The field of tumor immunology has made important scientific advances during the past 10 years. One major advance has been the identification of major histocompatibility complex (MHC) class I-restricted human tumor antigens that act as targets for cytotoxic T lymphocytes (CTLs), at least in vitro (1). Much of the pioneering work has been performed in melanoma, where a number of tumor antigen genes have been isolated and shown to fall into two main groups based on their pattern of gene expression. One group includes the MAGE/GAGE/BAGE gene families, which are expressed by a variety of different tumors but not by normal cells in adults (with the exception of testes) (1). The normal functions of the proteins encoded by these genes remain obscure. The second group includes genes encoding tyrosinase, MART-1, TRP-1, and gp100, which are normally expressed in pigmented cells and whose expression is maintained in tumors derived from melanocytes (2). These are lineage-specific differentiation antigens that are not mutated or overexpressed in melanoma cells. That, in some cases, these antigens were identified by use of CD8+ tumor-infiltrating lymphocytes (TILs) isolated from patients’ tumors was an early indication of an important paradigm that has evolved as a result of these data. The new concept is that tumor antigens are often normal self-proteins to which tolerance can be broken. This concept, in turn, leads to the hypothesis that, under normal circumstances, tolerance exists only for dominant epitopes, while the T-cell repertoire directed against cryptic (or subdominant) self-antigens remains largely intact. Other examples have recently been described. For instance, overexpression of normal cellular proteins in tumor cells, which results from gene amplification or transcriptional enhancement [i.e., HER2/neu (3)] or from stabilization of protein half-life by point mutation [p53 (4)], appears to be important both in the genetic evolution of the cancer cell and in increasing the presentation of peptides derived from these tumor antigens. In the case of p53, an important recent finding is that T-cell immunity can be efficiently directed at nonmutated epitopes within the p53 protein (4), which greatly increases the likelihood that multiple MHC class I molecules will be able to bind and present antigenic peptides derived from p53.

Another class of tumor antigens consists of cell surface glycoproteins expressed on normal epithelium that are secreted into the bloodstream at high levels in cancer patients. These are the carcinoembryonic antigen (CEA) and prostate-specific antigen (PSA). At first glance, it would seem that the biology of these molecules would preclude them as effective targets for antigen-specific T cells. After all, one would predict some degree of central tolerance to these antigens would be induced during development. Furthermore, since immunity directed against high levels of circulating antigen does not exist de novo, it seems likely that a state of peripheral tolerance has been induced. It would also seem likely that uptake and presentation of circulating CEA and PSA by professional antigen-presenting cells ought to occur in cancer patients; yet, immunity against these proteins is not apparent, suggesting that a state of tolerance exists. Nevertheless, previous work (5) studying responses to these tumor antigens has indicated that the T cells isolated from patients with circulating CEA can be induced in vitro to recognize HLA-A2 (MHC class I)-restricted CEA peptides.

The possibility that self-antigens on tumors may be immunogenic was pursued by Correale et al. (6) in this issue of the Journal in experiments designed to identify HLA-A2-restricted T-cell epitopes in peptides derived from the PSA protein. After scanning the amino acid sequence of PSA for peptides with appropriate anchor residues, four HLA-A2-binding peptides were identified by use of the T2-cell-line-binding assay. Peptides designated PSA-1 and PSA-3 were tested for their ability to induce peptide-specific CTLs from peripheral blood lymphocytes isolated from normal control subjects and one prostate cancer patient. T-cell lines generated from all three donors responded to PSA-3, while only one PSA-1-specific line was obtained. The CTLs lysed T2 cells pulsed with the cognate peptides, and, perhaps more importantly, also lysed the HLA-A2* prostate cancer cell line LNCaP. The cell surface phenotype of the T-cell lines was quite varied: two lines were predominantly CD8+, one was a mix of CD4+ and CD8+, while, in the third,
CD4/CD8 double-positive cells predominated. For establishment of these lines, six to seven in vitro stimulation cycles were required, each consisting of 5 days of co-culture with peptide, followed by another 11-day culture in interleukin 2 (20 IU/mL) containing medium. Thus, 96-112 days of culture were required to obtain the PSA-specific CTL lines!

The main conclusions to be derived from the PSA data are that the T-cell repertoire is not totally depleted of PSA-reactive T cells and that at least one prostate cell line processes and presents the peptides appropriately. From these data, it is tempting to hypothesize that it should be possible to generate CTLs directed against virtually any self-protein by careful selection of MHC-class-I-binding epitopes and prolonged in vitro stimulation. What is the best way to select peptides for in vitro analysis? In melanoma studies, investigators have used elegant molecular biologic techniques to let melanoma-specific TILs search out genes encoding their cognate antigens (2). In the absence of TILs, selecting peptides by MHC-binding assays is appropriate, although, as discussed below, T cells reactive against weak- or intermediate-affinity peptides are more likely to be represented at a higher frequency in the peripheral T-cell repertoire than high-affinity binders.

Vaccine trials using the newly discovered tumor antigens have recently begun. Preliminary data indicate that HLA-A1-restricted, MAGE-3-specific CTLs have been induced by subcutaneous administration of MAGE-3 peptide, even without the use of adjuvants (7). Similarly, CTLs specific for one of three gp100 peptides were enriched in patients vaccinated with peptide in incomplete Freund’s adjuvant (8). Refinements in peptide vaccines are likely to include the addition of T-helper epitopes, covalent attachment of lipid moieties, and modification of anchor residues to increase the affinity of MHC binding while maintaining T-cell receptor-specific determinants. While peptide vaccines have the attractive feature of focusing the T-cell response to epitopes that might represent minor determinants within the larger protein, the potential for the induction of anergy, even against epitopes that are immunodominant using other methods of vaccination, has been demonstrated in some systems (9). Peptide-pulsed dendritic cells or recombinant viruses encoding tumor antigen genes may provide alternative or synergistic methods to overcome this potential problem.

As stated above, the use of potent vaccination techniques, such as peptide-pulsed dendritic cells, recombinant viruses, and peptide–adjuvant mixtures, is likely to induce significant increases in peripheral CTLs in cancer patients. Furthermore, it is reasonable to speculate that the increase in peripheral CTL frequency will not lead to clinical benefit. What is missing? It may be instructive to contrast naturally induced immune responses directed against infectious agents (which are often highly successful) with our attempts to induce immunity against tumors (which have been largely unsuccessful). Immune responses to infectious agents are begun by activation of the macrophages, neutrophils, and natural killer cells of the natural or innate immune system, which act to limit initial growth of the microbe (i.e., by phagocytosis or interferon production) and also to prime subsequent responses by antigen-specific lymphocytes of the adaptive immune response (10). Thus, the type of subsequent T- and B-cell responses, including the production of TH1- versus TH2-type cytokines, are tailored to the type of pathogen (11). In contrast to infectious processes, very little is known concerning the in vivo response of the innate immune system to evolving neoplasms in patients with cancer, although it is generally assumed that evolution of a TH1-type cellular immune response is critical for attaining therapeutic benefit (11). Clinical researchers will have more control of this aspect in the vaccine setting, where the type of immune responses can be dictated by the choice of adjuvant or cytokine administered with the antigen.

Sites of active infection are characterized by the selective infiltration of leukocytes and the secretion of soluble products that combine to generate the local physiologic processes that characterize the inflammatory milieu. This inflammatory response may be one critical feature of infectious processes that distinguishes them from tumor deposits. Activated macrophages and endothelial cells secrete a variety of cytokines and chemokines at inflammatory sites that profoundly affect the ability of T cells to initiate and mount an effective effector response to the invading pathogens. The initial step in the adaptive immune response occurs when tissue dendritic cells, the professional antigen-presenting cells responsible for activation of naive T cells, take up antigen and process it for MHC presentation (12). The local inflammatory milieu induces dendritic cells to shift their role from efficient phagocytes to that of professional antigen presenting cells, whose critical role in activating naive T cells occurs after their migration to regional draining lymph nodes (12). A critical aspect of this T-cell-antigen-presenting cell interaction in the lymph nodes is the presence of signal 2 (costimulation through CD28-B7 interactions) required for activation of naive T cells (13). This costimulation must occur in the setting of the lymph node, since recognition of signal 1 (T-cell receptor triggering) by naive T cells in peripheral tissues (in the absence of signal 2) can lead to anergy (13). T-cell activation events include increased expression of cytokine receptors and adhesion molecules, secretion of an assortment of cytokines and chemokines, and proliferation of antigen-specific clones. Activated T cells exit the lymph node and circulate through the vascular tree, where interaction with receptors on the endothelium regulates their final destination (14). Whereas naive T cells migrate exclusively between secondary lymphoid organs until activated by professional antigen-presenting cells bearing cognate antigens, under appropriate conditions, activated T cells (and also memory or “experienced” T cells) will extravasate into tissues where they perform effector functions, such as killing virally infected cells and regulating the ongoing inflammatory process.

The interaction of circulating T cells (and other leukocytes as well) with the endothelium occurs in an orchestrated manner that permits leukocytes to enter the local environment and results in inflammatory processes (14). The primary adhesion of leukocytes to endothelium occurs despite the shear forces caused by blood flow in small vessels. Initially, this adhesion involves rolling or tethering mediated by reversible binding of the selectins (carbohydrate-binding proteins) expressed on leukocytes (L-selectin) and endothelial cells (E- and P-selectins) to their ligands and by some integrin–ligand interactions (15). T-cell recruitment to sites of inflammation appears to depend on interaction of the L-selectin and α4β7 integrin expressed on T cells with L-selectin ligands (i.e., peripheral lymph node addressins,
including GlyCAM-1 and CD34) and mucosal addressins (e.g., MAAdCAM-1) expressed on the inflamed endothelium. For tight binding and arrest to occur, a triggering step is required to activate integrins on the leukocyte surface. Inflammatory signals make this second step possible. Cytokines, such as interleukin 1, tumor necrosis factor-α, and interferon gamma induce a number of changes in the local endothelium, including increasing the expression of ICAM-1 and VCAM-1 (16). Activation of T-cell integrins is mediated by the binding of specific chemokines (chemoattractant cytokines) produced at inflammatory sites to G-protein-coupled seven transmembrane chemokine receptors on T cells (17,18). At least 25 species of chemokines have been isolated, some of which (i.e., MIP-1β, MCP-1, and RANTES) are produced by a variety of cells at inflammatory sites, including macrophages and endothelial cells. The interaction of the heparin-binding domains of chemokines with the proteoglycan-rich glycocalyx of endothelial cells concentrates them appropriately for interaction with chemokine receptors on rolling leukocytes (18). This interaction results in the rapid activation of T-cell integrins αβ1 (VLA-4) and αLβ2 (LFA-1) and their association with the cytokine-activated endothelial ligands VCAM-1 and ICAM-1, respectively (15). Tight adhesion, arrest, and T-cell migration into the inflamed site then result, and effector functions ensue. These effector functions include further T-cell production of cytokines and chemokines that amplify and modify the evolving reaction and augment the killing capacity of pathogen-infected cells by CTLs.

The failure of antigen-specific T cells to migrate to, expand, and eliminate tumor cells may be attributed not only to the weak T-cell-stimulating capacity of known tumor antigens but also to the lack of an effective inflammatory milieu at the tumor site. It is known that the process of tumor neovascularization results in abnormal vessel architecture, including disruption of the basement membrane in small tumor vessels (19). The ability of T cells to perform normal surveillance functions by rolling along the tumor endothelium is unknown, although there are data to suggest that various defects in the normal interactions between leukocytes and the endothelium may occur in tumors. In a rat mammary carcinoma model, both the flux of rolling leukocytes and the density of adherent leukocytes were significantly reduced in tumor microvessels relative to what was observed in adjacent normal microvessels; this was true under normal conditions and after the administration of bacterial lipopolysaccharide and tumor necrosis factor-α (20). The induction of tumor endothelial cell VCAM-1 expression can be inhibited by soluble factors that are produced by the tumor and act at the transcriptional level (21). The pattern of inflammatory infiltration has been studied in islet tumors that arise in rat insulin promoter-SV40 large T-antigen transgenic mice (22). Hyperplastic islets were extensively infiltrated with inflammatory cells, including T-antigen-specific T cells; however, adjacent tumors were devoid of lymphocytic infiltrates. The lack of lymphocytic infiltration was associated with the absence of L-selectin ligands and MAAdCAM-1 on tumor endothelial cells. Therefore, in this model, the lack of expression of leukocyte vascular addressins was associated with tumor progression (22).

Other obstacles are present at tumor sites that prevent activated T cells from performing effector functions as described above. In comparison with normal tissues and organs, tumors often have increased interstitial pressure that acts to prevent the influx of many substances from the vasculature into the tumor. Some tumors secrete transforming growth factor-β, which is inhibitory for many leukocyte functions, including adhesion and transendothelial migration (23). Inhibitory molecules that inhibit the function of T cells that do manage to gain access to tumor deposits are expressed by tumor cells. These inhibitory molecules include the Fas ligand, which can induce apoptosis in activated antigen-specific T cells (24), and the epithelial mucin DF3/MUC-1, which similarly induces apoptosis in activated T cells (25). Interestingly, soluble Fas ligand is detected in the serum of melanoma patients (24), although its physiologic role, if any, is unclear, and soluble DF3/MUC-1 was shown to induce T-cell apoptosis in vitro (25). These data relate to the work of Correale et al. (6) by raising the possibility that soluble CEA and/or PSA could have T-cell-inhibitory functions; this was not investigated in the current work with in vitro-activated PSA-specific T cells. These data also suggest that, even if antigen-specific T cells are activated, peripheral mechanisms are in place to re-establish tolerance or at least to prevent any brisk auto-regulatory enhancement of the inflammatory process. Could this explain why some melanoma patients demonstrate patchy vitiligo? Why does the apparent T-cell attack directed against normal melanocytes come to a halt? Was peripheral tolerance re-established? It is difficult to invoke a process of high-zone tolerance (exhaustion) that occurs in some instances of viral infection (26). Other obstacles to successful immunotherapy have been well documented, including the reduction of tumor antigen expression, the loss of MHC restriction elements, and a decrease in TAP (peptide transporter) activity in some tumor cells and aberrant signaling pathways in T cells in tumor-bearing animals and patients (27).

The current state of the field of tumor immunology indicates that T cells reactive against tumor antigen epitopes, which are in fact normal self-proteins, can be found in vivo (in TILs) and are inducible in vitro (albeit using high concentrations of peptide and often multiple rounds of stimulation). The repertoire against these epitopes has not been deleted (consistent with the notion that central tolerance does not create holes in the repertoire but only dents); but, either peripheral tolerance to these self-antigens has been induced (but can be broken under the right conditions) or, in the absence of signal 2, peripheral T cells simply ignore these antigens. Thus, the question arises as to whether tumor immunology is best viewed in the context of the self–nonself discrimination model of immune reactivity or whether the key to triggering an immune response is by the sensing of danger (28) to the host (such as tissue destruction during an infection). If immune activation in response to infection reflects the body’s appropriate recognition of “danger,” then it appears that tumors mask their inherent danger from physiologic detection systems. During infectious processes, the cellular and molecular correlates of danger have been discussed above, i.e., the invoking of a local inflammatory response with the activation of the innate immune system, the secretion of cytokines and chemokines, and the induction of the adaptive immune system that focuses the attack on specific targets. Thus, danger is manifested by the inflammatory process itself, and the link to antigen-specific T-cell responses requires the activation of local tissue dendritic cells as described. The biologic function of dendritic cells places
them at the key intersection between innate and adaptive systems, responding to inflammatory signals by migration to draining lymph nodes, where activation of naive T cells occurs. Cancer, especially in its early stages, does not signal danger; the appropriate inflammatory milieu is not induced, and downstream events including dendritic cell–T-cell interactions in regional lymph nodes do not occur. Infectious agents inherently express multiple, strong T-cell epitopes (in the most profound cases, superantigens) that have not been “tolerized,” which leads to amplification of the local inflammatory process as activated T cells enter the site and secrete a panoply of soluble mediators. Although TILs capable of lysing autologous tumor cells can be found in some tumor types (2), apparently the activation state of these cells is not sufficient to propagate and expand local inflammation. This insufficiency may reflect the subdominant nature of tumor antigen epitopes, which are weakly stimulatory at the concentrations of antigen encountered in vivo, and the lack of stimulation of the T-helper arm of the immune response. According to the danger model, tumor-antigen-reactive T cells would rarely if ever encounter their cognate antigens presented by dendritic cells (because there is no inflammation to initiate the process), and, therefore, they would remain in a state of peripheral tolerance, or they would merely ignore the low level of antigen presented by normal or tumor cells (28). Concepts inherent in both the self–nonself and danger models of immune regulation are relevant to tumor immunologists. The danger model may be of greater use for conceptualizing the future course to be taken by this field: it places less emphasis on understanding the specificities of individual T-cell receptors, and it invokes more pressing issues, such as how to orchestrate processes involved in inflammation, dendritic cell activation, and T-cell activation to focus on the tumor site. Converting tumor sites disseminated throughout the body into inflammatory sites may now be the critical issue. Whether tumor antigen-specific T cells will be sufficiently reactive to propagate the inflammatory process and mediate systemic antitumor effects remains to be determined. Oncologists and their patients know that malignant tumors, responding to inflammatory signals by migration to draining lymph nodes, where activation of naive T cells occurs. Can-

References


