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Induction of Anti-Self-Immunity to Cure Cancer

Meeting Review

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A quiet but profound revolution in theoretical framework, a paradigm shift, now has infused the consideration of therapies in autoimmune diseases and cancers, as exemplified at the Jennifer Jones Simon Foundation workshop on Tumor Antigens as Self-Antigens, held in Los Angeles, California, on January 26–27, 1995.

It is becoming accepted that a large set of antigenic determinants of the self have not induced self-tolerance (reviewed by Sercarz et al., 1993) and that these peptide determinants furnish target structures for autoimmune attack (reviewed by Lanzavecchia, 1995) and could provide potential targets for immune responses directed against tumors. It is not necessary to seek mysterious nonself- or neoself-antigens expressed by the tumor. The bulk of the lively and open discussion, characteristic of these free format workshops with no fixed presentations, was modulated very capably by A. Tobin (University of California, Los Angeles), a neurobiologist, and focused on how to initiate, maintain, and regulate antitumor autoimmunity, which could translate into effective treatment in cancer clinics.

Are there in fact certain tumor-related determinants that can be rendered into crucial targets of attack by the immune system? The pertinent focus is on suitable antigen processing and subsequent presentation by major histocompatibility complex (MHC) class I and class II molecules expressed by tumor cells. Any of the peptides that is bound in the groove of an MHC molecule on tumor cells provides a potential target determinant for attack by the immune system. The peptides bound by the MHC molecules of all cells, including tumor cells, are derived from endogenous cellular (or viral) proteins, and the antigen-processing machinery of the cells manages to display certain antigen determinants to ambient T cells. Tumor cells are distinct in that they possess additional oncoproteins that either are overexpressed owing to dysregulation or are mutated and have thereby conferred the tumor phenotype to these cells, to developmental antigens reexpressed during the process of tumorigenesis, or to passenger mutations in nononcogenic proteins that result from the loss of mechanisms that maintain genomic stability. Using T cell clones that are specifically able to kill the tumor as detectors for immunogenic tumor-derived determinants bound to MHC molecules of the tumor, several investigators have shown that T cells indeed recognize peptides from endogenous normal (and occasionally mutant) self-proteins. T. Boon and colleagues (van der Bruggen et al., 1991), in their pioneering studies, drew a page from bacterial genetics and cloned genes encoding tumor antigens recognized by CD8 T cells specific for human melanomas. Melanoma antigen 1 (MAGE-1) to MAGE-3 were the original family of human melanoma-specific antigens that were molecularly defined in this way and were followed by isolation of determinants on tyrosinase, gp100, and Melan-A-MART-1 recognized by antimelanoma T cells (reviewed by Houghton, 1994). It is interesting that the *MAGE* gene family is not expressed in any normal adult tissue except testis, but is expressed in a large proportion of other human tumors (including small cell lung carcinoma, breast cancers, and colon carcinoma) and perhaps represents developmental antigens reexpressed during the process of tumorigenesis. Tyrosinase, gp100, and Melan-A-MART-1 are normal self-proteins specific to the melanocyte lineage and T cells specific for determinants on each of these antigens can be found in a large majority of melanoma patients (reviewed by Houghton, 1994; Pardoll, 1994). It is thus becoming clear that there is no special group of proteins that can be dubbed tumor antigens, and the distinction between self-antigens and tumor antigens is rapidly vanishing. Thus, as summed up by Tobin, we have entered an era in which "we either know the tumor antigens or know how to know them."

But can the immune system make a T cell response against all the peptides bound to MHC molecules of a tumor cell? Part of the answer lies in the availability of the T cell repertoire membership directed against MHC-bound determinants and the proportion of T cells rendered tolerant in each individual. It is now thought that only well-expressed self-determinants are efficient in tolerance induction. In addition, on every self-antigen, there are sequestered determinants that do not succeed in inducing tolerance but that, under the circumstances of severe inflammation and its attendant cytokine milieu, can be displayed in an immunogenic context. Among the diversity of self-reactive T cells that evade negative selection, tumor-specific members can be mobilized under conditions of heightened awareness by the immune system—in which MHC molecules, surface adherence molecules, and costimulators are up-regulated and become available for possibly killing interaction along with newly displayed, previously "cryptic" self-antigenic determinants (reviewed by Sercarz et al., 1993; Lanzavecchia, 1995). Autoimmune disease on one hand and the existence of tumor-specific cytolytic T cells recognizing self-peptides in the cancer patient on the other are a testimony to the enormous potential resources inherent in the positively selected T cell repertoire, directed to the cryptic self. For example, T cells specific for peptides of self-protein tyrosinase can be isolated from normal individuals, which can attack and kill melanoma cells from human lymphocyte antigen (HLA)-matched cancer patients (Visseren et al., 1995). What may be critical is the density of the up-regulated peptide–MHC complexes, as well as the expression of costimulatory signals that influence activation of the otherwise silent tumor-specific T cell repertoire existent in the cancer patient. The task at hand is how to generate and manipulate

these signals so as to engage these antitumor, antiself T cells in an attack on the tumor (see below) without causing damage to neighboring tissue. Lessons still to be learned from the analogous initial stimuli that activate the hardy, pathogenic, self-directed responses found in autoimmunity could potentially be applied to the specific initiation and maintenance of response against such dangerous internal enemies as tumors. The use of immunogenic peptide determinants from self-proteins that are either specifically expressed (for example, MAGE antigens) or overexpressed (for example, HER-2/neu; see below) in the tumor will possibly activate an immune activity that exclusively damages the tumor.

It is well established that the immune system gets propelled only upon ligand recognition in a context of heightened expression of costimulatory molecules, adhesion molecules, and HLA molecules on antigen-presenting cells (APCs) (as described above), or in P. Matzinger's (National Institutes of Health, Bethesda, Maryland) words, in the context of "danger" (reviewed by Matzinger, 1994). Adjuvants can transform a weak stimulus into one that signals danger, the only state to which the system has evolved responsiveness. If a dangerous stimulus is one that produces a costimulatory milieu, Listeria monocytogenes works as the Paul Revere of danger, warning adjuvants that might render the immune system tumor-ready. One reason for this could be its penchant for inducing macrophages to produce interleukin-12 (IL-12), a cytokine that is a potent activator of T helper type 1 (Th1) cells (reviewed by Mosmann et al., 1991) and cytotoxic CD8 T lymphocyte cells (CTLs). In another experiment by Noguchi et al. (1995), a mutant peptide of p53 injected along with IL-12 was able to destroy completely an established tumor in a tumor-bearing host, whereas the same peptide or IL-12 alone was totally ineffective at killing the same tumor. J. Berzofsky (National Institutes of Health, Bethesda, Maryland) mentioned similar results indicating that IL-12's remarkable effectiveness was noteworthy when used in vitro with normally unresponsive T cells from HIV-positive individuals (Clerici et al., 1993). IL-12 thus was touted as an ultimate weapon in tumor therapy, an "atomic missile" that could help initiate response in the intended direction with minimal side effects. Given the heterogeneity among tumors, different cytokines might be effective in killing different tumors.

The effectiveness of heightened expression of costimulatory molecules and cytokines in antitumor immunity is also evident from studies using whole tumor cell vaccines. Genetic manipulation of tumors designed either to enhance the presentation of tumor antigens or to provide enhanced costimulatory signals to T cells has been a route adopted by immunologists to increase immunogenicity of whole tumor cells (reviewed by Pardoll, 1993; Moudgil and Sercarz, 1994). Tumor cells transfected with B7 genes to provide enhanced costimulation were clearly more potent immunogens than parent tumors. Similarly, vaccination with tumor cells transduced with granulocyte–macrophage colony-stimulating factor (GM–CSF) induced long-lasting, antitumor immunity, involving both CD4 and CD8 T cells. This effect was attributed to the ability of GM–CSF to promote differentiation of dendritic cells, which are the most potent APCs for activating both class I and class II restricted T cells (Dranoff et al., 1993).

There was rather complete consensus that a key to inducing the most potent killing response to tumor seemed to be the coactivation of both the $CD8^+$ CTLs and the $CD4^+$ Th1 cells specific for the tumor. A known CTL determinant covalently linked to a T helper determinant and injected with either Listeria or IL-12 emerged as one effective way to achieve this scenario. Injection of a CTL epitope along with a helper epitope in combination with Listeria or IL-12 seemed a reasonable alternative. D. Pardoll (Johns Hopkins University, Baltimore) described an experiment, done in collaboration with Y. Paterson (Johns Hopkins University, Baltimore), in which simple immunization with Listeria expressing a model tumor antigen induced regression of established palpable tumors expressing the model antigen (Pan et al., 1995). They postulate that this is due to the sequential residence of Listeria in both the class I and class II compartments of a macrophage, which could possibly induce efficient peptide loading into both the MHC class I and class II pathways.

Do self-peptides associated with tumors actually induce T/B cell responses in real life? This was answered by M. Cheever (University of Washington, Seattle), whose group has shown that some patients with breast cancer have an existent immune response to HER-2/neu. T cells specific for peptides of HER-2/neu have also been demonstrated by Peoples et al. (1995) in patients with breast and ovarian cancers. HER-2/neu is a growth factor receptor homologous to epidermal growth factor receptor, which is overexpressed in 25%–30% of patients with breast cancer as well as some patients with colon, pancreas, gastric, and ovarian cancer. Tumor cells from breast cancer patients display a 40- to 50-fold excess of this molecule relative to the very low levels in normal tissue, making this a target self-molecule of choice for raising a possible therapeutic target for antitumor autoimmune response. In patients with overexpressed HER-2/neu, both T cells and immunoglobulins reactive to HER-2/neu could be demonstrated, thus indicating that self-tolerance has been circumvented. Rodent studies have identified vaccine regimens capable of inducing anti-HER-2/neu immune responses in naive hosts. Rat and human HER-2/neu proteins are highly homologous (89%). Immunization of rats with 15-mer peptides identical to the natural sequence of both rat neu and human HER-2/neu proteins elicited immunity specific for both proteins. Clearly, no significant self-tolerance had been established toward many determinants on this self-protein. Others have used peptides from different tumor-related self-proteins such as p53 (Yanuck et al., 1993) or Ras (Peace et al., 1994) and found them highly immunogenic.

Fortunately, it is not always necessary to identify the effective cryptic peptide determinants on the self-proteins in order to induce an effective immune response. For example, with the self-antigen tyrosinase in a vaccinia virus construct, P. Greenberg (University of Washington, Seattle) was able to elicit both CD4 and CD8 antitumor T cell clones in melanoma patients of six different haplotypes.

In a study by C. Melief (University Hospital, Leiden, The Netherlands) with a virally induced tumor, vaccination with the whole tumor induced a vigorous $CD8^+$ T cell response to two codominant adenovirus determinants. Surprisingly, treatment with these peptides resulted in enhancement of tumor growth, presumably because of down-regulation or competition with a more efficacious response induced by the tumor to the same determinants. In contrast, Melief reported another example using the human papilloma virus 16 (HPV-16)-induced tumor, where vaccination with the whole protein (recombinant oncoprotein E7 in a vaccinia virus vector) or the tumor itself failed to raise any immune response at all. However, vaccination with the right peptides of E7 consistently raised protective T cell immunity, thus pointing to the significance of trying heterogeneous strategies, including use of both the whole proteins or their cryptic peptides (or both) to raise effective immunity.

One proponent of exploring the humoral as well as the T cell–mediated arms of the immune response in the fight against tumors is H. Wigzell (Karolinska Institute, Stockholm). He reported results from a Swedish study of colorectal carcinoma patients treated with a monoclonal antibody (MAb1) against a cell surface glycoprotein epithelial cell adhesion molecule (EPCAM) overexpressed by colorectal carcinoma cells. Riethmuller et al. (1994) previously reported that treatment with this antibody alone prevents micrometastasis in such patients, and now in two cases, when administered along with GM–CSF, complete remission resulted. Interestingly, Wigzell and colleagues also administered anti-idiotypic antibody (Ab2, recognizing MAb1) in an alum adjuvant in six of their colorectal carcinoma patients, with a very positive clinical outcome in each. Of these patients, five not only made an antibody (Ab1′) that recognized Ab2, but furthermore induced a T cell response (delayed-type hypersensitivity [DTH] measured by skin granulomas, in vitro IL-2, and interferon-γ [IFNγ] synthesis) specific for EPCAM. As Ab2 images of self-antigens may be subjected to unique processing and network regulation quite distinct from that of the antigens per se, it remains a task for the future to determine the efficacy and possible exploration of this approach.

Why some patients have cancer despite an immune response to tumor antigens is probably due to a multitude of factors. Survival of many tumors in the face of measurable antitumor cellular activity (much as in parasite systems) underlines the importance of regarding the living tumor as more than an aggregate of tumor cells. The tumor can help to establish its own microenvironment by altering the expression of MHC molecules or the levels of other activities affecting immune induction, such as peptide transporter molecules, thus preventing the display of peptide determinants on their cell surface. We must learn details about the local milieu of the tumor, trafficking of cells in and out, the true nature of tumor-infiltrating lymphocytes (TILs), and APCs at sites of tumor deposition in order to exploit methodologies to skew responsiveness at the tumor sites in the desired direction.

At the other end of the spectrum, induction of antitumor, antiself-immune reactivity could cause simultaneous autoimmune pathogenesis: this was another obvious concern that was discussed at length in this meeting. A majority of self-antigens that have been described as target antigens for antitumor T cells are expressed at varying levels by normal tissue. For example MAGE-1 and MAGE-3 are expressed in testes, MART-1/Aa and gp100 are synthesized in the retina and normal melanocytes (reviewed by Houghton, 1994), and HER-2/neu is expressed at lower levels in all tissues. Several strongly encouraging results were described in this regard. In a study reported by Kawakami et al. (1994), patients after having received in vitro expanded melanoma-specific TILs that recognize peptides from gp100, Melan-A-MART-1, or both, although showing a sporadic occurrence of vitiligo did not show any adverse ophthalmologic effects. Depigmentation is reported to be associated with a good prognosis in melanoma patients, an indication that T cells were successful in attacking the tumor. A similar report has been made of a patient who, after having received tyrosinase-specific TILs, developed neither depigmentation nor any retinal damage (Visseren et al., 1995). In a Johns Hopkins University trial, renal carcinoma patients, after having received GM–CSF-transduced tumor cell vaccine, showed a DTH reaction not only against the renal carcinoma cells but also against normal epithelium derived from the unaffected kidney: there was, however, no functionally apparent autoimmune damage to the normal kidney in these patients (D. Pardoll). Analogous results were reported by Greenberg and colleagues who constructed transgenic mice expressing an envelope (env) protein of Friend murine leukemia virus under the regulatory genetic elements of immunoglobulin (Hu et al., 1993), albumin, or myosin to direct tissue-specific expression of *env* in lymphocytes, liver, and muscle. The env protein is expressed at high levels by a Friend virus–induced erythroleukemia (FBL) of B6 origin. These *env*-transgenic mice were tolerant to the (whole) env protein. However, adoptive transfer of env antigen-specific T cells (derived from nontransgenic mice) into the transgenic mice that harbored FBL resulted in complete eradication of FBL without any sign of autoimmune injury in any of the lymphoid (Hu et al., 1993) or other tissues expressing *env*. The lack of autoimmune damage did not reflect lack of *env* expression in transgenic lymphocytes for recognition by T cells, since transgenic lymphocytes functioned effectively in vitro as stimulators for env-specific T cells. The relatively increased level of expression of these immunogenic determinants in the tumor cells accompanied by unique inflammatory conditions existing at the tumor site may account for this selective tumor-specific destruction. These data seem consistent with recent experimental evidence that both a critical density of MHC–peptide complexes as well as a critical set of costimulatory/accessory molecules is necessary to activate T cells; in the absence of these signals, T cells either fail to respond or become anergic. The challenge ahead is to define these signals further and to learn to manipulate them toward a beneficial immune response in the cancer patient.

What would be an intelligent strategy for choosing the self-peptides (or mutant self-peptides) expressed on the tumor cell that are likely to most effectively immunize the host to make a specific assault on the tumor? The

current choice is to use peptide determinants that are recognized by T cells that are readily isolated from the cancer patient. This ensures that the peptide binds to the MHC and that the peptide-specific T cell repertoire exists in the individual. A. Coutinho (Institut Pasteur, Paris) and I. Cohen (Weismann Institute, Rehovot, Israel) emerged as proponents of considering a second level of complexity arising for example from the T cell receptor (TCR)-centered regulatory circuits of the immune system (see below). They postulated that prior encounters of regulatory circuits with different internal and external stimuli will affect the outcome of an immune response. This proposition is consistent with the notion expressed earlier by A. Mitchison (Deutsches Rheuma Forschungszentrum, Berlin) that "every gene in the body is an immune response gene" with a potential influence on immune responsiveness: these gene products could be enzymes involved in processing, surface adherence molecules, cytokine genes and receptors, et cetera, and genetic regulatory elements controlling their expression.

Most experimentally induced autoimmune diseases are self-limiting, and Cohen focused on this issue in the context of his view of the coordinated TCR-centered immune regulation of self-recognition (reviewed by Cohen, 1992). The organism has developed a natural, vigorous self-reactivity toward a key group of self-antigens and determinants, precisely so that it can focus regulatory cells capable of recognizing target structures on the TCRs of the effector population. Myelin basic protein (MBP), heat shock protein, and tyrosinase are three examples of such antigens, as normal individuals have benign self-reactivity to these proteins. These TCR-centered regulatory circuits have been characterized using MBP in both mouse and rat models: mouse and rat T cells specific for MBP use conserved α and β chains (reviewed by Nanda and Sercarz, 1993) in their TCR structures. The connectivity of these MBP-reactive T cells to regulatory circuits was revealed by the demonstration that peptides of their TCR structures (without introduction of MBP in the animal) can activate CD4 and CD8 regulatory cells and prevent subsequent activation of T cells specific for MBP (reviewed by Kumar and Sercarz, 1993). A similar interpretation was offered for an experiment from Melief's laboratory: an excellent tumor-specific response could be induced by immunizing the host with irradiated tumor cells expressing p53, and the antitumor T cells in this host recognized a wild-type peptide of p53. However, if the animal were immunized with the same peptide, no antitumor CTL response was raised. One explanation for this could be involvement in a hard-wired circuit resulting in activation of a strong down-regulatory system upon its introduction. Another explanation for the result of the p53 experiment of Melief's could be that the particular peptide chosen for vaccination might have had partially antagonistic properties in signaling the TCR. The interaction of antagonists and partial agonists of the TCR has already been considered as a regulatory device (P. Allen, Washington University, St. Louis) in autoimmune contexts (reviewed by Sette et al., 1994). In the context of an antitumor response, it is perhaps worthwhile to plan to inhibit crucial regulatory populations while simultaneously seeking very strong agonists that would energetically activate the T cell system.

Passive immunization with tumor-specific CD8 T cells along with IL-2 as a cytokine to replace CD4 helpers is a more traditional strategy (described above) that is successfully being used in cancer patients. Greenberg is investigating several sophisticated avenues in this area. One strategy (being pursued with S. Lupton, Targeted Genetics, Seattle is to transfect the CD8 T cells directed against the tumor with the IL-2 gene linked to the regulatory elements of genes that get activated upon target recognition, such as IFNγ. This would result in IL-2 production following encounter with the tumor in CD8 transfectants with the hybrid IL-2 gene. The tumor-specific CD8 T cells would thus be propagated upon activation, resulting in maintenance of effective immunity against the tumor without the requirement for CD4 helper T cells or potentially toxic exogenous IL-2. Another highly efficient avenue they have pursued is to construct chimeric growth factor receptor molecules in which the extracellular domains of the α and β chains of the GM–CSF receptor are linked to the cytoplasmic domains of β and γ chains, respectively, of the IL-2 receptor (Nelson et al., 1994). CD8 T cells normally do not have receptors for GM–CSF, but upon activation they produce GM–CSF. The CD8 transfectants that express the chimeric receptor chains can proliferate to GM–CSF as a result of intracellular signaling via its IL-2 moiety, thus rendering GM–CSF as a potential autocrine growth signal. Additionally, transfectants made using either of the above strategies can also be constructed to contain a suicide gene, thymidine kinase (HSV–TK), that can be used to ablate these CD8 cells in vivo when the job of tumor eradication is complete, to avoid autoimmune damage caused were there to be $CD8^+$ T cell reactivity for self-antigens.

Closing Remarks

It was evident from the discussion at the workshop that students of autoimmunity, parasite immunity, and tumor immunity have many shared interests. How self-destructive T cells became initially activated and propagated during the autoimmune disease still defies explanation. It is, however, being actively studied, and a solution to this puzzle should be readily adaptable to the cancer problem. Aside from virus-induced tumors, effective immunization against tumors represents an activation of natural autoimmunity to a set of self-antigens that had not induced tolerance during development or in the periphery. In the same vein, parasites, perhaps just like tumors, seem to create a barrier: they escape in the face of specific and fully capable T cells. Understanding the nature of this barrier may reveal what needs to be overcome in the cancer patient to allow the activation of T cell responses specifically to kill the tumor. Thus, the task ahead is to exploit the resource of the protected, nontolerized T cell repertoire and to learn how responses within it can be fostered by appropriate antigen presentation and costimulation. To paraphrase

Pogo, "we have identified the enemy and it is us!" What is left is to seduce the available immune repertoire into effective action.

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