Why then don't we get more tumors? There are two possibilities. One is that we simply don't. The immune system has little to do with it. The other is that the immune system functions by containing tumor-forming viruses rather than by eliminating the tumors themselves.

There is evidence both for and against the view that a functioning immune system has a suppressive effect on the incidence of tumor formation. For example, cyclosporin-treated patients have an increased incidence of many different tumors, most of them connected to viruses such as EBV and papilloma (113), yet nude and SCID mice get very few. In fact, the incidence of tumors in nude mice goes up if they are thymus grafted, the tumors in that case being mostly of T cell origin (114). It therefore appears that mice deal with tumors in the absence of an immune system, yet humans do not. However, this species difference may actually be an environmental difference. Since both nude and SCID mice are maintained in pathogen free environments, they seldom come in contact with tumor forming viruses. Nor are they often out in the sun. The differences between humans and mice suggest that the beneficial effect of the immune system may be to contain the viruses themselves rather than the tumors they form. Once a tumor forms, there is little the immune system can do. NK cells, with their ability to kill MHC negative targets, may play some role in the battle against tumors that lose their MHC expression, but their main function is probably to deal with cells infected by viruses, such as adenovirus, that inhibit MHC class I expression as a way to escape CTL lysis. Tumors are simply not something that the immune system is good at dealing with and, since most of them occur after the breeding age, the pressure to evolve mechanisms to deal with them has probably not been very strong.

Yet, in spite of their nonimmunogenic nature, it should be possible to immunize against any tumor carrying antigens not found on normal cells. The immunization might be as simple as an injection of disrupted tumor cells in adjuvant. It might be necessary to add carrier determinants to activate helper cells, or to inject professional APCs that have been fed with the tumor cells (or their antigens if they are known). However, because a tumor may well induce tolerance to its antigens, the immunization should be done early, while the tumor is small, or it should be done a reasonable

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time after the tumor is removed in order to give the thymus time to repopulate the periphery with potentially tumor-specific T cells.

Taken all together, the data from experiments with APC-depleted or intact transplants, transgenic mice, or with particular cell types such as T killer cells, B cells, keratinocytes, and various tumors support the notion that peripheral tissues can tolerize T cells specific for their antigens. Thus, autoreactive T cells that are activated by professional APCs presenting self-antigen will later become tolerized by the self-tissue itself. In this way, T cells need not discriminate between self and foreign antigens. They discriminate between second signals instead, and by following the first and second laws of lymphotics, they refrain from causing irreparable damage to peripheral tissues.

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T CELLS THAT HAVE A DUAL SPECIFICITY FOR FOREIGN AS WELL AS SELF PEPTIDES These cells have a different effect from purely autoreactive cells in that they may be carried for longer periods of time because of their cross-reaction to foreign antigen. Therefore there may be cases in which a significant amount of transient auto-destruction is seen. Sometimes, as in the case of rheumatic fever, irreparable damage is done during the infection by cross-reactive antibodies that decline when the infection is over (115). At others, such as adult infection with LCMV, the temporary damage is enough to kill the individual. The characteristics of the target tissues are important in these cases, as small organs are more likely to be irreparably damaged than large organs or than those that are turning over rapidly. Once the antigen is cleared however, these cross-reactive cells should rest down and, like the purely autoreactive cells described above, will then be tolerized by the self-tissue.

There is the slight possibility that these cells may even be useful. Because antigen specific lymphocytes are rare, primary responses can be difficult to start. B cells partially solve the problem by recruiting many rather low affinity IgM producers which switch to other Ig classes and to higher affinities as the response progresses. T cells do not have the same option. However, they may enhance their primary responses by recruiting every possible reactive cell, regardless of whether that cell is strictly specific for the foreign antigen or cross-reacts on a self-molecule. Because the responders are usually not monoclonal, and because the focus is on the foreign antigen, not the self-molecule, the percentage of autoreactive T cells specific for any particular self-antigen will be a small proportion of the total, and little damage should be done to any one self-tissue. Thus, during a primary response, when the immune system must mobilize as many cells as possible, it may trade a small amount of auto-destruction for the gain in efficiency brought about by the greater number of responding cells.

PART V: FURTHER TOPICS

Anergy

If I were designing the immune system and wanted to ensure against reactions to self-tissues, I would not do it by pushing cells into a long lived anergic state. At best, if they maintain their anergic state, such cells are a useless drain on resources, since they must function well enough to stay alive, turn over their membranes, replenish their energy sources, etc. At worst, should they break from their somnambulant state, they are destructive. I would instead choose to let them die. Why then have so many immunologists decided that peripheral tolerance equals anergy? On the basis of what data? Let's look first at B cells.

The report in which the term "anergy" first received its cellular meaning (116) showed that mice tolerant of FITC had a normal number of FITC binding B cells, yet made no plaques. This was taken as evidence that the FITC specific B cells were present but "anergic." A close look at the data, however, shows that only about 1% of the antigen binding cells make plaques; thus all of the relevant B cells could have been deleted unnoticed. Let's now skip forward eight years to mice transgenic for HEL and an anti-HEL antibody in which the B cells are said to be anergic. These poor B cells can hardly do anything (117). Their numbers are down approximately two-fold. Their life span is short, and if transferred out of their tolerogenic environment, all but a few will die. In both the normal and transgenic mice, the "anergic" B cells can be pushed to make small responses if stimulated with strong doses of mitogens or repeated doses of antigen plus T cell help, and this has been taken as evidence that the anergic state is reversible. My interpretation of these data is that the B cells are dead or moribund, not anergic. After all, B cells do not simply implode when they see antigen. Effective antibody responses would be almost impossible if B cells were not able to wait a certain period of time for T cell help after receiving signal one. The data from both the normal and transgenic mice are compatible with the idea that the tolerized B cells are dying, that death may take a couple of days, and that 20 million new B cells are made daily to replace them (21).

What about T cells? From studies on some T cell clones, it appears that signal one without co-stimulation does not lead to death but to an "anergic" state in which the responding T cell cannot make IL-2 or proliferate but can make γIFN, IL-3, and IL-4 (118). However, not all clones are anergizable. Most of them simply die if offered antigen on cells that cannot deliver co-stimulation. The primary distinction between clones that die and those that merely become anergic is in their susceptibility to starvation from IL-2 depletion. If stimulated to produce IL-2 by antigen plus APC,

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most clones die if they are then washed 48 hr later and recultured without IL-2, but anergizable clones are special: they survive this treatment. This resistance to death by IL-2 withdrawal is a very useful property. Since the cells don't die, they can be used to study various aspects of T cell activation at the biochemical level, and they have been used extensively to dissect the events that lead to IL-2 production (118). However, the existence of these resistant variants is not evidence that a state of "anergy" contributes to in vivo tolerance.

So then, what of the in vivo data? It was reported that the same chemically treated APCs that induce anergy in a (resistant) T cell clone can also induce tolerance in vivo (119). This is an interesting finding that fits with the view that signal one in the absence of signal two leads to tolerance, but there is no evidence that the tolerance was due to anergy. It might as easily have been deletion. Next we have the superantigens, large doses of which generate massive proliferation followed by death of the majority of responding cells; the remaining cells are unresponsive to the superantigen for some time. Although these data seem straightforward, some questions need to be answered before they can be accepted as compelling evidence for in vivo anergy. First, what is the evidence that the surviving "anergic" cells responded to the superantigen in the first place? Perhaps the reason they survived the treatment is that they were intrinsically unreactive by virtue of their TCR α chains (120, 121). Did they proliferate? If one injects BrdU along with the superantigen, are the surviving cells labeled? Second, there is some evidence that recently primed cells do not respond to superantigens, although they respond normally to conventional antigens (122). Perhaps a cell's recent history predisposes it to respond differently to conventional and superantigens (123).

Until these questions are answered, I will continue to follow the experiments with anergizable clones because of the light they shed on T cell activation pathways, and those with superantigens because the superantigens are evolutionarily very interesting, but one should wait a while yet before adding anergy to the list of in vivo tolerance mechanisms.

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Exhaustion

Tolerance by non-APCs may account for the cases where anti-viral killers die off during the course of an overwhelming infection (124). During a normal infection, the specific killers differentiate from virgin to experienced cells and expand before the infection gets out of hand. As the infection progresses, a few killers may be tolerized by recognition of infected non-APCs, but these will be far outnumbered by those being activated. However, when enormous initial doses of virus are given (not a situation that would normally occur in the wild), several things can happen. First,

the non-APC cells of the lymph nodes may become infected and tolerize virgin cells. Second, the killers elicited in the primary stages may eventually kill every APC. Third, the large numbers of infected peripheral cells will become a source of tolerogen for the experienced cells. Thus the "exhaustion" may simply be due to presentation of antigen in the absence of signal two

Deviation and Suppression

At the beginning of this essay I mentioned that one of the three main questions in immunology concerns how the mode of response is determined. Not all modes of an immune response are useful, for example, antibodies to intracellular parasites or killers to bacterial toxins. On the flip side, not all modes of autoresponses are harmful, like T helpers and antibodies to intracellular (or denatured) self-proteins, or killers against serum components. Thus, control over the mode of response can be as effective a mechanism for preventing self destruction as control over its specificity. This area of study has exploded recently with the realization that T helper cells and their lymphokines control at least some aspects of the response mode (125).

There are those who argue that control of the response mode has nothing to do with tolerance, and in a way they're right. When a response shifts from IgM to IgA, we call it a class shift, not tolerance of the IgM producers. Similarly, if an antigen that normally induces Th1 and killer cells can be made to induce Th2 and B cells instead, it is a shift in mode, not tolerance of the Th1 cells. From the viewpoint of self–non-self discrimination, the individual is not tolerant; it still responds. However, from the perspective of the discrimination of danger, the shift in mode allows the immune system to prevent self destruction in a more subtle and efficient way than by deleting every possible reactive cell. It need only suppress the dangerous ones, leaving the rest available to fight external attacks.

What governs the shift from one mode of response to another is currently an area of extensive investigation and rightly belongs in a different review. I have only a couple of comments. First, a shift in mode can be extremely stable. Experiments with Leishmania (126, 127) showed that it takes about a week to set the mode and that, once set, it persists. Second, this stability may account for many suppressive phenomena. If the mode is controlled by a population of cells that have switched, for example, from Th1 to Th2 or visa versa, then it will be transferable by these cells to new individuals (128). Third, several different lines of experimentation would be best explained if the mode were at least partially controlled by the APCs presenting the antigen. This may account for such examples of tolerance as that seen in animals given UV irradiation before skin painting with

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DNFB (129), neonatal animals made tolerant of allogeneic or xenogeneic cells or proteins, animals depleted of CD4 cells or in which CD4 helper activity was blocked before transplant, or animals given two grafts of thymic epithelium carrying different antigens (130–134). These cases all exhibit one form of suppressive activity or another, and evidence that at least some of the suppressive activity may be due to a shift in the response mode comes from studies showing that injections of IL-2 or of blocking antibodies to IL-4, which can shift a response from Th2 to Th1, can prevent the induction of neonatal tolerance (135–137).

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Initiation and Maintainance of Autoimmune Disease

First let me make a distinction between autoimmune reactions and autoimmune disease. An autoimmune reaction, to my mind, is a normal part of any response. It will tend to be greater early in a primary response than in the late primary or secondary response because the autoreactive cells will eventually be tolerized by the presence of the autoantigen in the absence of second signals. For example, the early primary response to Epstein Barr virus contains large numbers of dual specificity antibodies that disappear as the response progresses (138); the response to Streptococcus elicits heart-specific autoantibodies which disappear as the infection clears (115); and many otherwise specific sera contain low level "background" IgM antibodies. Autoantibodies may come from several sources. They may be due to low affinity B cells that cannot be activated by the normal concentrations of auto-antigen, to B cells specific for intracellular antigens, to new mutants, or to new B cells. For T cells, there is the low-level killing that is often seen against uninfected syngeneic targets in CTL tests and at least part of the activity seen in the syngeneic MLR. These autoimmune reactions may be harmful, harmless, or even useful, but they eventually disappear as the autoreactive cells become tolerized.

If T and B cells both adhered perfectly to the rules and were rendered tolerant by the recognition of signal one without signal two, rampant autoimmune disease should not occur. But, since there are several levels of control, e.g. circulation, activation, cell division, reversion to a resting state, apoptosis, etc, there are several different ways to go wrong. The genes involved in generalized autoimmunity, as for example in the lpr mouse or human Lupus, could include those genes controlling any one of these functions. It has been suggested, for example that the Fas/Apo-1 and/or Bcl-2 genes, which control apoptosis induced by some, but not all signals (139–142), may be involved in a generalized deregulation of normal apoptotic pathways in the immune system and thus may lead to generalized autoimmune responses.

I would like to suggest an alternative, though not mutually exclusive,

possibility. It is really an extended version of Bottazzo's (143) idea that ectopic expression of MHC class II could expose the immune system to antigens it had not encountered and was not tolerant of. From several lines of experiments, we now know that aberrant MHC expression is not sufficient to initiate responses. It takes a cell that can deliver signal two, and I would argue that ectopic expression of MHC on a non-APC should actually lead to tolerance. However, there is a difference between the details of an idea and the intuition behind it. The important feature of Bottazzo's model was that autoimmunity may not be a defect in the immune response but in the expression of antigen, either in its concentration, location, or the way in which it is presented. This is not the same as the popular view that an autoimmune disease may be initiated by a disruption of immune regulation during the response to a foreign antigen that crossreacts with self. Although a foreign antigen may initiate the anti-self response, the difference here is that the deregulation, if any, occurs with the self-*antigen*, not with the responding lymphocytes.

One possibility may be that expression of the self-antigen is deregulated. For example, it has been suggested that the Fas mutation found in MRL-lpr/lpr mice may be involved in deregulating normal lymphocyte death such that many different sorts of autoimmune cells fail to be deleted normally (139). However, it may also affect other cell types. Suppose, for example, that cells dying by apoptosis are usually efficiently scavenged. Their internal components would not come in contact with B cells. However, if something went wrong with normal apoptotic death, B and T cells might well be exposed to levels of internal components they were not tolerant of. If the abnormal death continued, there would be no way to stop the response. Since MRL lpr/lpr mice do worse than lpr mice of other backgrounds, there are also other genes involved in the disease. Some of these might also be involved in the abnormal presentation of antigen rather than in governing immune responses, e.g. could plasma levels of ribonucleases be low in MRL mice such that they would not be able to handle an overload? Another example is seen in the case of *Myesthenia gravis* where the ectopic expression of fetal acetylcholine receptor in the thymus may similarly trigger a continuous but normal immune response because of its sensitivity to thymopoietin (144).

Yet another possibility could be that the disease may actually be directed not at a self-antigen but at a slow or low lying infective agent (3, 145, 146). Although this is not strictly an autoimmune reaction, it may easily mimic one and, if the infectious agent were difficult to isolate, could be taken for true autoimmunity. This could hold for organ specific diseases if the infection were localized, or for generalized diseases if the infection were, for example, a mycoplasma that acted as a constant or perhaps cyclic

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source of extracellular nuclear material. Waldmann had yet another suggestion (2). If peptides compete for MHC molecules in any cell, then the immune system is only tolerant of the subset that wins. Should the concentration of any one of these proteins change, because of infection, environmental trauma, or genetic reasons, then its MHC slot might be taken by a peptide that the immune system was not tolerant of. The deregulation of one peptide might thus cause the display of different peptides in different tissues, serving as a source of multi-organ autoimmunity.

The point of all these possibilities is that many autoimmune diseases may not be due to dysfunction of the responding cells but, as Bottazzo first suggested, to the inappropriate presentation of antigen, whether it is inappropriate because it is a deregulated self-antigen or a slow, low lying, infection.

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Recirculation and Autoimmunity

Virgin T cells seem to circulate from lymph node to lymph node rather than to extravasate into tissues (88). Since this shortens their pathlength, it increases the efficiency with which they scan for antigens and professional APCs. It also prevents them from being tolerized by infected peripheral tissues before they can be activated by an APC. As usual, however, the increased efficiency has a cost in that lymphocyte flow may influence an organ's susceptibility to autoimmune destruction. I pointed out above that a small organ will be inefficient at tolerizing cells specific for its antigens. The same holds if the rate of circulation through it is low. In liver, unlike most organs, there is intimate contact between blood and hepatocytes (111). They should be (and are) good tolerogens, but the endothelial cells lining blood vessels in islets, thyroid, and other endocrine organs may not normally allow easy passage of lymphocytes, and thus tolerance induction could be a slow and uneven process, leaving these organs susceptible to autoimmune attack during a strong response to a cross-reactive antigen or in cases where the endothelial cells may be defective (147, 148).

Haptens

Although we have a mechanistic reason for the immune system's inability to respond to isolated haptens, the viewpoint that the immune system discriminates self from non-self does not offer an evolutionary rationale. After all, haptens are non-self. However, since isolated haptens are not destructive unless they bind to something, there is no reason, from a danger discrimination viewpoint, to respond to them. The same holds for silicone, boiled bone, bits of metal, rocks, food, and other innocuous substances.

Costimulatory Signals

Finally, since naive and experienced cells respond differently to B cells but professional APCs can stimulate both, there must be a difference in the costimulatory signals involved. The study of activation in truly virgin T cells may therefore reveal cellular pathways distinct from those demonstrated in T cell clones and hybridomas. In particular, since activated B cells, which do not stimulate virgin T cells, express B7/BB1, the ligand for CD28 and CTLA-4 molecules on T cells, and HSA, another potential costimulatory molecule (reviewed in 149), there must be at least one other interaction between B and T cells that has not yet been found. In addition, the co-stimulatory signal for killer cells may well be different from the one given to helpers.

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PART VI: WHAT IS DANGEROUS?

This has been surprisingly difficult for me to work out. So many things are dangerous, like bacterial toxins, viruses, worms, and fast growing tumors, yet so many similar things are not, like beneficial bacterial secretions, nonlytic viruses, and rapidly dividing tissues like skin, gut, and bone marrow cells. How can the immune system distinguish? I have no single answer but, because the danger discrimination viewpoint has turned out to be so useful, I have tried to see how the immune system *could* do it, even if this is not the way it does do it. There are several possibilities, and they differ depending on whether we want APCs to be constitutively able to activate T cells.

If Professional APCs Are Consitutively Co-stimulatory for Virgin T Helper Cells (The Simplest Strategy)

If T helper cells specific for tissue restricted antigens can be tolerized by the tissues themselves, e.g. by γIFN induced class II/peptide complexes, then APCs can be constitutively active. In this scenario, dendritic cells are always in the process of circulating through tissues, picking up what they find and taking it to lymph nodes for perusal by T cells. Since T cells are tolerant of the APCs themselves, and since any autoreactive T cells specific for peripheral tissues can be tolerized by those tissues, the only lasting immune responses will be against captured foreign antigens.

If Professional APCs Are Inducible (A More Efficient Strategy)

If APCs cannot constitutively deliver co-stimulatory signals, but are activated only when needed, they can serve as tissue specific tolerizers (see

above), and they waste no energy on useless responses. In this scheme, *Danger* equals tissue destruction.

If a virus were to infect a cell, make a few copies of itself without harming the host cell, and move on, it would do no damage and there would be no need to eliminate it. Ephraim Fuchs suggested that we might even want to encourage such viruses in case they carry useful genes. Similarly, if a bacterium were to harmlessly colonize an organ, there would be no reason to attack it, and if it supplied some needed nutrient, there might even be reason to nurture it. We are, of course, full of commensal and ex-commensal organisms. Retroviruses jump in and out constantly, sometimes leaving bits of themselves behind. Bacteria colonize some internal and external organs, contributing enzymes for digestion and blood clotting, protection against other bacteria, even flavoring our individual scents, and over time some have become inseparable intracellular organs. There is no reason to rid ourselves of such organisms. How then can we distinguish harmful from harmless or beneficial ones? One mechanism would be to turn the immune system on only when cellular damage occurs.

Cell death is not always a sign of parasitic attack. It is a normal event during embryonic development, formation and death of hematopoietic cells, maturation and expulsion of oocytes, etc. In these cases death is controlled, usually apoptotic, and the dying cells are phagocytosed rather than lysed into the surroundings. However, since unprogrammed cell death does not usually occur by apoptosis, it could serve as a clue to local APCs that unplanned destruction is occurring. The signal could be active or passive.

1. Active signs of distress:

Cells stressed by sudden changes in their condition, such as temperature or infection, elaborate a series of heat shock proteins designed to aid their recovery (150). If one of these acted as a stimulator of dendritic cells, immune responses would only be initiated when they were needed. This scheme has the disadvantage that an invading organism could inhibit the pathway by, for example, blocking production of the stress protein.

2. Passive signs of distress:

Any internal molecule that is normally not secreted, be it cytoplasmic, nuclear, or membrane, could act as an activator of dendritic cells when released. Thus any cell damage would be noticed, whether the cells had an opportunity to synthesize new proteins or not. Any cut, bruise, infection, in short, any trauma, would activate the APCs and initiate an inflammation. If the trauma were noninfectious, a small autoreactive response might be initiated, but this would soon dissipate,[12] leaving nonspecific

[12] Occasionally, however, it might reveal itself as an autoimmune phenomenon such as trauma induced uveitis.

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inflammatory mechanisms to clean up. In contrast, an infection would elicit a full blown immune response and be cleared. If the relevant intracellular protein were absolutely necessary for cell life, this pathway would be very difficult to block.

CODA

I have found, for myself, that it is time to change viewpoints. For the greater part of a century, immunologists have been steeped in the tradition that the immune system's primary goal is to discriminate between self and non-self, and consequently we have been mostly preoccupied with the specificity of lymphocytes (151) or, as we might now call it, Signal One. We have spent a great deal of energy working out the structure of antibodies and T cell receptors, the genes that generated receptor diversity in species as different as mice, chickens, and sharks, and the chemical nature of the antigens recognized by these receptors, from the minimal size of carbohydrates seen by antibodies to the crystal structure of MHC/peptide complexes seen by TCRs. We probed the repertoires of mice and guinea pigs that were genetically incapable of responding to certain antigens and discovered IR genes. We probed the repertoires of mice that responded too well and discovered superantigens. Over time, we also began to see that single lymphocytes couldn't do the job alone: Jerne introduced cellular interactions (6), Bretscher and Cohn invented the second signal (17), and Lafferty and Cunningham started the process of bringing APCs onto an equal footing with lymphocytes (18). Though it is often expressed in different ways (216, 202), most of us no longer think that each lymphocyte stands alone, making its own decisions.

Looking from the perspective that the driving force behind immunity is the need to recognize danger, we are led to the notion that the immune system itself does not stand alone. It is not simply a collection of specialized cells that patrol the rest of the body, but an extended and intricately connected family of cell types involving almost every bodily tissue. Tolerance no longer resides solely with deletion vs persistence of single lymphocytes; rather it is seen to be a cooperative endeavor among lymphocytes, APCs, and other tissues. Memory no longer resides with long-lived lymphocytes but in their interactions with antibodies, antigen, and follicular dendritic cells. Immune response modes are governed by interactions between lymphocytes, APCs, basophils, mast cells, and all of their lymphokines (and perhaps the parasites themselves, as they try to influence the immune system). By these networks of co-operating cells, the immune system can be alerted to danger and destruction without ever needing to consider the question of self vs non-self. It can contain myriads of auto

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and foreign reactive lymphocytes, each ready to respond and each ready to be tolerized if necessary. In this way it has the strength to destroy the things it needs to destroy, the tolerance to leave others alone, and the ability to tell the difference.

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