*seminars in* **IMMUNOLOGY**, Vol. 12, 2000: pp. 231–238
doi: 10.1006/smim.2000.0236, available online at http://www.idealibrary.com on IDEAL

# Danger: the view from the bottom of the cliff

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To bring a measure of cohesiveness to the collection of papers and discussions in this issue, Langman and Cohn offered a guideline to the questions to be considered. In this series of articles we, as a group, have been set the task of determining what are the important criteria that give the immune system the ability to rid the body of pathogens, using quite destructive effector mechanisms, without also destroying the host. As a starting point, Langman and Cohn proposed that the ability is derived from the first of two decisions that the immune system must make when confronted with antigen. The two decisions being:

1) A self-nonself (S/NS) discrimination (to keep from destroying self).

2) The magnitude and class of the response (to choose an effector mechanism that is effective in ridding the pathogen).

Regarding the first decision, they previously suggested that an immune system cannot use a nonspecific universal glue to fight pathogens without killing its own body in the process. T and B cell receptors must therefore be specific, so that they can make a self-nonself discrimination. In biology, however, it can be imprudent to use words like 'cannot'. A universally non- specific defense mechanism could work if self tissues had the capacity to regenerate rapidly and completely, because any damage done to self tissues would be quickly repaired. It would only be necessary to turn it on and off carefully. Thus, it is at least theoretically possible to have immunity without either receptor specificity or self-nonself discrimination. However, specificity is also important for memory, and our immune systems seem to have chosen this path.[a] Nevertheless, to say that specificity is important is different from saying that a discrimination between self and nonself is necessary. We have therefore re-stated Langman and Cohn's questions, attempting to remove any assumptions about mechanism.

When faced with an antigen, the first decision the immune system must make is:

1) Shall I respond?

   If the answer is yes, there are three more questions:

2) How strongly?

3) With what effector class?

4) Where?

And a fifth, all encompassing question:

(5) How can I do this without destroying the tissues that I am meant to protect?

By asking the questions this way, one leaves room for all the models. For example, the first decision is an on-off switch. S/NS models neatly combine it with the fifth decision, suggesting that both are made on the basis of self-nonself discrimination. In their most basic form,[1,2] they suggest that T cell self-nonself discrimination is set by the timing of antigen exposure (early in ontogeny = tolerance, later = immunity). Because T cell help is critical for B cells, timing of antigen exposure thus ultimately controls both T and B cell responses. Although this view is attractive, as it provides a model that explains many immunologic phenomena, it requires additional assumptions to explain observations such as: 1. Lack of rejection of the fetus. 2. Presence of autoreactive T cells in normal individuals. 3. The

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*1044–5323/00/030231+ 08/$35.00/0*

[a] However some tissues, liver and skin, for example, may take advantage of their regenerative ability to recover from immune mediated damage

need for adjuvant to induce immune responses. 4. Immunocompetence of T cells early in ontogeny. 5. The existence of T-independent antigens.

A newer S/NS model, proposed by Janeway,[3] explains many of the thorns in the side of the old version. Janeway's infectious-nonself (INS) model shifts control away from the adaptive immune system's somatically generated S/NS discrimination to a germline-encoded S/NS discrimination made by the innate immune system, suggesting that APC must be activated, through pattern-recognition receptors specific for evolutionarily distant infectious agents (such as bacteria), before they can present antigen in a stimulatory fashion to T cells. The model is consistent with the lack of rejection of fetuses (no bacteria to activate APCs), the need for adjuvant to induce immune responses (adjuvants stimulate APCs) and the immunocompetence of T cells early in ontogeny. However, new thorns have replaced the old ones. For example, why are transplants rejected (no infectious agents to stimulate APCs)? What induces and maintains autoimmunity? Why does the immune system spontaneously reject some tumors (no infection)? Why do viruses stimulate immunity? Why do germ- free animals have serum immunoglobulin? How do non-bacterial adjuvants, such as alum, work?

Rather than go further into other models (geographic, network, homunculus etc.), each having its strengths and weaknesses, hereafter we will concentrate on the Danger model (with its own strengths and weaknesses) and how it answers the above five questions.[4,5] Briefly, the Danger model shifts control of immunity to the tissues that need protection rather than the cells that protect them. From this viewpoint, we find that the thorns in the S/NS model can be removed. In fact, the S/NS discrimination itself can be removed and replaced with the view that the body cares more about what is dangerous (damaging, toxic etc.) than what is nonself.

On what basis could a person choose between models? We believe that a good model should fulfil certain criteria. It should have a short set of clear assumptions, highlighting those that are hidden or commonly held. It should explain as much of the existing data as possible without the addition of situation-specific assumptions. It should make clear predictions and, if possible, be of some practical use.

We will therefore divide this essay into three parts. The first describes our view of the primer problem (what activates the first T helper?: the question underlying the difference between the S/NS, INS and Danger models) and lists the assumptions of the Danger model, pointing out which are fundamental to many models and which are unique. The second section briefly lists immunological phenomena that the model explains, showing how it deals with the immune system's first and fifth decisions listed above. In the third section, we will take a stab at explaining the other three decisions.

## The primer problem

The Danger model, like many models described in this volume, stands on the shoulders of the associative recognition model elaborated by Cohn, Bretscher and Langman.[1,6] Like their model, the Danger model holds that:

1) Thymocytes are deleted to antigens presented in the thymus (though the two models postulate different mechanisms).

2) There exist tissue specific antigens not expressed in the thymus.

3) A mature resting antigen-specific B- or killer T-cell (naive or memory) that recognizes antigen in the periphery is predisposed to die but can be rescued and activated by a second signal from an effector T helper.

4) An effector T cell does not last forever. The activated state is short and they either die or become resting memory cells. Thus, an immune response does not continue in the absence of repeated stimulation.

5) The helper and the cell it helps are linked by antigen.

6) T helper cells themselves also need two signals for activation.

In this last assumption lies the primer problem. If helpers need help, who helps the first helpers? Langman and Cohn suggest that resting T helpers periodically become spontaneously activated such that they can deliver help to other T helpers. To allow for peripheral self tolerance, they suggest that the time taken to spontaneously become an effector is sufficiently long so that a new autoreactive T cell would encounter its self antigen, and die before becoming an effector. The Danger model, and the INS model of Janeway, solve the primer problem in a different way, by borrowing from Lafferty and Cunningham[7] the assumption that:

7) The critical second signals for T helpers are costimulatory signals from APCs.

To allow for peripheral self tolerance, both models assume that:

8) Only activated APCs can deliver appropriate costimulation.

9) Activated APCs are short lived. Thus, continuous immunity requires continuous or repeated APC activation.

However, this simply sets the primer problem back one step. If T helpers are stimulated only by activated APCs, what activates the APCs? Here the INS and Danger models differ. Unable to abandon S/NS discrimination, the INS model suggests that APCs are activated by exogenous signals, the conserved structures of evolutionarily distant microbes. The Danger model suggests that:

10) APCs can be activated by endogenous alarm signals from distressed or damaged bodily tissues.[b] Alarm signals may come in two forms: pre-packaged and inducible. Any structure that is normally found inside but not outside cells (such as DNA, RNA, mitochondria, highly mannosylated nascent glycoproteins, etc.) may act as a pre-packaged alarm signal. Inducible alarm signals may include such molecules as HSPs and IFN$\alpha$. This assumption is now supported by evidence that necrotic, but not healthy or apoptotic, cells can release factors that activate resting dendritic cells *in vitro*, and serve as adjuvants for the priming of immunity *in vivo*.[8]

Although this solution to APC activation is only a small step away from other suggestions, it is a step that drops the model off a cliff. If APCs are activated by endogenous alarm signals, then self versus nonself ceases to be the decision driving an immune response.

We are often asked whether deletion in the thymus imparts the Danger model with a S/NS distinction. The answer is no, deletion in the thymus imparts a thymus–non-thymus distinction and the thymus is far from a complete definition of self. Such questions, however, illuminate the main difference between S/NS models and the Danger model. The Danger model separates two functions that S/NS models combine, the on-off switch versus the need not to autodestruct. In the Danger model, definitions of thymic self, peripheral self, evolutionary-self or any other self are not what controls the decision to respond. That function is handled by alarm signals. The Danger model could therefore have relied solely on tissue regeneration to prevent self destruction. However, the induction of tolerance through the death of a few lymphocytes is a more efficient method of preventing self destruction than is massive tissue death and subsequent regeneration. Thus, we postulated that each bodily tissue induces tolerance to the antigens it expresses. Except for APCs, this is a simple task. The tissue simply does not offer costimulatory signals, thus provoking the death of autoreactive T cells. APCs, however, are hampered in their ability to induce tolerance to themselves because they have the capacity to costimulate T cells. Thus, there must be another way to induce tolerance to APC antigens. This, we suggested, is the reason for thymic deletion. If thymocytes delete upon recognition of antigen, even if the antigen is presented with costimulatory signals, then tolerance to APC antigens will be induced. As a side-effect, this step also induces tolerance to a large chunk of other self antigens that are not tissue specific. In the terms of Langman and Cohn, this is a time and space model. The first step (thymic deletion) establishes tolerance to APCs and the second (peripheral tolerance) establishes tolerance to other tissues. This second step also allows the Danger model to drop the assumption, common to many S/NS models, that all self antigens need be expressed prior to immunological maturity. It need only postulate that they will be present in the absence of Danger. Antigens that appear late in ontogeny (fetuses and milk in mammals? Post metamorphosis antigens in frogs etc.) are no problem provided they are presented in the absence of Danger. A time component enters with infection, because any existing autoreactive T cells will be activated along with those specific for the pathogen and will continue to be reactivated while infection persists. However, when the pathogen is cleared, the response stops because there are no more Danger signals to restimulate it. The reactive T cells then die or rest to become memory cells, a state in which they are again susceptible to tolerance induction. With time, as the autoreactive memory T cells traffic and come in contact with selfantigens in the absence of costimulation, many will be eliminated.

[b]Endogenous alarm signals may also act as stimulators of T independent responses an idea that we're working on.

In this way, an immune system acting under Danger model principles categorizes antigens into those associated with Danger (to be attacked) and those not associated with Danger (to be tolerated) and the latter set includes most self antigens because self is usually not dangerous. However, this set of antigens also includes resident commensal and other organisms that do no damage, because they will not elicit costimulatory APCs and the T cells specific for their antigens will become tolerant. We would have been happy to call this a form of self-nonself discrimination that includes as self anything that causes no damage. However, as Cohn points out, using words that are already in general use opens the way to misunderstandings that result when people have different definitions for the same words (for example, positive selection, a step in thymocyte differentiation, is often confused with MHC restriction, a description of the MHC oriented specificity of the TCR). So we have chosen to emphasize the difference. The point is that no matter how the definition of self is made, the most important distinction between S/NS models and the Danger model is whether this definition is used to determine whether a response will occur. In the Danger model, endogenous alarm signals govern that decision. Thus, self need not be ignored and nonself need not always be fought. This distinction allows for all of the following. First, the definition of self can change as the body changes, matures, procreates and grows old. Second, the definition is never final. As long as the thymus continues to produce, there will exist T cells specific for peripheral self antigens. Should self become consistently dangerous (e.g. if an infection persists, or if mutations in genes governing programmed cell death cause cells to die badly) autoreactive T cells will not become tolerant. This sort of alarm-driven autoimmunity may lie behind certain autoimmune diseases. Third, some seemingly harmless organisms may be deadly. For example papilloma virus, which arrives long after the immune system is mature and lives quietly for years, inducing little if any immunity, occasionally promotes cervical cancer.[9] The immune system does not respond to these and consequently leaves itself open to mistakes. The lack of response to these sorts of organisms and tumors is difficult to explain from a standard S/NS viewpoint in which the immune system attacks anything that did not exist prior to its maturity. However, they are integral to the Danger model. Fourth, if self need not be ignored, then useful autoreactivity is possible (see below).

The Danger model requires one more assumption.

11) The immune system does not throw fuel on the fire: immune system-mediated damage does not trigger further activation of T cells (for example, killers kill by inducing apoptosis rather than necrosis in their targets, antibody does not kill cells necrotically because of complement inhibition factors, etc.)

Assumptions (10) and (11) are unique to the Danger model. (10) is definitive. Should it turn out that purely apoptotic death stimulates immunity, or that immunity can be initiated without APC activation, then the Danger model, in its current form, is wrong. Assumption (11) is a bit less definitive. Many tissues can regenerate. If it turns out that some forms of immune-mediated destruction do indeed activate APCs, we will ask about the consequences before throwing out the model.

## The list of explained phenomena

Many other models offer explanations for some of the following phenomena. One nice feature of the Danger model is that it covers all of them, and it does so without adding any new situation-specific assumptions. For space considerations, the explanations below are *exceedingly* brief. More detailed explanations for many of these can be found in ref 4 and 5.

### *(1) The need for adjuvant*

Adjuvants usually cause damage. This causes a release of alarm signals that activate APCs, which then take up the local antigens and display them along with costimulatory signals to T cells. The exceptional adjuvants that do not cause damage are likely to be endogenous Danger signals or their mimics, such as IFN$\alpha$, HSPs, DNA, RNA, poly I:C etc.

### *(2) Lack of reaction to developmentally late arising self-antigens*

Mammals pass through puberty. They lactate and age. Frogs go through metamorphosis. The new antigens that appear in these cases do not elicit destructive immune responses, though, in Xenopus, tadpole T cells make mixed lymphocyte reactions against adult stimulators.[10] However, cell death during metamorphosis is programmed, usually apoptotic cell death. Although new antigens appear, there are no alarm signals to activate the APCs. Thus, T cells specific for

these antigens die. The same holds for puberty and lactation. As long as the tissue is healthy, there are no alarm signals to trigger a response and tolerance will occur.

***(3) Lack of rejection of allogeneic fetuses***

During development, cells die by programmed cell death. Although many antigens may be available to maternal T cells, there are no alarm signals to initiate a response. If the fetus should become infected, however, the maternal immune system will respond to clear the infection, perhaps resulting in concomitant miscarriage.

***(4) Rejection of transplants***

A transplant cannot be performed without generating damage. Surgeons cut, burn, perfuse in ice-cold saline (a heat shock), and send organs around the country on helicopters. Further damage occurs upon reperfusion. Damage is done to both donor and host and will consequently elicit alarm signals and activate both donor and host APCs.

***(5) Lack of response to tumors***

Tumors are generally healthy cells that do not send alarm signals and do not activate APCs. As they grow, they will not elicit immune responses and, in fact, will slowly induce tolerance to any tumor-specific T cells passing through. Although the centers of large, solid, poorly vascularized tumors may eventually begin to starve, starvation death is usually apoptotic, and this death will not elicit an immune response until the amount of death overloads the local scavenging capacity. At this point dying cells will fall apart, local APCs will be alerted by alarm signals, and a response may ensue. The few remaining tumor-specific lymphocytes will then attempt to clear the tumor, but by this time it is a case of too little too late.

***(6) Graft versus host disease***

At any time, individuals living under normal environmental stresses will inevitably contain a number of activated APCs able to present their own antigens to allo-specific T cells. Irradiation and other insults to the hosts may increase this number. A prediction from this view is that sterile, undamaged hosts should elicit less vigorous GvH reactions.[11]

***(7) Autoimmunity***

We have divided autoimmunity into four categories, one of which is unique to the Danger model. Although it has been thought that autoimmunity is due to defects in the tolerance process and, though some autoimmune diseases may indeed be caused by such defects, our view is that tolerance, in the best of circumstances, is never complete. For B cells, tolerance to intracellular components is difficult. For T cells, peripheral tolerance requires antigen encounter, and this will occur more readily in some tissues than others. The efficiency of tolerance induction will vary with the size of the tissue, the rate of T cell traffic through it, and the levels at which its antigens are displayed in draining nodes by unactivated APCs. Large, unbarricaded tissues (liver, skin, etc.) will induce tolerance efficiently whereas small or barricaded organs (islets of langerhans, brain) will do so much less efficiently. Thus, the tolerance process is constant but never fully accomplished. We have therefore moved away from the question 'how do we break tolerance?' to ask 'what activates the autoreactive cells and what maintains their response?'

a) An undetected infection: the response is directed entirely against an undetected pathogen, but it destroys the infected organ. This is essentially similar to the response to LCMV, where killers kill virus-infected cells.

b) Molecular mimicry: an environmental pathogen cross reacts with a self tissue-antigen. The autodestruction is initiated by the pathogen and continues while the infection persists. It subsides when the pathogen is cleared, only to be reinitiated when the infection reoccurs. This category differs from the first in that the disease has an autoimmune component and the pathogen need not therefore be in the organ that is destroyed. Essentially similar to rheumatic fever, this may be the category for some 'flaring' diseases.

c) Bad death: many genes govern normal programmed cell death. Mutations in any of them, including mutations in the scavenging process, could lead to the release of alarm signals. Non-pathogenic environmental agents that are toxic and cause cell damage could also be involved. In this category there need not be a foreign antigen involved. The autoimmune response occurs because of cellular distress.

d) The wrong class: The antibodies and cytokines released during an immune response are highly potent molecules that can wreak enormous havoc, and some tissues are more sensitive to certain cytokines than others. For example, the skin survives the onslaught of TNF and IFN$\gamma$ made during a DTH response, but a DTH in the eye or gut (where the normal response is TGF$\beta$ and IgA, not TNF and IFN$\gamma$) can destroy it.[12,13] The presence or absence of an autoimmune component is not the primary question here. The problem is a defect in class regulation that results in a harmful effector response. A switch in class may alleviate the disease.

## The remaining decisions

If tissues control the activation and inactivation of immune responses, might they also be able to control the strength, effector class, and location of those responses? Below we offer some suggestions, pointing out any new necessary assumptions as they come up.

### *Strength*

(no new assumptions needed). The strength of an immune response will vary proportionately with the amount of damage (which determines the number of activated APCs) and the frequency of potentially responding T cells (which determines the number of initial reactants). The same antigen might thus elicit different strengths of responses, depending on its association with different amounts of danger. 'Strong' antigens, such as allogeneic MHC, might elicit no response if there is no danger, such as in the case of the fetus, and weak antigens might elicit good responses if given with damage-inducing (or mimicking) adjuvants.

### *Effector class*

(including privileged sites, oral tolerance, the effect of immunization route, and 'useful' autoreactivity). Cohen was right when he pointed out that the Danger model, in its original form, did not deal with the control of class.[4] It was not meant to. It was originally designed to cover the on-off switch, not the control of class. However, the Danger model's solution to the on-off switch, that the immune response is not controlled solely by cells of the adaptive or innate immune system, but rather by cells of all bodily tissues, also reveals a new possibility for class control. We suggest that tissues not only determine whether an immune response is needed, they also influence the effector class that the response will take. We need one additional assumption here:

12) Tissues protect themselves by generating signals that control the class of response, either by influencing lymphocytes directly, or indirectly through the APC.

There is a general belief that class is tailored to the pathogen (e.g. the immune system generates CTL to fight viruses and IgE to fight worms) and there is evidence that some pathogens are able to direct the class of response. However, our view suggests that each tissue has a certain set of effector classes that it prefers, either because they are the most effective at that site, or because they do less damage to the tissue than other classes of response. This part of the model is new and not yet completely worked out. At the moment, we tend to think of these preferred responses as default responses that may be influenced by signals from other tissues or from the pathogens themselves.

It has long been known that the route of administration can affect the class of an immune response, a phenomenon that is not covered by any self-nonself model but that is easily explained if tissues control class. Privileged sites, including the anterior chamber of the eye, the testis and the hamster cheek pouch, are an extreme case where the administration of antigen does not appear to result in a response at all.[14] In particular, fully allogeneic transplants are accepted in these sites without the need for any immunosuppression. It is fairly easy to understand the lack of response in the anterior chamber of the eye, where blood cells would hamper transmission of light. But the hamster cheek pouch? This pocket, used as a carrier for food, is certainly not maintained as a sterile environment. Why on earth should it be a privileged site? The answer may come from Streilein's study of the eye[13] showing that its inability to reject grafts is not due to an absence of response but to a shift in the effector class. The eye is a site where IgA is particularly effective but where a DTH response can do severe damage, even if it is not directed against eye antigens[15] To help ensure that IgA is made and DTH responses are not, the anterior chamber epithelial cells produce TGF$\beta$ and VIP, cytokines that influence the production of IgA[16] and its secretory piece and help to shutdown a TH1 response.[17] The gut behaves similarly, containing both epithelial and

T helper cell populations that produce TGF$\beta$ and direct the immune response into a class that has been termed oral 'tolerance'.[18,19]

Tissues may use different mechanisms to set class. Although epithelial cells may make immunomodulatory cytokines, others could arise from specialized migratory cells. For example, mice (and cattle) produce dendritic epidermal $\gamma\delta$T cells that migrate to the skin and recognize a stress-induced molecule on keratinocytes and produce keratinocyte growth factor as well as IL-2 and IFN$\gamma$.[20] In this way, the skin nudges immunity towards a DTH, a locally effective class of response. In human gut, there are autoreactive V$\delta$1 T cells, responding to MHC-like molecules that carry no peptides and use heat shock promoters as controlling regions.[21] The thymus, bone marrow and liver contain NK1 T cells, accounting for up to 40% of T cells in the liver. These cells also seem to be autoreactive, recognizing a CD1 molecule that is expressed by activated, but not resting dendritic cells,[22] and produce copious amounts of IL-4, a cytokine that nudges local immunity into the TH2 class.

Returning to the hamster cheek pouch, like the eye and the gut, it may simply be a mucosal tissue that promotes a useful and non-destructive class of immune response. We would predict that careful study of brain and testes would reveal the same pattern. In a sense, this is a mechanism of tolerance. It does not depend on a S/NS discrimination because both anti-self and anti-foreign responses can be destructive when executed in the wrong class. It is just one more example of the control that tissues exert over the immune response.

### *Location*

Effector T cells must often leave lymph nodes and search the body for their target antigens. Although activated T cells can traverse into many tissues, they preferentially enter sites of inflammation, where local distress signals change the adhesive properties of vascular endothelium.[23] Thus, danger is involved not only in the initiation of responses but also in targeting their execution. In the absence of distress signals, effector T cells that have been activated by vaccination may not reach the appropriate places. This may be one of the reasons that some vaccinated tumor patients show mixed responses, shrinking some tumor metastases while neighboring metastases continue to grow. We do not presently know which alarm signals are involved in the activation of APCs and vascular endothelium, though parsimony might drive us to postulate that they could be the same. If they are not the same, parasites might manipulate them to circumvent (or encourage) local traffic of activated effector cells, usurping a role that normally belongs to the tissue.

## Selection pressures

It is often pointed out to us that eukaryotes might find it useful to have pattern-recognition receptors for conserved structures on prokaryotes as a quick-response mechanism to protect themselves from invasion. This may be true. However, protection by elimination is not the only response that might be of evolutionary advantage. There are many instances where the nurture of an evolutionarily distant organism might be of more use than its elimination. Leguminous plants, for example, nurture the nitrogen-fixing bacteria in their root nodules and some squids and fish harbor bacteria that produce light and assist in the development of the fish's light-producing organ.[24,25] Each of our cells, in fact, is a combination of long entwined pro and eukaryotic partners. Thus, we suggest that PRRs may have evolved in several ways. They may be receptors for endogenous molecules that pathogens have learned to use for their own purposes, in the same sense that CD4 and CR2 evolved to bind to MHC class II and complement C3d, not HIV and EBV. They may be receptors for endogenous molecules that, like endorphin receptors, have a fortuitous cross-reaction for an environmental agent. They may be 'shepherding' receptors for common commensal microbes, inviting microbes into the appropriate places or ensuring that they remain there. Finally, they may have evolved as part of an arms race with a common dangerous pathogen. If we view these pressures as a response to danger, rather than as a general xenophobic aversion to nonself, we can step back and see the vertebrate immune system as an extended family of communicating bodily tissues that allows an individual to be an environment as well as to live in one, ignoring nonself that is harmless and welcoming nonself that is useful. Thus, by incorporating the associative- recognition-model's assumption that:

> antigen recognition without a second signal predisposes a lymphocyte to death

plus Lafferty and Cunningham's suggestion that:

> the second signal for a T helper cell is costimulation from an APC

plus Janeway's proposal that:

> only activated APCs can costimulate

and our own proposal that:

> APC activation is induced by endogenous alarm signals,

the Danger model allows for a definition of self that is dynamic and non-exclusive: a living, changing, definition that allows us to come out of the cold war attitude of 'us *versus* them' to a new view of global inter-relationships.

## Acknowledgements

We would like to thank Albert Bendelac and Bana Jabri for reading and commenting on the manuscript.

## References

1. Langman RE, Cohn M (1996) A short history of time and space in immune discrimination Scand. J Immunol 44:544–548
2. Bretscher PA (1999) A two-step, two-signal model for the primary activation of precursor helper T cells. Proc Natl Acad Sci USA 96:185–190
3. Janeway CA Jr (1992) The immune system evolved to discriminate infectious nonself from noninfectious self. Immunol Today 13:11–16
4. Matzinger P (1994) Tolerance, danger, and the extended family. Annu Rev Immunol 12:991–1045
5. Matzinger P (1998) An innate sense of danger. Semin Immunol 10:399–415
6. Bretscher PM, Cohn M (1970) A theory of self-nonself discrimination. Science 169:1042–1049
7. Lafferty KJ, Cunningham A (1975) A new analysis of allogenic interactions. Aust J Exp Biol Med Sci 53:27–42
8. Gallucci S, Lolkema M, Matzinger P (1999) Natural adjuvants: endogenous activators of dendritic cells. Nat Med 5:1249–1255
9. Frazer IH, Thomas R, Zhou J, Leggatt GR, Dunn L, McMillan N, Tindle RW, Filgueira L, Manders P, Barnard P, Sharkey M (1999) Potential strategies utilised by papillomavirus to evade host immunity. Immunol Rev 168:131–142
10. Du Pasquier L, Blomberg B, Bernard C (1979) Ontogeny of immunity in amphibians: changes in antibody repertoires and appearance of adult major histocompatibility antigens in Xenopus. Eur J Immunol 9:900–906
11. Heidt PJ, Vossen JM (1992) Experimental and clinical gnotobiotics: influence of the microflora on graft-versus-host disease after allogeneic bone marrow transplantation. J Med 23:161–173
12. Nilsen EM, Lundin KE, Krajci P, Scott H, Sollid LM, Brandtzaeg P (1995) Gluten specific, HLA-DQ restricted T cells from coeliac mucosa produce cytokines with Th1 or Th0 profile dominated by interferon gamma. Gut 37:766–776
13. Streilein JW, Ksander BR, Taylor AW (1997) Immune deviation in relation to ocular immune privilege. J Immunol 158:3557–3560
14. Streilein JW (1995) Unraveling immune privilege. Science 270:1158–1159
15. Hendricks RL (1997) An immunologist's view of herpes simplex keratitis: Thygeson Lecture 1996, presented at the Ocular Microbiology and Immunology Group meeting, October 26, 1996. Cornea 16:503–506
16. Coffman RL, Lebman DA, Shrader B (1989) Transforming growth factor beta specifically enhances IgA production by lipopolysaccharide-stimulated murine B lymphocytes. J Exp Med 170:1039–1044
17. Letterio JJ, Roberts AB (1998) Regulation of immune responses by TGF-beta. Annu Rev Immunol 16:137–161
18. Gonnella PA, Chen Y, Inobe J, Komagata Y, Quartulli M, Weiner HL (1998) *In situ* immune response in gut-associated lymphoid tissue (GALT) following oral antigen in TCR-transgenic mice. J Immunol 160:4708–4718
19. Mitchison A, Sieper J (1995) Immunological basis of oral tolerance. Z Rheumatol 54:141–144
20. Boismenu R, Hobbs MV, Boullier S, Havran WL (1996) Molecular and cellular biology of dendritic epidermal T cells. Sem Immunol 6:323–331
21. Groh V, Steinle A, Bauer S, Spies T (1998) Recognition of stress-induced MHC molecules by intestinal epithelial gammadelta T cells. Science 279:1737–1740
22. Bendelac A, Rivera MN, Park SH, Roark JH (1997) Mouse CD1-specific NK1 T cells: development, specificity, and function. Annu Rev Immunol 15:535–562
23. Wu KK, Thiagarajan P (1996) Role of endothelium in thrombosis and hemostasis. Annu Rev Med 47:315–331
24. Graf J, Ruby EG (1998) Host-derived amino acids support the proliferation of symbiotic bacteria. Proc Natl Acad Sci USA 95:1818–1822
25. Montgomery MK, McFall-Ngai M (1994) Bacterial symbionts induce host organ morphogenesis during early postembryonic development of the squid Euprymna scolopes. Development 120:1719–1729