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Is cancer dangerous to the immune system?

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The hypothesis of immunologic surveillance of neoplasia is predicated on the theory that the immune system is capable of discriminating self from foreign antigens, and that tumor-specific antigens are regarded by the immune system as nonself. We propose here an alternate view, that the immune system has evolved to detect danger by employing 'professional' antigen-presenting cells as sentinels of tissue distress. In this model, cancers do not appear dangerous to the immune system, so that the default response of T cells to tumors is to be turned off. We discuss the implications for immunotherapy of malignancy.

Key words: antigen-presenting cells / immune surveillance / immunologic tolerance / tumor immunology / T lymphocytes

IN THE FIRST HALF of this century, infectious diseases were the major cause of human suffering and death. Medical research was devoted almost exclusively to the conquest of microbes, and the objectives were quite clear — find a way to kill the organisms directly, or augment the body's natural defenses against the pathogens. Within this historical context it is not at all surprising that the basic research program of immunology was concerned with how the immune system responds to exogenous stimuli. The theoretical edifice of immunology, constructed around mid-century largely by the Australian Frank Macfarlane Burnet, was based largely upon Darwinian concepts of the struggle for survival and evolution by natural selection. Burnet's paradigm has had a profound and lasting influence on the direction of immunology.[1] But with the advent of vaccines and antibiotics, the threat to humanity posed by infectious diseases abated, and many immunologists are now concerned with the control of 'endogenous' diseases such as autoimmune diseases and cancer. Burnet regarded cancers as 'not self', and postulated that a major function of the immune system is to seek out and destroy new cancers as they arise.[2] This view of cancer as nonself gained wide acceptance, and a substantial effort has gone into the development of tumor 'vaccines'. But, with this century coming to a close, and the results of trials of tumor vaccines less than impressive, it seems reasonable to ask whether a self/nonself paradigm that makes sense in the context of the struggle for survival against pathogens, is as well-suited to approach the relationship of the immune system to a genetic disease. We argue that it is time to abandon the self/nonself discrimination paradigm and to adopt a more global perspective, one in which the need to defend against lethal pathogens and the need to avoid lethal autoimmunity are equally balanced. In our view, which we term the 'danger' model,[3] we propose that to avoid autoimmunity, the default reaction of T cells to antigens on non-hematopoietic tissues is tolerance, and that it is the role of blood-derived 'professional' antigen presenting cells, particularly dendritic cells, to detect and report to T cells situations of dangerous tissue distress. Because we propose that tissue cells induce tolerance in susceptible T cells, we predict that the default immune response to tumor-specific antigens occurring on such tissues is tolerance as well. We hope that by focusing our attention on the question of what is dangerous rather than what is nonself, it shall be possible to devise more effective strategies to generate an immune response against cancerous tissue, and we close by suggesting some strategies. But first, we would like to outline the origins of self/nonself discrimination and explain why, in our opinion, the paradigm is no longer tenable in the light of the available evidence.

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Origin of the self/nonself discrimination theory

How could Burnet have come to the conclusion that cancers are regarded by the immune system as nonself? This conclusion was a necessary consequence of his model of how the immune system defines the

self. He was impressed by Ray Owen's observation that dizygotic twin cattle, whose placental circulations are interconnected, are hematopoietic chimeras and tolerant of each other's tissues.[4] Seizing upon this observation, Burnet proposed that the immune system defines the 'self' before birth and predicted that allogeneic cells injected into a fetus or neonate would automatically be regarded by the immune system as self and therefore tolerated.[5] A necessary consequence of this model is that antigens making their first appearance after the immune system has reached maturity would automatically be regarded as nonself and attacked. Capitalizing upon Burnet's self marker concept and the discovery of lymphocytes as the mediators of immune responses, Joshua Lederberg, a molecular geneticist on sabbatical in Burnet's laboratory, proposed in 1959 the first theory of self/nonself discrimination.[6] The model was quite simple: lymphocytes are born in a state in which antigen recognition leads only to inactivation, but then mature to a state where antigen recognition leads only to activation. Since birth was taken to be the rough divider between immunological immaturity and maturity, the theory meshed nicely with Burnet's model. The hypothesis of immune surveillance of neoplasia, first proposed by Lewis Thomas[7] but championed by Burnet,[2] was a natural outgrowth of the Lederberg theory of self/nonself discrimination. Because most tumors arise after the immune system has reached maturity, any unique antigens expressed by the tumors should be regarded as nonself. Therefore, a major function of the immune system is to survey the body for the development of malignancy and to eliminate tumors as they arise. A critical feature of the immune surveillance hypothesis is that the default reaction of the mature immune system to new antigens is activation, and so the major question posed to cancer researchers by this hypothesis was how tumors managed to sneak through this surveillance mechanism.

Because the immune surveillance hypothesis relies upon the 'activation only' state of the mature lymphocyte to identify tumor-specific antigens as foreign, the hypothesis remains plausible only so long as the Lederberg theory of self/nonself discrimination remains valid. Unfortunately, it was not so long before this theory ran into serious trouble. In 1970, Weigert *et al* found that antibody molecules secreted by a B cell undergo sequence variation *after* activation of the B cell by antigen.[8] To Peter Bretscher and Mel Cohn, this meant that a B cell specific for and activated by a foreign antigen could mutate its immunoglobulin receptor and become an autoreactive cell. Because an autoreactive cell in an 'activation only' state posed a grave risk of autoimmunity, they proposed that the options of activation and tolerance must both be available to a lymphocyte throughout its lifetime. Bretscher and Cohn therefore put forth a 'two signal' model of lymphocyte activation, in which antigen receptor ligation is an 'off' signal to the lymphocyte, unless accompanied by a second signal delivered by a lymphocyte specific for a distinct epitope on the antigen.[9] This model received a slight but highly important modification by Kevin Lafferty and Alistair Cunningham[10] in order to account for T-cell activation and the phenomenon of MHC restriction (Figure 1). In their model, signal 2 was delivered not by another antigen-specific cell but from the antigen-presenting cell. To account for MHC restriction, they proposed that ligation of the MHC on the APC was a necessary condition for the upregulation of signal 2, dubbed the 'lymphocyte costimulator'. Moreover,

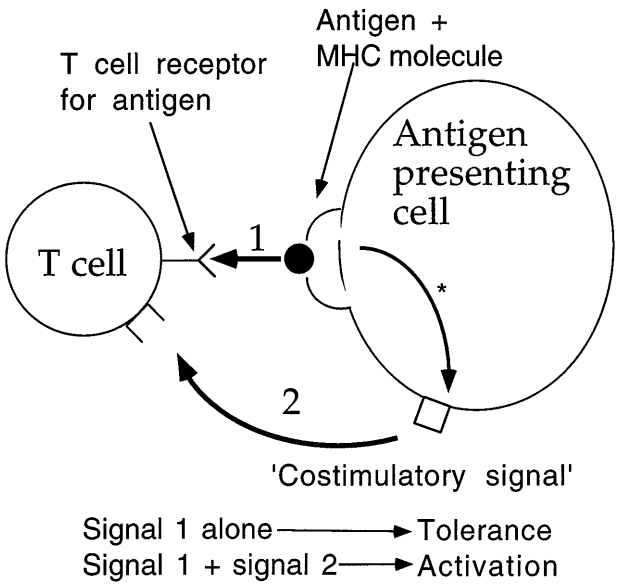


Figure 1. The Lafferty/Cunningham two-signal model of T-cell activation. Signal 1 is generated by ligation of the T-cell antigen receptor by a complex consisting of the antigenic peptide presented in the groove of the major histocompatibility complex (MHC) molecule. Engagement of this complex generates a signal within the antigen-presenting cell that leads to the expression of the 'lymphocyte costimulator', which provides signal 2 for T-cell activation. In this model, the requirement for MHC ligation for signal 2 delivery accounts for the phenomenon of MHC restriction of T-cell responses. Expression of the lymphocyte costimulator is limited to cells of hematopoietic origin, accounting for the ability of 'passenger leukocytes' to trigger solid organ allograft rejection.

they proposed that the capacity to deliver signal 2 was limited to cells of hematopoietic origin. This model has two profoundly important implications. First, if the Lafferty/Cunningham model is valid, then the self/nonself discrimination paradigm breaks down, because APCs do not possess antigen-specific receptors and so cannot discriminate self from nonself. Second, because in their model only APCs of hematopoietic origin can activate T cells, it follows that tumors of non-hematopoietic tissues cannot initiate an immune response.

The demise of self/nonself discrimination

We undertook a rigorous test of the Lafferty/Cunningham model by examining the capacity of B cells to serve as antigen presenting cells for T-cell responses *in vivo*. Previous studies had shown that heavily irradiated resting B cells induce tolerance when presenting antigen to a $CD4^+$ T-cell clone,[11] and one of us (P.M.) had shown that B cells are unable to activate the naive precursors of helper T cells *in vivo*.[12] The first experiment showed that metabolically inactivated B cells behave as if they deliver signal 1 in the absence of signal 2, and the second experiment showed that B cells are unable to provide both signals for activation of naive T cells *in vivo*. The direct prediction of the Lafferty/Cunningham model is that if unirradiated B cells can present antigen but cannot activate T cells *in vivo*, then they must be inducing tolerance. We tested this by examining the capacity of purified B cells from C57BL/6 (B6) male mice to induce cytotoxic T cell (CTL) responses to the male-specific histocompatibility antigen, H-Y, in unprimed or H-Y primed syngeneic females.[13] B6 females can be immunized to H-Y antigen by injection of syngeneic male dendritic cells; that is, they can later generate CTL *in vitro* upon stimulation with male cells, and they reject syngeneic male skin grafts much faster than do unprimed female littermates. But when unprimed B6 females were first injected with purified syngeneic male B cells, they were rendered tolerant of H-Y; that is, they were incapable of generating anti-H-Y CTL upon subsequent immunization and were unable to reject syngeneic male skin grafts. Importantly, the activation state of the B cell was irrelevant to the outcome: activated male B cells, which could stimulate T cell clones *in vitro*, were nonetheless tolerogenic APCs for H-Y *in vivo*. In contrast, females that had first been primed to H-Y by an injection of male whole spleen cells (which contain dendritic cells) could not be rendered tolerant to the male antigen by an injection of male resting B cells; in fact, many of the primed mice appeared to be boosted by the injection of male resting B cells. These results are compatible with the interpretation that both resting and activated B cells induce tolerance in naive T cells but restimulate memory T cells. Tolerance can also be induced in memory T cells.[14,15] For instance, memory CTL specific for minor histocompatibility antigens or for the nonclassical MHC Class I antigen, Qa-1, can be rendered tolerant by seeing their antigen in the absence of help provided by $CD4^+$ T cells.[14]

What is the significance of these results? First, they challenge directly the paradigm of self/nonself discrimination by the immune system. This is because mature, naive T cells are rendered tolerant of any antigen, self or foreign, that is presented exclusively by B cells. Second, the results also cast doubt on the immune surveillance hypothesis, because they show that the option of tolerance induction is also available to mature, postnatal T cells. Thus, the appearance of new antigens, such as tumor-specific antigens, after the immune system has reached maturity, need not result in a destructive immune response. In fact, our results predict that B-cell leukemias and lymphomas induce tolerance in any naive T cells that are specific for any unique antigens that such tumors may express.

Arriving at a new model by filling out the rules for T-cell activation and tolerance induction

The results of the experiments examining B cells as APCs suggest that there are two critical parameters controlling the outcome of a T cell–APC encounter: the type of APC, and the differentiation state of the T cell. However, these experiments only examined two types of APCs, dendritic cells and B cells, and only two differentiation states of the T cell, naive and experienced (memory). What are the rules for immature thymocytes and effector T cells? Are tissue cells, such as lung, liver, colon, thyroid, muscle, nerve, etc., able to provide signal 2 for T cells at any stage of their differentiation?

Developing thymocytes appear to be uniquely tolerance susceptible. In fact, one of us (P.M., with Sylvie Guerder) has found that even dendritic cells, generally regarded as the most potent immunogenic APCs in the periphery, induce tolerance when presenting antigens to immature thymocytes.[16] Tolerance can be induced in developing thymocytes by many

Table 1. Antigen-presenting cells* for T-cell activation and tolerance induction

	T-cell differentiation state			
	Immature thymocyte	Resting naive T cell	Resting experienced T cell	Activated effector T cell
Potentially activating (or triggering) cells	None	Dendritic cells Activated macrophages?	Dendritic cells Macrophages B cells	All†
Tolerizing cells	All	B cells Tissue cells	Tissue cells	None

*Cells are categorized according to their capacity to provide signal 2: 'Professional' APCs (dendritic cells, possibly activated macrophages) can provide signal 2 for naive and experienced T cells; semi-professional APCs (B cells) provide signal 2 for experienced T cells only; non-APCs (non-hematopoietic tissues) cannot provide signal 2, and so tolerize resting naive and experienced T cells.
†Whereas resting T cells require 2 signals for activation, activated effector cells need only to be triggered (signal 1 alone) to mediate their function (killing, interleukin secretion, etc.).

types of APCs, including other thymocytes,[17] thymic epithelium,[18,19] even pancreatic islet cells.[20] Immature thymocytes behave as if they are incapable of receiving signal 2, so that inactivation is the obligatory outcome of antigen encounter for T cells at this stage of development.

Activated effector T cells exhibit the opposite behavior, in that they do not require signal 2 to be triggered to mediate their effector function, such as cytolysis. This statement follows from the observation that there is no tissue of the body that is immune from T-cell mediated rejection when transplanted across histocompatibility barriers.

Tissue cells can therefore serve as the targets of *activated* T cells. But can they provide the stimulus for their activation? Here, Kevin Lafferty and his colleagues have provided some critical information. For instance, thyroids grafted into allogeneic recipients are promptly rejected. However, when thyroids are depleted of 'passenger leukocytes' by prolonged culture of the graft in high oxygen concentrations, the grafts are readily accepted by allogeneic recipients. The status of the graft is tenuous at first, as injection of donor spleen cells leads to prompt rejection of the graft. Later on, however, rejection cannot be initiated by injection of donor spleen cells, suggesting that the graft has induced tolerance to the antigens it presents. A similar phenomenon is observed with liver allografts, but here the grafts do not even need to be depleted of passenger leukocytes. Transplantation of an unmanipulated liver into an allogeneic rat usually sparks a rejection 'crisis', but tolerance ultimately ensues in most recipients, and the graft stabilizes.[21] These results suggest that tissue cells may indeed function as toleragenic APCs for both naive and resting memory T cells. The results are also compatible with the original 'passenger leukocyte' hypothesis of George Snell, which states that transplant rejection is contingent upon the presence of contaminating lymphoid cells which migrate to draining lymphoid organs and provide the initial activation stimulus for alloreactive lymphocytes.[22] According to Lafferty, these would be the cells capable of providing the 'lymphocyte costimulator', and in more modern terminology the cells capable of providing signal 2.

Table 1 represents our synthesis of the available experimental information on T-cell activation and tolerance culled from the fields of basic and transplantation immunology. The difference between potentially activating APCs and tolerizing APCs is, in our opinion, the inability or ability to deliver signal 2. These differences are of course irrelevant for both thymocytes and activated effector T cells, because the former can't see signal 2, and the latter don't need it. We also note the phrase 'potentially activating' because, as noted before, memory $CD8^+$ T cells are rendered tolerant when they encounter antigens in the absence of $CD4^+$ T-cell help. $CD4^+$ T cells may deliver help to $CD8^+$ T-cells by inducing signal 2 on the APC.[23]

The danger model of the immune response

We have stated firmly our view that our experimental results on the immune response to H-Y invalidate the notion of self/nonself discrimination by the immune system, and have provided a simple yet comprehensive set of rules governing the T-cell activation/tolerance decision in Table 1. So what is the true organizational nexus of immune function, if not to discriminate the self from the foreign? Consider for example the fate of a virus that infects liver cells. If the virus were to

remain in liver cells only and do no harm, the immune system would have no reason to eliminate it. Any viral antigens expressed on the surface of the cell in the form of viral peptide–MHC complexes would, by the rules outlined in Table 1, induce tolerance in any viral antigen-specific T cells that might happen to wander by. But now consider the consequences of cellular damage by the virus. Death of the infected cell would result in danger signals as well as release of the cell's contents into the extracellular medium. Neighboring cells, including dendritic cell precursors, would become activated to pick up viral antigens and would home to lymph nodes, where they could provide both signal 1 and 2 to virus-specific $CD8^+$ T cells. These T cells would thus become activated and differentiate into effector CTLs, whereupon they would travel to the liver and deliver their lethal hit to any cell expressing viral antigens (recall that signal 2 is not required by the activated effector cell). This process would continue for as long as cells were being destroyed by virus. Thus, the only difference between the two viruses mentioned above, and the nature of the immune response to them, is the extent of tissue destruction caused by the virus. By these considerations, the true role of the immune system becomes apparent: it is to detect and react to situations of dangerous tissue distress.

What is dangerous?

Early on we stated that a new paradigm of immunology must take into account both the need to react to dangerous pathogens as well as to minimize the risk of dangerous autoimmunity. We presented a model in which the signals that initiate an immune response emanate from stressed or dying cells, relayed through dendritic cells, which then travel to the local lymph node to alert T cells to imminent danger. But cell death is taking place constantly in the normal, unperturbed organism; in fact, it is essential for normal tissue homeostasis. Examples of normal cell death include the massive apoptosis that occurs in the thymus as part of the selection process, B-cell apoptosis in germinal centers following the generation of an antibody response, involution of breast tissue that occurs with each menstrual cycle, and the constant turnover of cells of the hematopoietic system or the epithelium of the gastrointestinal tract (reviewed in refs 24,25). In this last circumstance, dying cells are simply shed into the lumen of the gut and it is quite possible that the immune system is blind to this occurrence. But how does the immune system distinguish the novel antigens that are released as a consequence of pathologic versus physiologic cell death? The answer to this question depends upon whether the default response of the immune system to new antigens presented by dendritic cells is ON or OFF. In other words, is signal 2 on a dendritic cell inducible or constitutively expressed? If the default immune response to new antigens is OFF, then a specific signal is required to induce costimulatory signals on professional APCs. If the default response is ON, then one of two events must occur during physiologic cell death to prevent autoimmunity from being triggered. First, to prevent autoimmunity against tissue-specific antigens, the apoptotic tissue cell may possess a mechanism to prevent its antigens from being captured and presented by professional APCs. Alternatively, apoptotic cells must somehow suppress the immune response, either by directly turning off signal 2 on professional APCs or by secreting molecules that have globally immunosuppressive properties, for instance TGF-β. We favor the idea that the default response of the immune system is OFF, and that a 'danger' signal is required to activate dendritic cells to move to secondary lymphoid organs to alert T cells to ongoing tissue damage or distress. This would explain the need for adjuvant in the generation of immune responses to most soluble protein antigens,[26] and would obviate the need for a mechanism of suppressing immune responses to antigens released from cells dying physiologically.

What is (are) the danger signal(s)?

Danger signals may be constitutively present within cells or induced only upon stress, such as infection, temperature shift, hypoxia, or trauma. If there are constitutive danger signals, cells that are dying physiologically must prevent this signal from acting upon professional APCs. This may be easily accomplished if normal physiological death induces uptake by phagocytic scavenger cells. For inducible signals there is the danger that an infectious agent may shut off their synthesis, or that they may not be synthesized fast enough in response to certain types of cellular damage. However, an appropriate mix of danger signals would be difficult to circumvent.

Cells of the innate immune system, such as granulocytes and macrophages, possess receptors for conserved microbial molecules by which pathogens can be recognized and engulfed. Charlie Janeway has

argued that these microbial pattern receptors, for instance the LPS receptor, have been adopted by cells of the adaptive immune system to serve as inducers of signal 2.[27] He therefore postulates that danger equals infection, and that the immune system will regard new antigens as being nonself if they are presented in association with engagement of these pattern receptors. Heat shock proteins, synthesized by cells in response to a variety of stressors, such as heat, trauma, and infections,[28] may also be mediators of a danger signal. Recently, Srivastava has shown that gp96, a peptide-binding heat shock protein residing in the endoplasmic reticulum, plays a critical role in the phenomenon of immunologic cross-priming,[29,30] whereby antigens produced by one cell are presented on another cell to induce an immune response. If the danger model is correct, then all immune responses to tissue-specific viruses must occur through the phenomenon of cross-priming, so gp96 itself would be a good candidate for the danger signal.

Tumor immunology in light of the danger model

The clinical literature is littered with anecdotes regarding the potential role of the immune system in the surveillance and elimination of cancer. Though these observations make little sense when approached from the paradigm of self/nonself discrimination, many can be interpreted more easily in the framework of danger discrimination. The first such phenomenon is that of 'spontaneous' regression of cancer — that is, the shrinking or disappearance of tumor in the absence of any specific anticancer therapy. The factors most commonly associated with spontaneous regression as reported in the literature have been concurrent acute bacterial infections, administration of bacterial vaccines, or the removal of at least some of the tumor or its metastases.[31] In compiling 449 cases reported in the world literature of spontaneous regression associated with concurrent bacterial infection, Nauts found that regression was most commonly associated with suppurative infections, particularly erysipelas (a superficial spreading infection of the skin) from *Streptococcus pyogenes*.[31] But what is most interesting is that an intimate contact between bacteria and tumor cells was found to be necessary for the inhibition of tumor growth.[32] In 1966, Everson and Cole reported 176 cases of spontaneous regressions.[33] Interestingly, 71 (40%) of the patients experiencing regression had some type of operative trauma, usually within a few months of the regression.[34] In 43 patients, excision of the primary tumor was followed by regression of metastases; 22 of these had palliative nephrectomies for kidney cancer. Twenty-three patients experienced regression following biopsy or partial excision of the tumor.

According to a self/nonself discrimination paradigm, tumors avoid immune surveillance by hiding from the immune system. Trauma to the tumor bed or infection at the site of the tumor would expose the immune system to tumor-specific antigens for the first time, leading to 'spontaneous' regression. According to the danger model, trauma or infection would provide the stimulus to activate professional APCs near the tumor, and these APCs would present tumor antigen along with the appropriate signal 2 to naive, tumor-antigen specific T cells. So how do the two models differ? Remember that, according to the immune surveillance hypothesis, the default response of mature T cells is activation, whereas in the danger model the default response of T cells is inactivation, and a danger signal is constantly required to sustain the immune response. Therefore, according to the self/nonself discrimination paradigm, once the immune system has been activated by tumor-specific antigens, the T cell response to the tumor should be sustained until the tumor is eliminated. But according to the danger model, the response would proceed only so long as danger signals are present. In the absence of a danger signal, memory T cells encountering the tumor-specific antigen on the tumor cell would receive signal 1 in the absence of signal 2 and become tolerized. In fact, spontaneous regressions are by no means synonymous with cure. Rather, cure is the exception rather than the rule.

The occasional regressions that accompanied bacterial infections did not go unnoticed by physicians, several of whom introduced infections into their cancer patients in the hopes of obtaining a tumor response. This technique, referred to as nonspecific cancer immunotherapy, dates back to at least 1774, when a Parisian physician injected pus into the leg of a patient with inoperable breast cancer.[35] As the infection worsened, the patient's breast cancer disappeared. More than a century later Coley, noting the apparent salutary effects of erysipelas infections on cancer regression, formulated a preparation consisting of the soluble toxins from erysipelas and *Serratia marcescens*.[36] Although Coley's toxins clearly benefitted some patients, their use declined following the introduction of chemotherapy and radiation therapy. More recently, topical application of the BCG vaccine,

prepared from Mycobacterium bovis, has proven in randomized clinical trials to be an effective therapy to prevent recurrence of superficial bladder carcinoma.[37] We find these observations intriguing, and suggest that these 'nonspecific' immune stimulants may be providing the necessary danger signals to stimulate local APCs to initiate an immune response to tumor-specific antigens.

The danger model may also explain in part the phenomenon of concomitant tumor immunity, defined as the capacity of any animal bearing a progressor tumor to inhibit a second challenge with the same tumor.[38] In some but not all animal model systems, concomitant tumor immunity has been found to be mediated by $CD8^+$ T cells, which begins at day 6 after initial tumor inoculation, peaks at day 9, and then decays with progressive growth of the primary tumor.[39] Ex-vivo manipulation of the tumor prior to injection may serve to upregulate danger signals within the tumor, such that an immune response ensues upon inoculation. As the tumor recovers from the manipulations and begins to grow, however, the danger signals wane and the tumor begins to induce tolerance in the T cells that were initially active. However, these T cells are still capable of becoming activated to reject a challenge with the same tumor, again a source of fresh danger signals.

Strategies to enhance the immunotherapy of malignancy

The field of tumor immunology has witnessed short bursts of great excitement followed by longer periods of pessimism. Enthusiasm appears to be rising again and is justified for several reasons. First, we now know that cells are remarkably efficient at displaying their internal contents, in the form of peptide-MHC Class I and II complexes, for perusal by $CD8^+$ and $CD4^+$ T cells, respectively.[40] Second, many tumor-associated antigens have been identified[41] and their use in vaccines is currently the subject of many clinical trials. Third, although many types of cancer are resistant to both physiologic and chemotherapy-induced cell death, cytotoxic T cells are able to overcome this resistance quite readily.[42,43] Finally, a beneficial graft-versus-leukemia effect of allogeneic bone marrow transplantation[44] and donor leukocyte infusions for relapsed patients[45] has been conclusively demonstrated, illustrating that T cells, properly stimulated, can eliminate previously incurable cancers.

We believe that the danger model may offer the following insights for the development of more effective immunotherapy:

Keep the response going

An immune response to a tumor must not only get off the ground, it must *stay* off the ground. Spontaneous regressions and concomitant immunity may be two examples of anti-tumor immunity that ultimately fail because memory T cells are susceptible to tolerance induction.

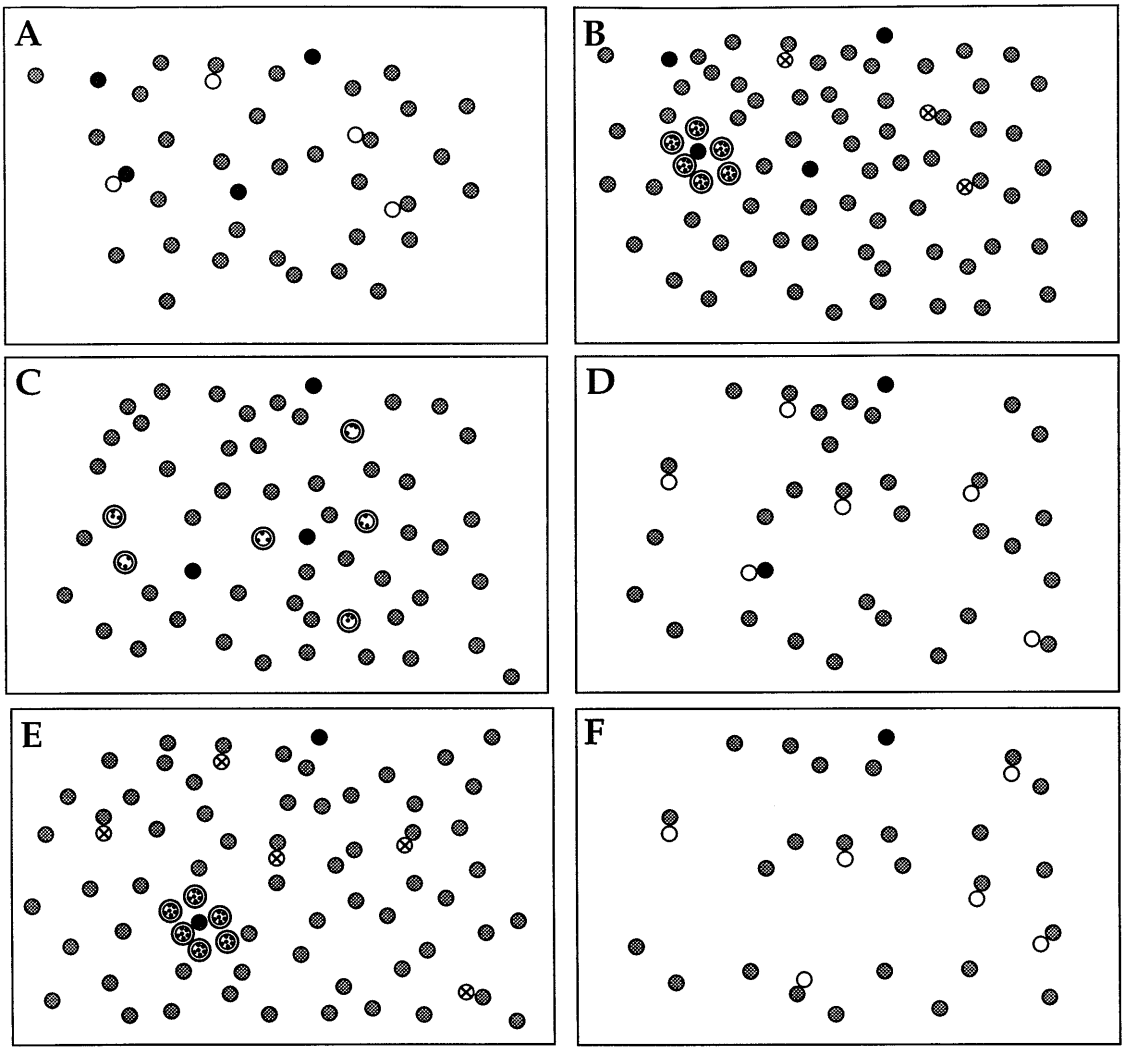
Vaccination protocols should therefore be modified. Since the tumor, unlike a virus, cannot by itself elicit danger signals, it will not be sufficient to vaccinate for tumors as we vaccinate against viruses. The activated effector CTL cannot be allowed to rest, lest they fail to eliminate the tumor, and then succumb to the toleragenic effect of the tumor. Therefore tumor vaccinations must be continued until it appears that the last tumor cell has been eliminated (and even then it might be a good idea to re-vaccinate periodically).

Another example of the failure of immunotherapy because of tolerance induction in memory T cells might be relapse of malignancy in an allogeneic bone marrow transplant recipient, despite the occurrence of graft-versus-host disease. As we have noted, the danger model predicts that all cells other than dendritic cells are toleragenic APCs for naive T cells. By this reasoning, the antitumor effect of allogeneic BMT is due solely to the immune responses to antigens that are shared by dendritic cells and tumor cells, and there can be no response to truly tumor-specific antigens, unless those antigens are captured by dendritic cells in the presence of appropriate danger stimuli. According to the danger model, the donor cells will continue to react against the recipient's tumor only so long as there are dendritic cells (and possibly macrophages and B cells, which can activate memory T cells) presenting shared histocompatibility antigens. If these cells are depleted or if danger signals wane, tolerance will result when memory T cells receive signal 1 in the absence of signal 2, on tissue or tumor cells. The ultimate failure of donor T cells to eradicate malignancy in bone marrow transplantation is illustrated in Figure 2. One way to prevent relapse following allogeneic transplantation may be to inject intermittently the patient's cultured dendritic cells, stored prior to the transplant. Since this is likely to increase the risk of graft-versus-host disease as well, it may be more advisable to reserve this strategy for the treatment of patients who

have relapsed and receive treatment with donor T cells. This may be particularly advisable for patients in whom 100% donor hematopoiesis is documented, because in such patients all the cells that are presenting recipient histocompatibility antigens are toleragenic.

Make sure the response is in the right class

The first decision a $CD4^+$ T cell makes when it encounters its antigen on an APC is whether to be turned on or turned off. If the T cell decides to become activated, it must then decide upon the



	A	B	C	D	E	F
Leukemia cell = ⊛	32	64	47	30	60	25
'Professional' APC= ●	4	4	3	2	2	1
CD8+ CTL precursor= ○	4	0	0	6	0	6
Effector CD8+ T cell= ◎	0	6	6	0	6	0
Tolerized CD8+ T cell= ⊗	0	3	0	0	5	0

effector cells or molecules that must be induced (macrophages, cytotoxic T cells, antibody with the appropriate isotype) to rid the organism of the inciting stimulus. An immune response inappropriately directed may be even worse than no response at all. Though the rules governing the regulation of immune effector classes are not yet established, it is possible that tissue cells play an important role in directing the class of immune response that has been generated. For instance, since IgA is particularly well-suited to protect the organism against bacterial infection in the gut, it stands to reason that colonic cells may produce cytokines, such as TGF-β, that favor its secretion. However, IgA may be totally ineffective in fighting colon cancer. The phenomenon of immunosuppression by malignancy may simply result from the secretion of molecules (for instance, secretion of TGF-β by colonic adenocarcinoma[46]) that would be secreted by its normal, nonmalignant precursor cell. In these cases, successful immunotherapy may require methods to divert the immune response into the appropriate effector class.

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Figure 2. An illustration of how a $CD8^+$ T-cell-mediated antitumor immune response can be initiated, mediate tumor regression, but abort at the memory T cell stage. (**A**) In allogeneic bone marrow transplantation, four donor T cells, reactive to recipient minor histocompatibility antigens (mHAs), are transfused into a hypothetical blood compartment containing four 'professional' APCs and 32 leukemia cells. (**B**) Three of the four T cells encounter mHAs on leukemia cells and are tolerized by signal 1 in the absence of signal 2. The fourth T cell is activated by a 'professional' APC, proliferates and differentiates to become six effector CTL, each of which can kill six targets. By this time, the leukemia cell mass has doubled to 64 cells. (**C**) Targets are killed at random. Midway through the killing process, 17 leukemia cells and one professional APC have been killed. (**D**) After the effector CTL have finished killing, there are 30 leukemia cells and two professional APCs remaining. (**E**) Five of the six CTL precursors encounter mHAs on leukemia cells and are tolerized. The sixth is activated, generating six CTL capable of killing 36 targets. Again, the leukemia cell mass has doubled. (**F**) At the conclusion of the second round of killing there are 25 leukemia cells and one professional APC remaining. This time, however, all six CTL precursors encounter mHAs on leukemia cells and are tolerized (not shown).

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