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A two-step, two-signal model for the primary activation of precursor helper T cells

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ABSTRACT **I present here a new model for the primary activation of precursor helper T cells. Observations demonstrate that the immune system learns not to respond to extrathymic, organ-specific self-antigens because of their early appearance in development. The immune system thus discriminates between peripheral self-antigens and foreign antigens and, when mature, usually makes an immune response against only the latter. Contemporary models for the activation and inactivation of T helper (Th) function do not account for such discrimination. The model proposed here is consistent with contemporary findings and incorporates a mechanism of peripheral self–nonself discrimination.**

A Mechanism of Peripheral Tolerance Exists

T cells specific for self-antigens are silenced in the thymus when they encounter an antigen (1). T cells specific for organ-specific antigens emigrate from the thymus and have the potential for causing organ-specific autoimmunity (2, 3). Hanahan and coworkers (4) showed that mice that first express a transgenic antigen in pancreatic cells early in ontogeny are tolerant of this "self" antigen, whereas mice that first express the antigen later recognize it as "foreign." The latter mice suffer pancreatic "autoimmunity" after the antigen's appearance, as expressed by antibody production and lymphocyte infiltration of the pancreatic islets (4). The immune system thus appears to discriminate self from nonself among peripheral, extrathymic antigens and to rely on the early and continuous presence of peripheral self-antigens as their defining characteristic.

Contemporary Models for the Activation/Inactivation of Precursor Helper T Cells Do Not Account for Peripheral Tolerance

The activation of naive precursor Th (pTh) cells requires the generation of two signals (5) according to contemporary models (Fig. 1). Signal 1 is generated after the interaction of the T cell receptor (TcR) with its ligand, whereas signal 2 is generated via an interaction between costimulatory molecules on the antigen-presenting cell (APC) and counterreceptors on the T cell, such as the well known B7–CD28 interaction. The generation of signal 1 alone leads to the inactivation (deletion/anergic state) of the pTh cell. Contemporary models can be divided into two classes. According to the Model of Constitutive Costimulation (6, 7), APCs, such as mature dendritic cells, constitutively express costimulatory molecules. Constitutive costimulation cannot be *centrally* involved in the decision between the activation/inactivation of pTh cells, *if* these processes are to be related to peripheral tolerance. Such a mechanism cannot favor the activation of pTh cells specific for foreign peptides and the inactivation of those pTh cells specific for self-peptides. This model cannot account for the observations concerning peripheral tolerance of Hanahan and coworkers (4).

The expression of APC costimulatory molecules is, according to other contemporary models, induced after the generation of a third signal. Janeway and coworkers (8, 9) postulated that the immune system does not discriminate self from nonself but rather "non-infectious self from infectious nonself" and that microbial products, often present in adjuvants, are *required* to act on innate defense mechanisms to generate the third signal that initiates an immune response. Matzinger (10) proposed that the third signal distinguishes danger from nondanger. According to these models, whether an antigen activates or inactivates available pTh cells is independent of whether the immune system has been previously and continuously exposed to this antigen from some time early in ontogeny. Rather, *only* the circumstances at a particular time are critical at *this* time. These models cannot account for the observations of Hanahan and coworkers (4).

These third-signal models seem implausible on general grounds. Many foreign antigens of nonmicrobiological origin, such as sterile xenogeneic red blood cells, are immunogenic when administered without adjuvant, in contradiction to what Janeway (8) proposed. These red blood cells, self-antigens of species closely related to the animal immunized, are unlikely to activate innate defense mechanisms of the host (but not of the donor) to generate the third signal. Human rhesus factor-positive red blood cells are immunogenic in rhesus factor-negative humans and must, according to Janeway's view, generate the third signal. The possibility that some self-antigens generate third signals is unappealing, because one would anticipate, within the context of Janeway's proposals, that this would lead to organ-specific autoimmunity. The idea that injection of all these immunogenic antigens results in the generation of a danger signal, as required by the danger model, stretches credulity.

Introduction to the Two-Step, Two-Signal Model

In this paper I describe a new model for the activation/inactivation of naive pTh cells that is consistent with contemporary facts and incorporates a mechanism of peripheral tolerance.

The two-signal model for lymphocyte activation was proposed some years ago (11). In the modern context, it incorporates a potential mechanism of peripheral self-tolerance. Observations support the validity of this model for most B cells (12) and for at least some $CD8^+$ T cells (13, 14). However, the model, as it pertains to the activation/inactivation of pTh cells, cannot be reconciled with contemporary facts. A discussion of this model provides a convenient context for considering contemporary findings and the basis of the new model.

The original two-signal model postulated that the activation of a resting lymphocyte requires the antigen-mediated inter-

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Abbreviations: Th, T helper; pTh, precursor T helper; TcR, T cell receptor; APC, antigen-presenting cell; eTh, effector T helper; MHC, major histocompatibility complex.

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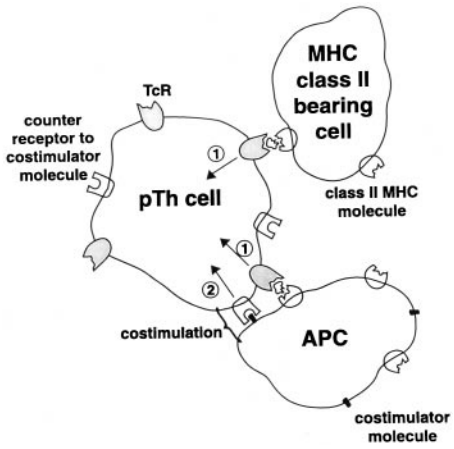

FIG. 1. Contemporary models for the activation of pTh cells. For a detailed explanation, see the text. The generation of signal 1 alone results in inactivation, whereas activation requires the generation of both signal 1 and signal 2 (costimulatory signal).

action of this lymphocyte with another "helper " lymphocyte specific for this antigen, whereas its inactivation occurs when this single lymphocyte interacts with antigen alone. These rules incorporate a process of peripheral tolerance. Lymphocytes specific for a self-antigen are eliminated as they are generated one (or a few) at a time, by virtue of the continuous presence of the antigen. In contrast, lymphocytes, specific for a foreign but not for any self-antigen, can accumulate in the absence of the foreign antigen. Once this foreign antigen impinges on the immune system, it can mediate interactions between the accumulated lymphocytes, leading to an immune response against itself. The interaction of antigen with a receptor of a precursor cell was envisaged to result in the generation of signal 1 that was inactivating when generated alone. The antigen-mediated interaction of a precursor cell with another antigen-specific cell, an effector T "helper" (eTh) cell, was postulated to result in the generation of another signal, called signal 2 (Fig. 2) and to the subsequent activation of the precursor cell. Signal 2 was envisaged to be short range. An intimate interaction between the two cells was thus required, rendering the activity of the eTh cell highly specific for the precursor cell with which it interacted. These postulates constituted the original two-signal model (11).

Four considerations/sets of observation suggest that the original two-signal model does not provide an adequate description of the activation/inactivation of naive pTh cells.

(*i*) **Recognition of Linked Epitopes in Cellular Interactions Involving T Cells.** Mitchison (15) showed in the late 1960s that the activation of an anti-hapten B cell by a hapten–carrier conjugate was aided by the presence of carrier-specific Th cells. Such help was postulated to require an antigen bridge between the two cells. These insights were incorporated in the two-signal model (Fig. 2). Effective help by carrier-specific T cells for the activation of hapten-specific B cells was observed only when the challenge was with the hapten–carrier conjugate and not when hapten and carrier were both present but unconjugated. This "recognition of linked epitopes" is central in ensuring the specificity of the activity of helper T cells. Thus, Th cells specific for an antigen Q can help only the antibody response to Q and not a response to a non-crossreacting antigen L in the presence of both Q and L.

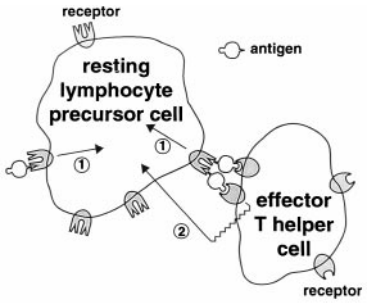

FIG. 2. The original two-signal model. The generation of signal 1 alone results in inactivation, whereas activation requires the generation of both signal 1 and signal 2.

This antigen bridge model appears inconsistent with the fact that $\alpha\beta$ Th cells recognize a peptide, derived from a nominal antigen, bound by a major histocompatibility complex (MHC) restriction element. How can there be a requirement for hapten–carrier linkage if the hapten–carrier conjugate must be degraded into peptides, unlinked to the hapten, before the carrier-specific $\alpha\beta$ T cells can recognize the "nominal" carrier? Lanzavecchia (16) resolved this paradox. He showed that hapten-specific B cells endocytose the hapten–carrier conjugate, process it to yield carrier-derived peptides that then bind to nascently synthesized class II MHC molecules. Peptide-loaded class II MHC molecules, subsequently expressed on the B cell surface, can be recognized by carrier-specific Th cells. This mechanism explains why carrier-specific Th cells can help hapten-specific B cells only if the hapten is attached to that carrier.

An even more acute problem arises if it is believed that the activation of pTh cells requires a pTh cell–eTh cell interaction mediated by the recognition of linked epitopes, as postulated by the original two-signal model.

(*ii*) **Constitutive Costimulation.** The Model of Constitutive Costimulation bears a superficial resemblance to the original two-signal model. The crucial difference is that the costimulatory signal (signal 2) is constitutively expressed, whereas its generation, according to the original two-signal model, follows the recognition of a second site on the antigen (Figs. 1 and 2). The original two-signal model does not account for the observations that led to the model of constitutive costimulation (6, 7).

(*iii*) **The Priming Problem.** Unprimed B cells do not secrete antibody, whereas their activated progeny do. eTh cells are required for the activation of Th cell-dependent B cells. However, eTh cells are normally not constitutively present at the level required to activate such B cells, and sufficient eTh cells must be generated through the antigen-dependent activation of pTh cells. Such activation of pTh cells requires eTh cells, according to the precepts of the original two-signal model. How in turn are these eTh cells generated? This reiterative problem is referred to as the "priming problem" (17). The priming problem must be faced by any attempt to provide a valid description of how immune responses are initiated.

(*iv*) **The Scarcity Problem.** Antigen-specific lymphocytes are scarce in unprimed animals. How can a primary immune response be initiated when this requires antigen to mediate a physically intimate interaction between two/several scarce specific cells? This scarcity problem is most acute for the subset of lymphocytes first activated during the course of a response. For example, scarce unprimed B cells may be efficiently induced if many eTh cells are generated after the efficient activation of pTh cells.

A Two-Step, Two-Signal Model for the Primary Activation of pTh Cells

The new model is depicted in Fig. 3. The model also postulates under what circumstances antigens can inactivate pTh cells.

(*i*) The activation of naive pTh cells to yield eTh cells occurs through a series of distinct steps.

(*ii*) The first step in the antigen-dependent activation of pTh cells specific for a nominal antigen Q is their proliferation on interacting with APCs that present Q and constitutively express costimulatory molecules, such as the B7 molecules. Such APCs are likely to be mature dendritic cells and/or macro-

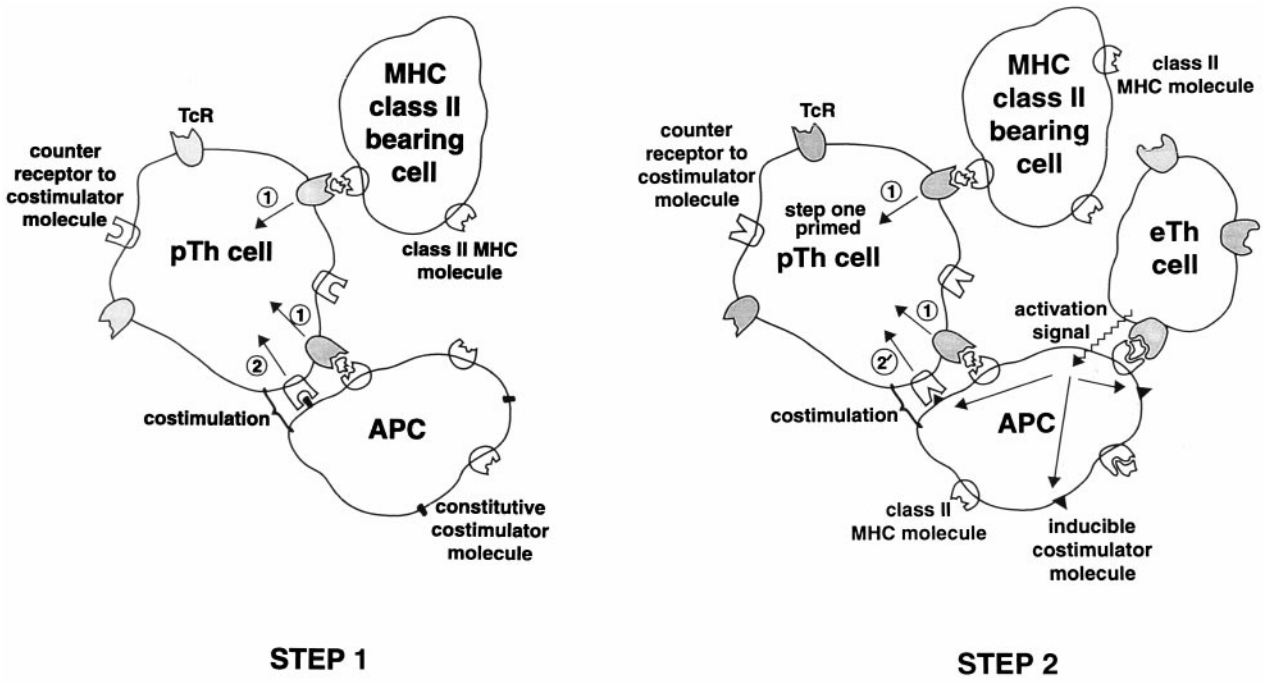

FIG. 3. A two step, two-signal model for the primary activation of pTh cells. For an explanation, see the text.

phages. pTh cells that have undergone this step are called step 1 primed pTh cells.

(*iii*) Step 1 primed pTh cells must go through (at least) a second step, step 2, to yield mature eTh cells. Step 2 requires the step 1 primed Th cells to interact with an activated, Q-specific B cell that acts as an APC. Activation of the B cell follows the binding by an eTh cell to B cell-presented peptides derived from Q. The activation of the B cell results in its expression of *inducible* costimulatory molecules, which are recognized by counterreceptors on the step 1 primed pTh cells. This interaction results in the delivery of a second signal to the step 1 primed pTh cells, distinct from the signal 2 of the first step, and referred to as signal 2′, for step 2. Thus, the completion of step 2 for pTh cells specific for Q requires the existence of eTh cells specific for Q, i.e., this constitutes a particular form of $CD4^+$ T cell/$CD4^+$ T cell cooperation.

The postulate that the APC in the second step is normally a *specific* B cell ensures that the activation of pTh cells by an antigen Q requires eTh specific for the *same* nominal antigen Q. The anti-Q B cell is postulated to endocytose the antigen Q efficiently but not antigens to which its receptors cannot bind. eTh cells specific for a Q-derived peptide, q_1, can help Q only to activate anti-q_2 pTh cells if the q_2 peptide is also derived from Q. This mandatory involvement of an activated B cell, or for an as yet uncharacterized cell with receptors specific for antigen and bearing class II MHC molecules, accounts for the requirement for the recognition of linked epitopes in the activation of pTh cells, for which there is evidence (see later). Consider the activation of Q-specific pTh cells when there are pTh cells also available for a non-crossreacting peripheral antigen P. The requirement for linked recognition allows Q to activate anti-Q pTh cells in the presence of anti-Q eTh cells without activating any available anti-P pTh cells even in the presence of both Q and P. This feature ensures that pTh activation is exquisitely specific. This specificity is guaranteed by virtue of the B cell receptor's affinity for unprocessed antigen. It would not be guaranteed if the APC was a macrophage, a cell less discriminating in uptake of antigen, or by an MHC class II-bearing T cell, because MHC-restricted T cells cannot guarantee the specific uptake of the two peptides, q1 and q2, derived from Q.

Further steps are likely required to achieve full activation of pTh cells involving, for example, signals generated by cytokines.

(*iv*) The interaction of antigen with a pTh cell without a completion of the process through step 2 results in the long term in the inactivation of the pTh cell. This proposal is supported by the general finding that antigen-dependent proliferation of T cells, after the first step, can sometimes result in their subsequent death or their acquisition of an anergic state (18, 19). Completion of step 2 may down-regulate the expression on the surface of the step 1 primed pTh cells of molecules such as CTLA-4, a counterreceptor to B7 molecules, whose engagement leads to apoptosis or down-regulation of the activity of $CD4^+$ T cells (5, 20).

(*v*) The initial multiplication of the pTh cell associated with the first step does *not* require specific cell–specific cell collaboration between scarce cells. This multiplication should more easily allow the specific cell–specific cell collaboration to take place that is required in step 2 of the activation process. The first step can be interpreted as a means of minimizing the scarcity problem associated with the second step.

(*vi*) The completion of step 2 requires the presence of anti-Q eTh cells. This raises the question of how the first Q-specific eTh cells are generated, i.e., how the priming problem might be solved. I describe the solution I favor.

The antibody response is susceptible to x-ray radiation (21) because of the radiation sensitivity of dividing cells, including B cells. The ability of pTh cells to be induced by antigen to yield eTh cells is similarly sensitive to irradiation, but the *activity* of eTh cells, once generated, is radiation resistant (22). This makes sense in that the generation but not the expression of eTh activity requires T cell multiplication. There is a spectrum of antibody-producing cells in a mature mouse not deliberately immunized. These cells maintain the level of serum Ig. Such on-going antibody responses are likely Th dependent, implying the existence of a spectrum of eTh cells in a mature mouse not immunized by an immunologist. Observations support this reasoning. Radiation-resistant, antigen-specific eTh cells can be detected in the spleen of unimmunized mature mice (23). How could such a spectrum of eTh cells arise?

The existence of a few eTh cells neonatally may, in the continuous presence of an appropriate variety of foreign antigens and spectrum of pTh cells, yield a wider spectrum of eTh cells, in accord with the precepts of the two-step, two-signal model. Thus, the priming problem may be reduced to understanding the origin of a few eTh cells neonatally. The difference between the neonatal pTh cell population specific for a foreign and for a peripheral self-antigen is in the number of cells available. The greater number of cells specific for a foreign than a self-antigen could allow the foreign but not the self-antigen to generate some eTh cells. This could occur if, for example, the foreign antigen was present and pTh cells were endowed with some basal eTh activity at approximately the time of birth (17).

(*vii*) This model incorporates a mechanism of peripheral self–nonself discrimination at the level of pTh/Th cells. In the absence of a foreign antigen, $CD4^+$ T cells specific for this antigen can accumulate, and a response can be mounted once this antigen impinges on the immune system. In the continuous presence of a peripheral self-antigen, first present before functional CD4 T cells develop, those $CD4^+$ T cells specific for the self-antigen will be inactivated as they are generated one or a few at a time. This model thus accounts for Hanahan's (4) obsevations on peripheral tolerance.

Evidence for the Two-Step, Two-Signal Model

Evidence for Constitutive Costimulation. "Passenger leukocytes" present in transplants are central to the transplant's rapid rejection on grafting to MHC-incompatible hosts. This finding was central to the formulation of the Model of Constitutive Costimulation. Such passenger leukocytes were identified as APCs that bore both the foreign MHC antigens and costimulatory molecules (7, 24). Further evidence from $CD4^+$ T cell clones suggested that the generation of signal 1 alone was a negative signal, rendering T cells refractory to subsequent attempts at activation (6). These observations provide evidence for the first step. They do not preclude the existence of further steps.

T–T Cooperation in the Activation of pTh Cells and Inducible Costimulatory Molecules. (*i*) The generation of T cells mediating delayed-type hypersensitivity requires T cell–T cell collaboration (22, 25). The activation of pTh cells, to yield radiation-resistant eTh cells able to aid the induction of precursor T cells whose progeny can mediate delayed-type hypersensitivity, also requires T cell–T cell collaboration. The generation of these eTh cells probably requires further T cell–T cell collaboration. Thus the generation of Th activity appears to involve a cascade of specific T cell–T cell interactions (26). All of these interactions require the recognition of linked epitopes (22, 26).

(*ii*) Mature T cells, able to react to organ-specific antigens, emigrate from the thymus (2, 3). The epitopes recognized by autoimmune T cells change with time in both type I diabetes and experimental allergic encephalitis. The first T cells generated in murine diabetes recognize one predominant epitope of a β-islet cell antigen, and with time there is an increase in the diversity of β-islet epitopes recognized. The generation of the T cells specific for the first epitope recognized can be blocked by administering in the neonatal period the appropriate antigen intrathymically. This also blocks the generation of the T cells normally subsequently generated and specific for other β-islet cell antigens (27). The induction of Th cells specific for a peptide of basic myelin protein, a self-antigen, by immunizing with the peptide in complete Freund's adjuvant results in the longer term in the generation of Th cells specific for other epitopes of this protein (28). The phenomenon, in which the epitopes recognized by Th cells against one autoantigen, or against epitopes on antigens present on a self-cell, increases with time, is called "epitope spreading." Epitope spreading is expected if the generation of Th activity requires pTh cell–eTh cell collaboration. The blocking of the generation of a cascade of T cells by blocking the generation of the first Th cells normally observed is in accord with this requirement.

(*iii*) The B7 molecules are constitutively expressed on mature dendritic cells, APCs often involved in the initiation of immune responses, and are sparse on resting but substantially present on activated B cells (5). The latter observations fit in with older studies demonstrating that APC function can be dramatically increased, or perhaps qualitatively altered, by activating the APC through exposure to antigen and antigen-specific MHC-compatible Th cells (29). A requirement for such activation of APC function is consistent with both the precepts of the new model (Fig. 3, step 2) and with the demonstration of T cell–T cell collaboration in the activation of pTh cells. The CD40 ligand is present on resting B cells, and its counterreceptor, CD40L, is present on activated $CD4^+$ Th but not on resting $CD4^+$ T cells. An interaction between CD40 and CD40L can result in the expression on the B cell of costimulatory molecules such as the B7 molecules and CD44H (30). The requirement that the APC in the second step be an activated specific B cell explains why the T cell–T cell interaction requires the recognition of linked epitopes, and this feature of the inductive process is regarded as critical to achieve specificity in the initiation of immune responses. How can the requirement for a B cell as the second-step APC be ensured mechanistically? It is likely that B7 molecules play a role as constitutive costimulatory molecules in step 1 and of an inducible costimulatory molecule in step 2. The B7 molecules must have different functions, and their engagement with counterreceptors on the pTh cell have different biochemical consequences for the interacting $CD4^+$ T cell when expressed on dendritic cells and on activated B cells. The full costimulatory effects of B7 molecules when on dendritic cells could be attributable solely to their interaction with counterreceptors on the pTh cell, whereas the different and full costimulatory functions of activated B cells might require, in addition to the interactions between B7 molecules and their counterreceptors, interactions between other receptors and their counterreceptors. The involvement of different receptors on different APCs, for which there are corresponding counterreceptors on pTh cells, can endow different APCs with different functional roles in activation.

Conditions for Inactivating T Cells. The new model predicts that a "natural" monovalent T cell antigen will inactivate its corresponding pT cells. A number of independent studies (31–33) showed that monovalent T cell peptides inactivate their corresponding pT cells when given to normal mice, so long as the peptide is not given in adjuvant, complete Freund's adjuvant in particular. Such a peptide, normally synthesized by an immunologist, is an "unnatural" monovalent ligand, because it will not be processed by the usual mechanisms and most likely externally decorates the class II MHC molecules of APC. It is unclear which APCs will preferentially pick up the peptide. If activated, nonspecific B cells do so, one would anticipate, within the context of the new model, that the peptide would be immunogenic. If resting B cells and other APCs, excluding activated B cells, do so, one would anticipate that the peptide would be tolerogenic. It would seem that there is a tendency for the latter situation to obtain, because peptides administered in saline usually inactivate their corresponding pTh cells (31–33). These studies with normal mice are in contrast with those with mice transgenic for a TcR that can recognize a defined peptide in the context of a host class II MHC restriction element. Administration of the appropriate peptide to such transgenic mice (34–36), or to their lymphocytes in culture (37, 38), often results in the activation of the corresponding pTh cells. A TcR transgenic mouse is different from a normal mouse in that there is a much higher frequency of the responding pTh/Th cells. Given this fact, it seems plausible that peptide- and B cell-mediated interactions of a pTh cell with eTh cells, of the kind depicted in step 2 of Fig. 3, might occur in TcR transgenic but not normal mice. This might explain the reported inability to inactivate the pTh cells of TcR transgenic mice when administering the peptide without adjuvant to intact TcR transgenic mice. Exper-

iments support this suggestion. Peptides can inactivate TcR transgenic T cells when it is arranged that such cells are less frequent, thus minimizing T cell–T cell cooperation (39, 40). These studies provide indirect support for the new model.

Conditions for Breaking Peripheral Tolerance. The original two-signal model explained why certain circumstances and related experimental conditions can result in immune responses to peripheral self-antigens (11, 41). Immunization with foreign antigens that crossreact with peripheral self-antigens can induce autoantibodies to the self-antigen. For example, immunization of rabbits with turkey thyroglobulin results in the production of antibody that binds to both turkey and rabbit thyroglobulin. The turkey-specific Th/pTh cells allowed those B cells specific for crossreactive epitopes on rabbit and turkey thyroglobulin to be induced by turkey thyroglobulin (41). In contrast, an insufficiency of Th/pTh cells specific for rabbit thyroglobulin normally prevents this self-antigen from inducing these same B cells.

The original two-signal model, and the new model, predict, for analogous reasons, that immunogenic foreign antigens that crossreact with peripheral self-antigens at the T cell level can induce T cells specific for the peripheral self-antigen. Indeed, it was argued that observations (41) provide indirect support for this prediction (17). Fortuitous crossreactions between microorganisms and self-antigens may sometimes lead to autoimmunity, and the crossreaction between group A streptococci and heart tissue is most probably responsible for the antibody to and the T cell-mediated inflammatory reaction against heart tissue seen in rheumatic heart disease. The most frequent way in which crossreacting antigens are generated is when cells bearing organ-specific antigens are infected by a virus or other intracellular parasite. It is not surprising in this context that a mouse expressing a transgenic viral antigen early in ontogeny in a peripheral organ will show no sign of organ-specific T cell autoimmunity but that such autoimmunity can be precipitated by the appropriate viral infection (42–45). The virally infected cells will crossreact with the organ-specific cell. Infection of mice with Theiler's virus can cause encephalitis (46). This does not require, according to the model proposed here, that viral antigens crossreact with brain antigens, as sometimes suggested (47), but merely that the virus can infect brain cells that bear peripheral antigens. In this case, epitope spreading can occur from viral to organ-specific antigens. An interesting study shows that immunization with an antigen that crossreacts with a self-antigen results, not unexpectedly, in the activation of B cells specific for this self-antigen. Passive transfer of these activated B cells, but not resting B cells (48), now allows the self-antigen to activate pTh cells specific for the self-antigen (49). This striking observation is in accord with the new model.

Variability in the inactivation of peripheral antigen-specific $CD4^+$ T cells may depend in part on differences in Th crossreactivity between these peripheral and foreign antigens against which there are on-going immune responses, such as those to gut flora.

Evidence That May Be Contrary to the New Model

Some observations support the likelihood of a central involvement of antigen-specific B cells in pTh activation (48–52), whereas others do not (e.g., ref. 53). A majority of these latter studies involve infectious agents and/or the use of certain gene knock out mice (54) that render the mice B cell deficient. This deficiency of B cells is probably "leaky" (55). It is also possible to envisage, thinking along the lines propounded by Janeway and coworkers (8, 9), that some infectious agents may interact with cells of the immune system to bypass the normal requirement for B cells in pTh activation. The evidence against a central involvement of B cells in the normal activation of pTh cells *may* be less compelling than sometimes inferred.

Experimental Distinction Between Different Models

Conditions Determining the Activation/Inactivation of pTh Cells: Timing Considerations. According to Matzinger (56), theories invoking a learning mechanism of self-tolerance predict that animals are perinatally susceptible only to acquiring tolerance and that the recently demonstrated ability of such animals to mount immune responses is therefore problematic for such theories. This reasoning is incorrect. Learning theories predict that an antigen, to be tolerated and regarded as self, must be present *before* the immune system gains competence to respond to the antigen in question. It was demonstrated years ago that animals gain immunocompetence before birth to certain antigens and that tolerance to such antigens could be achieved if the antigen was administered *before* this competence was acquired (57, 58). Moreover, the time when immunocompetence is achieved depends not only on the immune system but on the nature of the antigen. This point is conceptually critical, because it bears on whether organ-specific antigens can be "tolerogenic" throughout life. For example, according to the model proposed here, an antigen containing only one foreign Th cell epitope is most likely tolerogenic, even when administered to adults. Extrathymic self-antigens can be regarded as normally tolerogenic throughout life because there are so few lymphocytes specific for them at any one time. Matzinger and coworkers (56, 59) argued that the surgical process of collecting dendritic cells from a male mouse, involving trauma and the generation of a danger signal, means that such cells are activated to initiate a primary immune response. The administration of such activated male dendritic cells to female mice inevitably leads to the priming of the female mice to the male antigen according to her view point. A prediction of the model proposed here is that these male dendritic cells are not intrinsically immunogenic but can inactivate extrathymic male-specific pTh cells when administered to syngeneic female mice sufficiently early in ontogeny, i.e., at a time before they are immunogenic.

Conditions Determining the Activation/Inactivation of pTh Cells: Considerations Concerning the Nature of the Antigen. A prediction of the model proposed here is that the administration of a naturally monovalent $CD4^+$ T cell antigen will inactivate its corresponding pTh cells if given without adjuvant. Administration of a nonmicrobiological molecule P without adjuvant, which contains several $CD4^+$ Th cell peptides, will not inevitably result in inactivation, as Janeway's third-signal model would predict. If the number of foreign Th cell epitopes is sufficient, such immunization will lead to the cascade of eTh cell–pTh cell interactions that result in the activation of the pTh cells. Suppose P is immunogenic and is processed to yield nonself, Th cell peptides p1, p2, . . . pn. Administration of p_1 and p_2 in monovalent form, perhaps as peptides (see discussion above) will result in the silencing of the p1- and p2-specific pTh cells, with the corresponding specific eTh dying off because of their short half-life. In this case, the antigen P will in effect have fewer foreign sites in the exposed than in normal animals. It will therefore be more difficult to establish the cascade of Th cell–pTh cell interactions in exposed than in normal animals when immunizing with P without adjuvant; therefore, the activation of p3-, p4-, . . . pn-specific pTh cells will be more difficult to achieve. This kind of experiment directly tests critical features of the proposed model, and a positive result would be difficult to reconcile with the Model of Constitutive Costimulation, the Danger Signal Model, or the Infectious Nonself Signal Models.

Concluding Comments

The model proposed here bears on two general issues concerning the regulation of the immune response. It bears on the nature of the decision criterion determining whether antigens induce a cell-mediated Th1 response or an antibody Th2 response. A rational approach to analyzing the Th1/Th2 decision criterion requires a knowledge of the requirements for

the primary activation of resting $CD4^+$ T cells. For example, the model proposed here is compatible with the threshold hypothesis that attempts to describe this decision criterion (60, 61) but not with several other contemporary hypotheses. The second issue surrounds the specificity of the immune response. There are strong experimental reasons for supposing that the original two-signal model applies to the activation/inactivation of most B cells and at least some CD8 T cells. Th cells are in this sense the guardians over the behavior of these other subsets of lymphocytes. It seems incongruous to have exquisitely specific control over the activation/inactivation of B cells and $CD8^+$ T cells, provided by the exquisite specificity of $CD4^+$ T cells, only to have this control sabotaged by a less specific decision criterion operating at the level of the activation/inactivation of resting $CD4^+$ T cells. This consideration has been a main impetus for developing the proposed model and for regarding models, in which the initiation of an immune response is critically dependent on the generation of a third signal signifying infectious nonself or danger, as implausible. Finally, the new model may be regarded as bringing what we know about the activation of different subsets of lymphocytes into a common scheme. According to this view, resting $CD4^+$ T cells are unique in usually being the first antigen-specific clonal cell to be activated, and therefore the scarcity problem is more critical for their activation than it is for the activation of other lymphocyte subsets. The first step in the activation of pTh cells results in their multiplication, thereby minimizing the scarcity problem in the second step. The requirements for completing the second step in the activation of pTh cells means that the full activation of resting $CD4^+$ T cells to yield eTh cells shares with the activation of most B cells, and at least some $CD8^+$ T cells, the requirement for $CD4^+$ eT cells.

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