Integrating the quality of the cytotoxic response and tumor susceptibility into the design of protective vaccines in tumor immunotherapy

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The field of tumor immunology has enjoyed an explosion of knowledge about the molecular and cellular bases of immune regulation. The identification of antigens expressed by different types of tumors has been critically important, and the field of therapeutic cancer vaccine development is advancing rapidly. Currently, major efforts are focused on developing specific cancer immunotherapy strategies that rely on adoptive transfer of immune cells or the use of tumor-specific antigens (peptides) as vaccines. Such vaccines are expected to augment established anti-tumour immune responses and to induce de novo immunity or reverse tolerance. Major advances have resulted in several immunization strategies designed to boost immune responses to some tumor-associated antigens (1). Strategies involving various forms of peptides, either alone or in combination with different cytokines, adjuvant, or DCs, have been used to enhance specific immune responses. Although the identification of tumor antigens is a crucial step in the design of vaccination strategies, an effective anti-tumor vaccination should also aim to bring tumor antigens to the secondary lymphoid organ in appropriate amounts and within a specific time frame. Obviously, the difficulty consists in achieving such a fine balance in the dynamics and kinetics of antigen administration. Despite the enthusiasm for current vaccination strategies, it should be noted that tumor rejection in patients does not always follow successful induction of tumor-specific immune responses by cancer vaccines. Even if a strong and sustained cytotoxic response is induced, complex issues such as tumor evasion and selection of tumor-resistant variants remain.

In the current issue of the JCI, Molldrem et al. (2) provide further evidence of the ability of tumor cells to circumvent host anti-tumor defenses to ensure their survival and progression. The authors suggest the existence of a novel escape mechanism from tumor immunity by leukemia-induced selective deletion of high-avidity effector cells that have the greatest potency against chronic myelogenous leukemia (CML). These findings have significant implications for tumor immunotherapy strategies and raise further concerns with respect to what appropriate vaccine composition will efficiently induce protective immunity.

Molldrem et al. have previously demonstrated that cytotoxic T lymphocytes (CTLs) specific for a nine amino acid HLA-A2–restricted peptide, PR1 (derived from proteinase 3), are capable of killing leukemia cells and may contribute to the elimination of CML (3). Utilizing peptide and MHC tetramer technology, they now report the generation of both low- and high-affinity PR1-specific CTLs (PR1-CTLs) and demonstrate a correlation between the presence of high-avidity PR1-CTLs and the clinical responses after IFN-α treatment. While high-avidity PR1-CTLs were identified in patients with cytogenetic remission, suggesting that an effective immune response against CML may depend upon the expansion of these effector cells, only low-avidity PR1-CTLs could be identified in IFN-α-resistant patients. These observations suggest that although tetramer technology allows direct visualization and quantification of antigen-specific T cells and offers a powerful means to study specific T cell populations of interest, such approaches should be used in combination with functional assays to distinguish functional heterogeneity between reactive CD8+ T cells (4) and also to assess their therapeutic potential.

More importantly, the results indicate that specific deletion of peripheral high-avidity CTLs may occur in CML patients when the PR1 leukemia-associated self-antigen is overexpressed, suggesting that antigen dose is a determinant in the control of both CTL lytic potential induction and CTL survival. Nevertheless, it would be important to check whether CTL deletion is specific for PR1-CTLs or may be extended to other antigens expressed by CML tumor cells such as the junctional peptide BCR-ABL or the CML antigen CML28. It is also important to investigate whether the observed deletion is a widespread mechanism or if it is only related to haematological malignancies. Thus, because of the complexity of the CD8+ T cell response that can be elicited by vaccination with synthetic peptides (5), a precise definition of the targeted epitope, the corresponding peptide to be used as immunogen, and the dose of peptide are required to ensure the elicitation of an efficient CD8+ T cell response to tumor antigens. It is very likely that a therapeutic window of antigen concentration presented to T cells may be critically important to

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Nonstandard abbreviations used: chronic myelogenous leukemia (CML); cytotoxic T lymphocyte (CTL); PR1-specific CTL (PR1-CTL).
stimulate optimal immunity to tumors. The data of the current report suggests that with knowledge of how T cells differentiate into high- or low-avidity populations in response to antigen stimulation, we can begin to envision ways by which the T cell response can be manipulated in order to induce the right type of response and subsequently to improve the effectiveness of tumor vaccines utilized in immunotherapy.

The mechanism by which leukemia induces selective deletion of high-avidity PR1-specific T cells remains unknown. In this respect, the underlying mechanism resulting in lymphocyte apoptosis in response to high dose antigen merits exploration. Following this, we may have a better understanding of T cell reactivity and the prevention of T cell deletion, and be able to more judiciously devise specific reagents to interfere with dysregulated apoptosis during vaccination trials. In this regard, several crucial questions remain. Does this peripheral deletion involve activation-induced cell death by suicidal and fratricidal mechanisms involving the CD95 system at early stages after T cell receptor triggering? Which type of cytokines are produced by CD8+ T cells sensitive or resistant to antigen-induced apoptosis? How does IFN-α facilitate the emergence of PR1-CTLs and does it play a role in the regulation of their susceptibility to apoptosis? Does the CD3-ζ chain, reported to be impaired in peripheral blood T cells of patients with CML, play a role in T cell function and deletion in this particular model?

Clearly, this report further confirms that the CTL is only one of many players in the anti-tumor response and that the understanding of the functional interaction between the tumor and effector cells will be a key determinant in the rational approach to future tumor immunotherapy design. Although immune system-based approaches for the treatment of solid tumors have often focused on cytolytic effector cells such as CTLs, increasing evidence from studies in patients and cultured cells has raised the possibility that the induction of a cytotoxic response may be essential, but not sufficient, to control tumor progression (6). In this context, it must be emphasized that the infiltration of tumors by immune-competent T cells (such as CTLs) is not necessarily reflective of an antitumor response and might not even be a favorable indicator. Accumulating evidence points to the likelihood that tumor cell growth in vivo is influenced not only by the ability of CTLs to recognize and respond to the tumor, but also by the susceptibility of tumour cells to host-mediated anti-tumour immune responses (7).

In addition to chemo- and radioresistance, tumor cells can develop resistance to CTL-mediated cytoxic pathways. Besides resistance to perforin (8) and the granzyme B pathway (9), defective death receptor expression or signaling may contribute to the emergence of tumor resistance to cytotoxic cells and the subsequent survival and proliferation of tumor cells in vivo (10). Understanding the interplay between cancer-associated anti-apoptotic proteins may provide a novel avenue for selective manipulation of the sensitivity of cancer cells to cell-mediated cytoxicity, thereby improving the effectiveness of immunotherapy. Thus, immunotherapy approaches might well benefit by incorporating means to overcome tumor cell resistance to killing while generating more specific cytotoxic lymphocytes.

Furthermore, evidence has been provided indicating that tumor-specific T cell responses may prevent tumor cell growth, but may also select tumor antigen-negative and resistant variants in vivo. In a recent report, Schreiber et al. proposed the concept of cancer immunoeediting whereby the immune system not only protects the host against tumor development but also sculpts the immunogenetic phenotype of a developing tumor and can favor the emergence of resistant tumor cell variants (11). In this context, immunotherapy should incorporate the knowledge of the molecular pathways that regulate immunity but also the cross-talk between tumor and effector cells.

Immunotherapy of solid tumors is based on the premise that systemic stimulation of the immune system results in an effective local antitumor reaction. This is a somewhat simplistic view of the relationship between the immune system, the tumor, and its microenvironment (12). There are increasing indications that tumor cells play a crucial role in the control of immune protection (13) and contain many overlapping mechanisms to maintain their functional disorder and evasion of antigenic-specific immunotherapy. Therefore, in parallel to the efforts oriented towards the identification of potential candidate antigens for vaccination, closer attention should be paid to the development of more effective CTL-activating strategies to generate stronger and more sustained specific CTL responses. Clearly, much remains unknown about the functional interaction between the tumor and effector cells and further elucidation of basic immunology might in itself lead to a means of triggering and sustaining an immune response able to mediate the complete destruction of tumor cells. Immunotherapeutic approaches aimed at the induction of anti-tumor cytotoxic responses should also consider the resistance of tumor cells to cell death, since it is conceivable that reconstitution of normal apoptotic control might in itself represent an effective therapeutic strategy. The question remains, if the immune system of the host plays the music, does the tumor system call the tune?

The importance of calcium-dependent signaling in the heart has been appreciated for decades. For example, it is well accepted that intracellular calcium release from the sarcoplasmic reticulum (SR) is required for cardiac muscle contraction. Indeed, with each heart beat the calcium concentration in the cytosol of cardiac myocytes is elevated approximately 10-fold from a resting level of $\sim 100$ nM to $\sim 1$ $\mu$M.

Presumably, a defect in signaling that prevents effective elevation of cytosolic calcium would impair contractility as the contraction of heart muscle is directly determined by the level of calcium elevation during systole. Similarly, a defect in the removal of calcium from the cytosol during diastole would impair cardiac relaxation, which is critically important in that it allows the heart chambers to refill with blood in preparation for the next beat.

**Calcium and heart failure**

Indeed, an attractive hypothesis for the mechanism underlying cardiac muscle dysfunction during heart failure, the leading cause of mortality in the developed world, is that impaired calcium release causes decreased muscle contraction (systolic dysfunction) and defective calcium removal hampers relaxation (diastolic dysfunction). Given that the measurement of cellular calcium is relatively straightforward, the obvious experiment required to address this important issue is to measure calcium in heart muscle cells from failing hearts. Such measurements have been done in isolated cardiomyocytes and, though there is a fair amount of variability in the published reports, the data tend to support the concept of a decrease in SR calcium release and a defect in the termination of release. These results imply that there are presumably defects in SR calcium release in vivo. However, there are no data showing that calcium levels are chronically elevated in heart muscle in failing hearts. Such studies await the development of reliable techniques using calcium indicators with adequate signal to noise ratios and detection systems that will permit measurements of intracellular calcium in the living heart in intact organisms.

**Calcium and cardiac hypertrophy**

Another disease in which perturbations of calcium signaling have been alluded to is cardiac hypertrophy. Indeed, calcium elevation has been proposed as the trigger for cardiac hypertrophic signaling via the calcium-activated phosphatase calcineurin. Much attention has been focused on this possibility as a potential therapeutic target. Indeed, the initial studies identifying a role for calcineurin in hypertrophic signaling in the heart represented a tour de force, combining beautifully designed in vitro and in vivo studies that clearly demonstrated a physiologically important signaling system (1). An intriguing question is whether or not there are any clinical conditions in which one would actually want to treat (i.e. prevent) cardiac hypertrophy. Indeed, outside the cardiology community, cardiac hypertrophy is often lumped together with heart failure as though they are synonymous. This approach seems to add some excitement to the quest for a cure for cardiac hypertrophy by linking it to heart failure which, as mentioned above, is a leading cause of mortality (over 500,000 deaths per year in the US alone). Cardiac hypertrophy, on the other hand, rarely kills anybody and when it does, death is usually due to cardiac arrhythmias, not the hypertrophy per se. Indeed, most deaths linked to cardiac hypertrophy occur in individuals with inherited forms of the disease most often associated with mutations in one of the contractile proteins. These individuals exhibit abnormal pathology, including disorder in the usually well-ordered arrays.

See the related article beginning on page 617.

### Calcium and the heart: a question of life and death

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597