Breaking down the barriers to cancer immunotherapy

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Emerging insights into the mechanisms of activation and negative regulation of innate and adaptive immune cells are providing new opportunities for the development of safe and effective cancer vaccines.

Immunity against microorganisms comprises a highly orchestrated and integrated innate and adaptive immune response that represents a delicate balance between positive and negative regulatory pathways. The nature of tumor immunity is no less complex and is perhaps even more so because of the need to target what are for the most part self antigens. The data presented at the annual meeting sponsored by the Cancer Research Institute, Cancer Vaccines 2005: Barriers, Endpoints and Opportunities, held in New York City in October 2005, described recent advances in understanding the cellular and molecular basis of innate and adaptive immunity to tumors (**Fig. 1**) and opportunities for developing immune-based therapies for cancer (**Fig. 2**).

The first attempts to unleash the power of the immune system against tumors were undertaken by William Coley, who noted in the late 1800s that immune stimulation by microbes could result in the regression of tumors in some patients. Over the next 100 years or so, evidence both for and against the hypothesis of immune surveillance for tumors and for the prospects of developing immune-based therapies for cancer was reported. At Cancer Vaccines 2005, it was apparent that evidence for immune surveillance and immune responses to cancer continues to mount. In the 1950s, it was shown that the immune system could recognize and respond to tumor-associated antigens[1]. However, reports in the 1970s that nude mice do not demonstrate an increase in tumor incidence were considered by some to contradict the hypothesis of tumor immunosurveillance[2]. The hypothesis was 'rejuvenated' by Robert Schreiber (St. Louis, USA), who reported an increase in the incidence of chemically induced tumors, and a wider spectrum of tumors deficient in the tumor suppressor p53, in mice defective in responsiveness to interferon-γ (IFN-γ)[3–5]. Those studies also could explain results obtained using nude mice that, though deficient in lymphocytes, have IFN-γ-producing innate immune cells, such as natural killer (NK) cells. Papers from Mark Smyth (East Melbourne, Australia) have provided further evidence for immune surveillance and have shown that perforin-mediated killing is an important mechanism in tumor immunity[6–8]. At Cancer Vaccines 2005, Smyth presented evidence suggesting perforin functions as a tumor suppressor in mice that have a variety of molecular genetic defects, including *v-abl*-transgenic mice and mice deficient in *mlh1* (a DNA mismatch–repair gene) and *p53* that, when crossed with perforin-deficient mice, produce offspring that have increased plasmacytomas and lymphomas. An important issue is whether these mouse models

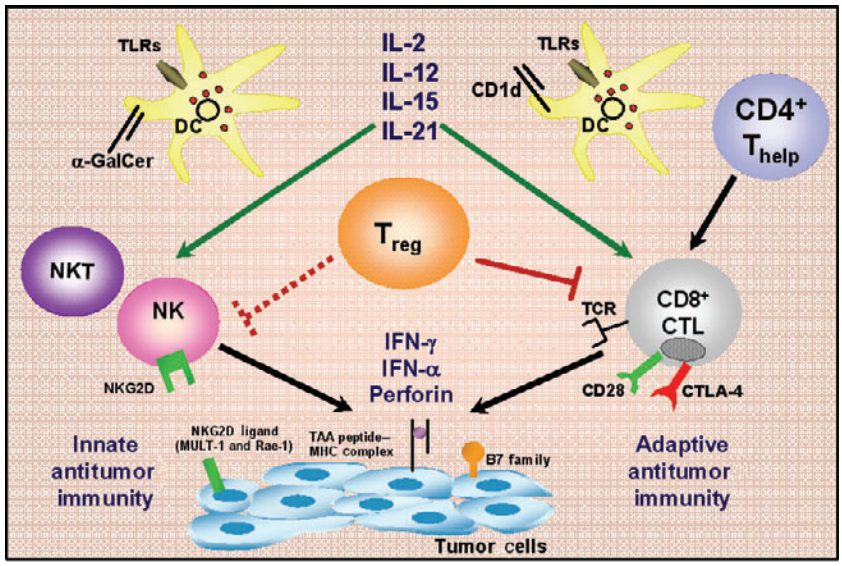

Figure 1 Innate and adaptive immune cell mediated tumoricidal activities. α-GalCer, α-galactosylceramide; DC, dendritic cell; TAA, tumor-associated antigen.

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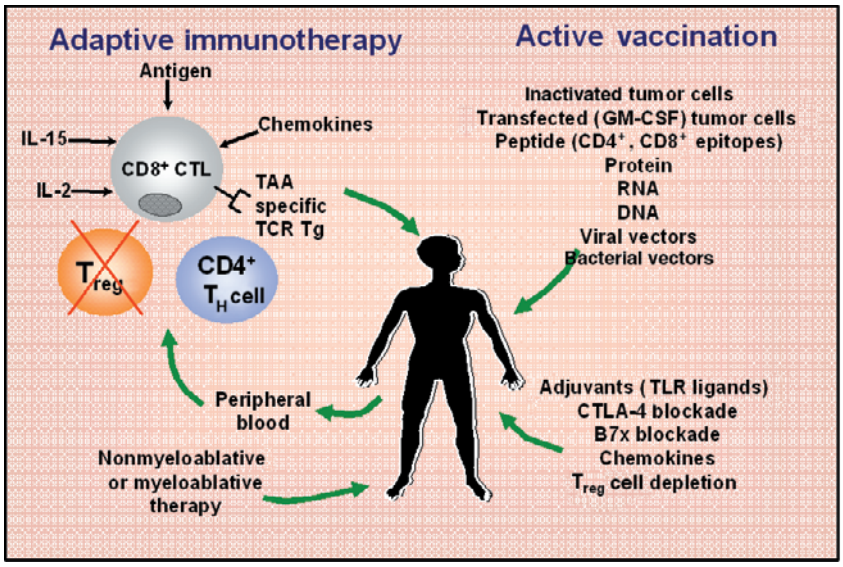

Figure 2 Opportunities for adaptive immunotherapy and cancer vaccines. Tg, transgenic; T_H cell, T helper cell.

relate to human disease. Notably, some patients with lymphoma may have mutations in the gene encoding perforin[9].

The meeting also featured data showing spontaneous and vaccine-induced tumor immunity in patients, as well as correlations between T cell infiltration of tumors and increased patient survival. However, despite some exciting and successful cases, most patients receiving cancer vaccines do not seem to mount an antitumor response, and even in those patients that do so, the response can be transient and less than robust and does not correlate with tumor regression. In isolation, the results of these clinical trials can be considered disappointing. However, responses obtained in some patients in clinical trials using a range of immune-based therapeutic approaches suggest that although the promise of cancer immunotherapy has so far remained mostly elusive, all is not lost. Data presented at this meeting providing insight into why immunotherapy fails in most cases, showing how an effective and integrated immune response can be induced and identifying the targets of adaptive and innate immune cell–mediated tumoricidal activity represent the most compelling reasons for forging ahead.

Innate antitumor immunity

The involvement of Toll-like receptors (TLRs) in the activation of innate immunity is well established. Bruce Beutler (La Jolla, California, USA) presented results of an unbiased mutagenesis screen in mice that is helping to define the signaling pathways that mediate activation of innate immune cells by various members of the TLR family. Several vaccines have incorporated adjuvants such as CpG and imiquimod that activate innate immune cells through specific TLRs. A greater understanding of TLR-mediated signaling will facilitate the development of more effective adjuvants, which may require targeting of particular TLRs or combinations of TLRs or their 'downstream' signaling pathways. New vaccine strategies are incorporating the increasing knowledge in this area. Jorge Galán (New Haven, Connecticut, USA), for example, is exploiting salmonella as a tumor antigen delivery platform based on its purported capacity to simultaneously deliver multiple antigens and activate innate immune cells via TLR-dependent and TLR-independent mechanisms (**Fig. 1**).

Several speakers addressed the molecular mechanism of NK cell–mediated tumor recognition and killing. One focus was on the NKG2D-activating receptor as well as the MULT-1 and Rae-1 families of NKG2D ligands (**Fig. 1**). Smyth reported that treatment with antibody to NKG2D (anti-NKG2D) increases tumors in a fibroscarcoma model to amounts equivalent to those in perforin-deficient mice. Notably, the tumors that develop in perforin-deficient and anti-NKG2D treated mice are mainly positive for the NKG2D ligand Rae-1, whereas tumors from fully immunocompetent mice are heterogeneous in Rae-1 expression. Smyth also demonstrated that perforin-mediated killing is required for rejection of tumors expressing Rae1β. Moreover, interleukin 2 (IL-2), IL-12 and IL-21 all enhance the killing of tumors expressing NKG2D ligand, and the effect of IL-21 is dependent on NKG2D. David Raulet (Berkeley, California) provided compelling studies on the involvement of genotoxicity in inducing NKG2D ligand expression. An array of genotoxic stimuli, such as gamma rays, ion radiation, ultraviolet A and drugs that induce DNA damage such as cisplatin, Ara-C and aphidicolin, but not other common cell stressors such as hypoxia, induce expression of NKG2D ligands of the MULT-1 and Rae-1 families. The studies presented by Raulet indicate involvement of the protein kinase ATM, which is activated in response to double-stranded DNA breaks, and ATR, which mediates replication stress pathways, in NKG2D ligand expression by normal cells in response to genotoxic stimuli as well as the constitutive expression of NKG2D ligands in tumors after DNA damage[10]. Those studies raise interesting issues regarding the possibility that chemotherapy and radiation therapy may lead to enhanced NKG2D ligand expression and increased sensitivity of tumors to NK cell–mediated killing. Alternatively, NKG2D ligand expression on normal cells may divert cytolytic activity away from tumor cells. Although the idea is still open to debate, NKG2D may also be involved in antitumor T cell responses. Andrey Shaw (St. Louis, USA) suggested that Rae-1 can enhance T cell responses to weak TCR agonists. These findings represent important advances in our understanding of NK cell–mediated tumoricidal activity, but NKG2D-independent mechanisms can be expected to be delineated in future studies. Finally, data presented by Ralph Steinman (New York, USA) indicated that mobilization of NKT cells by α-galactosylceramide on dendritic cells at the time of immunization with irradiated J558 plasmacytoma cells results in long-lasting $CD4^+$ and $CD8^+$ T cell immunity.

Barriers and checkpoints

Engagement of the cell surface molecule CTLA-4 can downregulate immune responses by inhibiting T cell proliferation[11,12]. CTLA-4 blockade enhances antitumor responses in mice, either alone, as in immunogenic tumors, or in combination with vaccines, in less immunogenic tumors[12]. CTLA-4 blockade is now being incorporated in clinical trials (**Fig. 2**). Steven Rosenberg (Bethesda, Maryland, USA) reported responses in a small percentage of patients with melanoma and renal cancer treated with anti-CTLA-4 alone. Perhaps not unexpectedly, however, the treatment was associated with grade 3 or 4 autoimmunity that was manageable in most cases with steroids. Although the number of responsive patients is still small, patients who experience complications of autoimmunity are typically those who also show clinical benefit.

Glenn Dranoff (Boston, USA) reported preliminary clinical studies from the Dana-Farber Cancer Institute showing that CTLA-4 blockade results in additional tumor destruction in patients that were previously treated with GM-VAX (autologous tumor cells engineered to express the proinflammatory cytokine GM-CSF). Initial results suggest that CTLA-4 blockade may induce antibodies that interfere with endogenous regulation of NKG2D-mediated killing. James Allison (New York, USA) reported that in a mouse melanoma model, the effects of CTLA-4 blockade are not associated with impaired regulatory T cell–mediated suppression but instead seem to be due to enhanced effector T cell proliferation due to rescue from cell autonomous inhibition.

New opportunities for blockade of checkpoints may emerge from other studies reported by Allison, who described a member of the B7 family, B7x, that is expressed on tumors of epithelial origin. B7x inhibits effector T cell function, including cytolysis, via an unidentified receptor. Its expression pattern suggests that exploring the therapeutic potential of B7x blockade may provide another exciting opportunity in the treatment of epithelium-derived tumors. Studies presented by Leiping Chen (Baltimore, USA) may extend that idea to targeting B7-H1, B7-DC and PD1 cosignaling pathways that have been linked to the regulation of the effector and memory phases of the immune response.

Regulatory T cells (T_{reg} cells) represent another recently appreciated barrier to effective tumor immunity (**Fig. 1**). Hiroshi Shiku (Mie, Japan) addressed the concern that some tumor antigens induce T_{reg} cells as well as helper $CD4^+$ T cells and therefore may have limited utility in cancer vaccines. However, Shiku suggested that these limitations may be overcome by immunization together with cytotoxic T lymphocyte (CTL) epitopes or administration of antibody to glucocorticoid-induced tumor necrosis factor receptor because they block the immunosuppressive activity of T_{reg} cells. Moreover, the function of T_{reg} cells is inhibited by IFN-γ. Alexander Rudensky (Seattle, USA) reported that the transcription factor Foxp3 is essential for T_{reg} cell specification and may therefore prove a useful target for enhancing antitumor immunity.

Recently developed imaging technologies allow direct visualization of the immune response in living cells and in intact tissues. One theme to emerge at this meeting was the importance of the quality and quantity of the immune response to tumor antigens. Shaw discussed data that combine imaging, signal transduction and computational modeling. His studies suggest that the half-life of the TCR-peptide-MHC complex is governed by the quality and quantity of receptor ligand, which thereby determine the balance between TCR triggering and degradation after 'immune synapse' formation. The data further indicate that although synapse formation may limit the response to strong agonists, it enhances the response to poor agonists by prolonging signaling. Applying similar approaches to tumor antigens in the future should provide a new level of understanding of the adaptive immune response to tumors.

Ulrich von Andrian (Boston, USA) used two-photon microscopy to capture images showing the 'kiss of death' delivered by cytolytic T cells to B cells expressing cognate antigen. Contact with TCR-transgenic CTLs induces loss of motility followed by a decrease in fluorescence intensity of adoptively transferred fluorescence-labeled B cell targets during cell death. However, CTL-mediated killing is reduced considerably in the presence of T_{reg} cells. CTLs can still establish contact with B cells and show no defects in their lytic granules, effector molecules or proliferative response in the presence of T_{reg} cells; however, degranulation is inhibited. Selective depletion of T_{reg} cells restores CTL activity. Although those results were obtained in a model system and require verification using nontransgenic T cells and tumor cell targets, the evidence indicates that CTL activity may be readily restored by depletion of T_{reg} cells, providing new opportunities for enhancing antitumor immunity in patients. Indeed, the potential clinical benefit of targeting T_{reg} cells in patients with ovarian cancer was discussed by Tyler Curiel (New Orleans, USA; **Fig. 2**). In a phase I–II dose escalation trial in patients with ovarian cancer, T_{reg} cells have been targeted using ONTAK (IL-2–diptheria toxin fusion protein), which results in a reduction in $CD4^+CD25^+$ T_{reg} cells and an increase in IFN-γ^+CD3^+ T cells in the blood. This trial was not designed to assess clinical efficacy, but future trials that do so will be of considerable interest. Rongfu Wang (Houston, USA) suggested another approach using TLR8 ligands to reverse the suppressive activity of T_{reg} cells.

Others addressed whether $CD4^+$ T cells are required for or enhance the generation of $CD8^+$ T cell responses. Ron Germain (Bethesda, Maryland, USA) described the dynamics of immune cell activity in lymph nodes. Alex Huang, for his group, showed that $CD4^+$ T cells enhance the accumulation of $CD8^+$ T cells in antigen-immunized lymph nodes. The enhanced $CD8^+$ T cell accumulation is prevented by neutralizing antibodies to CCL3 and CCL4. Those data suggest that vaccines designed to trigger $CD4^+$ T cells as well as $CD8^+$ T cells, or incorporating chemokines, may enhance the efficacy of cancer vaccines.

Cell transfer may perhaps be considered the most effective approach to immunotherapy so far (**Fig. 2**). Phil Greenberg (Seattle, USA) and Steven Rosenberg addressed the requirements for the *in vitro* generation of tumor-reactive T cells for use in adaptive immunotherapy. Greenberg demonstrated that 'adding back' $CD4^+$ helper cells during the initial priming event results in better survival and more robust production of IFN-γ, as well as enhanced cloning efficiency of activated $CD8^+$ T cells, compared with cytokines alone. Stephen Schoenberger (La Jolla, California, USA) also reported that the presence of $CD4^+$ T helper cells during the initial priming event endows CTLs with the capacity to expand their populations after secondary stimulation with antigen, whereas CTLs generated in the absence of T cell help undergo TRAIL (tumor necrosis factor–related apoptosis-inducing ligand)–dependent activation-induced cell death after restimulation with antigen. Greenberg also addressed whether it might be possible to 'rescue' self-reactive $CD8^+$ T cells that have been tolerized in the host. Tolerant TCR-transgenic $CD8^+$ T cells were collected from mice expressing the cognate antigen of the transgenic TCR on liver parenchymal cells. By moving the tolerized $CD8^+$ T cells to a nontolerizing environment (by adaptive transfer into wild-type mice), he demonstrated that antigen-reactive $CD8^+$ T cells could be 'rescued'. The tolerant state could also be overcome *in vitro* by treating the cells with IL-15 or high doses of IL-2. An important next step is to determine whether tolerized T cells from patients can be 'rescued' if cultured in defined conditions (for example, in the presence of IL-15) and used as effective 'weapons' in adoptive immunotherapy.

Rosenberg discussed results in patients treated with nonmyeloablative therapy followed by adoptive transfer of diverse populations of tumor-reactive $CD8^+$ T cells whose populations were expanded *in vitro*. About 50% of the patients that underwent adaptive transfer of autologous cells in this study showed objective responses independent of disease bulk and site[13]. However, populations of tumor-reactive cells could not be expanded from all patients and thus these results represent the outcome for a subset of patients. Furthermore, although the responses in some patients were prolonged, in others the responses were short lived. Efficacy was associated with persistence of oligoclonal populations of transferred T cells, whereas persistence of the transferred cells correlated with telomere length. Thus, adaptive immunotherapy continues to hold promise. However, the quality

of the T cell response must be optimized and maintained long term. The present protocols for generating T cells will need to be optimized and the treatments used to 'make room' for a sufficient number of tumor-reactive cells to expand and persist in patients must be modified. Both Greenberg and Rosenberg suggested that introducing high-affinity tumor-reactive TCRs into autologous cells before their reintroduction into the patient may also improve the quality of their antitumor response.

Targets of antitumor immunity

A wealth of tumor-associated antigens have been identified, and several have been used to generate vaccines used in clinical trials. It is not yet clear, however, which antigens will be most effective in inducing antitumor immunity. Tumor immunity may require vaccination with multiple target antigens. Furthermore, it may be critical to target antigens that are essential for cancer cell survival and that are expressed on most cancer cells. The existence of cancer stem cells, which may be the source of cancer cell renewal, indicates the targeted antigens may also need to be expressed on putative cancer stem cells, as discussed by Irving Weissman (Stanford, California, USA). One category of tumor-associated antigens, often referred to as 'CT antigens', was discussed by Andrew Simpson (New York, USA) who reviewed the progress made in defining the genetic regulation of these antigens and initial insights into the potential function of the most commonly expressed CT antigens. Based on the restricted expression of CT antigens on germ cells and cancer cells, it has been proposed that neoplastic transformation may recapitulate gametogenesis[14]. If indeed CT antigens confer some of the essential characteristics of malignancy on tumor cells and are expressed on cancer stem cells, as suggested, CT antigens could potentially be important therapeutic targets.

Monitoring patient antitumor responses

Danila Valmori (New York, USA) and Kunle Odunsi (Buffalo, New York, USA) presented the results of laboratory monitoring of spontaneous as well as vaccine-induced antitumor immune responses in patients in trials done under the auspices of the Cancer Vaccine Collaborative, a joint venture of the Cancer Research Institute and the Ludwig Institute for Cancer Research. Such analyses are providing clues about how to generate the 'most fit' T cell response by taking advantage of the relative benefits of peptide versus protein immunogens, as well as RNA, DNA and viral vector vaccines (**Fig. 2**). The results presented emphasize the potential advantage of using vaccines that will result in the generation of naturally processed peptides and provide both MHC class I– and MHC class II–restricted peptides to engage $CD4^+$ and $CD8^+$ T cells, respectively. A view is also emerging that monitoring tumor reactivity may prove the most informative and that more comprehensive monitoring as a means for assessing the quality of the immune response as a whole is impractical. Furthermore, although laboratory monitoring remains invaluable for learning more about the immune response in patients, many were of the opinion that it is now critical to move toward efficacy trials. In this context, some argue that it is essential to define clinical responses to immunotherapy strictly according to the standard criteria set forth by the World Health Organization and the RECIST criteria (defined by the National Cancer Institute) that are used to assess the efficacy of chemotherapy and radiation therapy regimens. Others are exploring the usefulness of expanding the criteria used to define clinical efficacy to accommodate ideas associated with immunotherapy, such as disease stabilization.

In summary, although cancer vaccines so far have shown limited success, the barriers to inducing effective tumor immunity are rapidly being defined. Advances in this area will continue to provide new opportunities for overcoming these barriers. Adaptive immunotherapy has arguably proven more successful, although the cellular and molecular bases for failure in most patients are less well understood. If the last proves to be an issue of 'too little of a good thing' even in a setting in which massive numbers of tumor-reactive cells are adoptively transferred, then it may be difficult to reach the ultimate goal with this approach. However, if it is because of the quality of the tumor reactivity of the cells or the presence of inhibitory populations, for example, then this approach likely holds much promise. The data presented at this meeting and from future studies should provide the foundation for designing the next generation of cancer vaccines that circumvent the natural barriers and checkpoints designed to protect against autoimmunity and chronic inflammation. The findings presented at this meeting also give much reason to believe that such vaccines will induce robust antitumor immune responses that may suffice to control the survival and growth of tumors. Future challenges include investigating whether immunotherapy is most effective as a combinatorial therapy with surgery, chemotherapy and/or radiation therapy. It was evident from the meeting that continuing the iterative process of combining basic research with patient-oriented research and clinical trials will greatly facilitate the development of cancer immunotherapy.

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