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Immunologic approaches to the treatment and prevention of cancer

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Immunology is advancing at a rapid rate and an increased number of investigators are providing important contributions to the field. Greater than 250 abstracts dealing directly with tumor immunology were presented at the 92nd Annual Meeting of the American Association of Cancer Research in New Orleans, LA, USA. All facets of tumor immunology were represented by excellent abstracts addressing dendritic cells, mechanisms of effective tumor immunity, immune defects in cancer patients, tumor antigens, cancer vaccines, and cancer immunotherapies.

Introduction
It was estimated that between 10,000 and 15,000 people attended the 2001 Annual Meeting of the American Association of Cancer Research (AACR). The diversified AACR has a strong tumor immunology membership, and this group submitted more than 250 abstracts for the meeting. Most of the tumor immunology abstracts could be categorized using the following descriptors: (i) dendritic cells; (ii) effector cells; (iii) cytokines; (iv) mechanisms of tumor immunity; (v) tumor antigens; and, (vi) immunotherapy. In general, most of the abstracts were of excellent quality, and this review highlights some of those presentations that are of particular importance and that cover some of the more important themes in tumor immunology.

Dendritic cells
Tumors evade immune recognition by preventing differentiation of dendritic cells (DCs). P Cheng and colleagues (Vanderbilt Cancer Center, USA) have identified a protein, myeloid-related protein 8 (MRP8), that may be involved in DC differentiation and tumor evasion. During normal maturation, DCs downregulate this protein. Immunosuppressive factors secreted by the tumor during culture block both DC differentiation and MRP8 downregulation. Increased MRP8 levels resulting from MRP8 transfection into DCs prevents differentiation. These results suggest that MRP8 may be a target of immunosuppressive factors produced by tumor cells.

Fusing DCs with tumor cell lines has been a common approach to generation of tumor-specific T-cells in vitro and in vivo. However, a limitation with this approach is that tumor cell lines are often difficult to establish and grow in vitro. LM Holmes and colleagues (Cancer Center of Greenville Hospital System, USA) have developed a new strategy of deriving instant dendritomas using freshly isolated primary tumor cells and peripheral blood mononuclear cell (PBMC)-derived DCs. Tumor cells are labeled with a red dye and DCs are labeled with a green dye. Following fusion, the resulting dendritomas are isolated by FACS. The dendritomas were found to express key antigen-presentation molecules, such as HLA class I and II. In vitro analysis shows that dendritomas generate tumor-specific T-cells that lyse primary tumor and secrete interferon (IFN)-γ. This strategy may be practical for many tumors in which sufficient tumor cells can be isolated, such as melanoma.

Effector cells
Claudia Rossig and colleagues (Baylor College of Medicine, USA) have taken a unique approach to generating tumor-reactive T-cells in vitro. A major problem with tumor antigen-specific T-cells manipulated in vivo is that activity is often low and the T-cells have a limited lifespan. Dr Rossig explained that Epstein Barr Virus (EBV)-specific T-cells are active in vivo for long periods of time due to continued antigen presence. The group transfected EBV-specific cytotoxic T-cell lines with a chimeric receptor protein consisting of the T-cell receptor β-chain fused to the variable domains of the neuroblastoma ganglioside antigen, GD2. The effector cell lines recognized neuroblastoma cells with IFNγ release and cytolysis. Responsiveness toward autologous B-lymphoblastic cell lines was still maintained, despite neuroblastoma responsiveness. Future studies are to be directed at the clinical, toxicological and immunological effects following infusion.

The magnitude of the tumor response is carefully controlled by immunoregulatory and suppressor cells. H Tanaka and colleagues (Cleveland Clinic Foundation, USA) described a regulatory role of CD25+ CD4+ T-cells. Anti-CD25 was used to deplete CD25+ CD4+ T-cells from regional tumor-draining lymph nodes. The remaining lymph node cells had >3-fold increased antitumor activity compared with lymph node cells without CD25 depletion. These results suggest that CD25+ CD4+ T-cells exhibit a suppressive regulatory role in response to tumor, and demonstrate that careful in vitro manipulation of the tumor-specific T-cells can result in an effective lymphocyte population for potential use in adoptive T-cell therapy.

Cytokines
Many cytokines having important antitumor attributes have been identified and characterized over the past decade. More recently, the importance of interleukin (IL)-18 in the antitumor response has been described. Zdenka Jonak and colleagues (GlaxoSmithKline plc, USA) are studying the role of IL-18, and reported that administration of IL-18 in both early- and advanced-stage murine tumor models resulted in tumor regression and the induction of memory cytotoxic T-
Mechanisms of tumor immunity

Dmitry Gabrilovich and colleagues (H Lee Moffitt Cancer Center, University of South Florida, USA) have previously shown that the number and function of DCs in the peripheral blood was dramatically reduced in cancer patients and found that this was linked to an accumulation of immature myeloid cells in the peripheral blood. Upon further analysis, they observed that approximately one-third of immature myeloid cells were immature macrophages and DCs. The addition of these immature cells into PBMC cultures greatly reduced antigen-specific recall responses to tetanus toxoid, as assessed by proliferation assays. Furthermore, mature DCs derived in the peripheral blood had increased ability to stimulate allogeneic T-cell responses when the immature myeloid precursors were removed. The authors suggested that causing accumulation of these immature myeloid cells may be a mechanism by which tumor cells evade detection by the immune system.

Tumor antigens

The identification of HLA-A and HLA-B peptides is important for modulating the cytolytic T-cell response to tumors. DG McNeel and colleagues (University of Washington, USA) identified 11 HLA-A2 binding peptides contained within the prostate cancer antigen, prostatic acid phosphatase (PAP). Natural immunity to these peptides was examined in 18 men with prostate cancer and 10 normal male individuals. A significant proportion of both normal individuals and prostate cancer patients recognized three of the HLA-A2 epitopes (PAP16-20, PAP12-20, and PAP35-40). Dr McNeel explained that the presence of naturally occurring immunity to these peptides suggests that they could be target epitopes for prostate cancer vaccines. Future studies will question the natural presentation of these antigens on prostate cancer cell lines.

KD Amos and colleagues (Washington University School of Medicine, USA) investigated seven HLA-A2 binding peptides contained within the breast tumor-associated antigen, mammoglobin A. Mammoglobin A is overexpressed in >80% of breast tumors. Two of the HLA-A2 peptides, FMQILYDSSL and KLLMVLMLA, were found to elicit CD8 T-cells in three out of seven HLA-A2+ normal volunteers. Dr Amos indicated that he is continuing to study the natural response to these peptides in order to determine if mammoglobin could be a potential target for a breast cancer vaccine. The continued discovery of tumor antigen HLA class I peptides will greatly facilitate the development of polyantigenic peptide-based cancer vaccines. Because CD8 T-cells require a concomitant T-cell helper, interest in identifying HLA class II epitopes of tumor antigens has increased. In one important example, Dr Chikamatsu and colleagues (National Cancer Institute, USA) identified an HLA-DR4 epitope, p53101234, of wild type p53. A bulk T-cell line, specific for p53101234, was generated from the peripheral blood of a healthy volunteer. The cell line reacted with HLA-DR4+ tumor cell lines, indicating natural presentation of this epitope. This could be an important addition to peptide-based immunotherapies targeting p53.

Immunotherapy

Joachim Schultz (Dana-Farber Cancer Institute Inc, USA) presented a seminar entitled 'On the way to a universal tumor vaccine'. Dr Schultz and colleagues are investigating the feasibility of developing a universal tumor vaccine that is effective at generating immunity regardless of the HLA genotype of the cancer patient. The ideal tumor antigen must have certain characteristics, for example they have to be: (i) expressed on all or a large majority of tumors regardless of origin; (ii) hidden from tolerogenic mechanisms; (iii) necessary for tumor growth and/or survival with no evidence of antigen-loss variants; and, (iv) have processed epitopes that can be recognized by both CD4 and CD8 T-cells. Dr Schultz and his colleagues have potentially identified one such tumor antigen, telomerase, and are investigating the therapeutic potential of UTAG-1, a human telomerase reverse transcriptase-specific peptide. Telomerase is a ribonucleic acid that maintains telomere length stability in all cancer cells. Telomerase confers immortality on cancer cells and immortal germ line cells; telomere shortening is associated with cell senescence. In preliminary murine studies, immunization against telomerase resulted in the ability of the mice to reject tumor. Epitopes restricted by both HLA-A2 and HLA-A3 have been identified and phase I clinical trials are being planned to assess safety of immunizing late-stage, high-risk cancer patients against telomerase.

Gopi Shankar and colleagues (Northwest Biotherapeutics Inc, USA) have conducted a phase I/II clinical trial evaluating the safety, immunogenicity and clinical response of CaPvax (Northwest Biotherapeutics Inc) in prostate cancer patients. CaPvax for prostate cancer consists of autologous DCs loaded with purified prostate-specific membrane antigen (PMSA). Vaccination was safe with negligible adverse reactions. A total of 91% (10/11) of patients had increased PMSA-specific antibodies following vaccination, while 80% developed varying T-cell responses to the vaccine. Although patient numbers were too small for statistical conclusion, 80% (four out of five) patients that generated high levels of immunity to the vaccine had
stabilized disease compared to 25% of the patients (one of four) that generated low levels of cellular immunity. Dr Shankar indicated that Northwest Biotherapeutics is interested in taking this immunotherapy to a phase III clinical trial to further ascertain clinical efficacy in the therapeutic setting.

An important issue for tumor vaccinologists is the threat of generating antigen-loss variants following immunization, which would circumvent the potential protective or curative effects of a vaccine. X Kang and colleagues (ImClone Systems Inc, USA) have designed and constructed a novel chimeric protein, hTRPx3, consisting of the human TRP-1, TRP-2 and tyrosinase proteins. hTRPx3 was tested in a preclinical model for the generation of immune responses. Both antibody and T-cell responses were elicited in mice following immunization. The vaccine was effective with many adjuvant preparations and could protect mice from tumor challenge.

Suppression of antigen-presenting cell activity in cancer patients is common. Sylvia Kiertscher and colleagues (University of California Los Angeles School of Medicine, USA) hypothesized that increasing the numbers of functional circulating antigen-presenting cells in cancer patients may improve endogenous antitumor immunity. The use of GM-CSF and IL-4 in combination resulted in generation of DCs in vitro. A phase I dose-escalation study, administering both GM-CSF and IL-4, was conducted in cancer patients with treatment-resistant disease and patients who refused treatment. A total of 21 patients were treated for 7 days in six cohorts, with varying doses of IL-4 (0.5 to 6 mg/kg/day) and a constant dose of GM-CSF (2.5 mg/kg/day). Following treatment, it was observed that the DC1 population (Lin-, DR+, CD11c+) increased substantially in the peripheral blood from 8 x 10^6 cells/ml to 8.7 x 10^7 cells/ml. This treatment also resulted in the increased expression of HLA-DR on the DC1. These results suggest that cytokine treatment may improve tumor immune responses directly in vivo through enhanced antigen presentation. Although regression of established tumor was limited with this approach, cytokine therapy coupled with vaccination may improve tumor-specific immunity.

In recent years, the use of novel antibody constructs to passively treat malignancy has increased. Oliver Press and colleagues (University of Washington, USA) reported results of a clinical trial evaluating the efficacy of radiiodinated anti-CD20 (I^131 anti-CD20; NeoRx) for the treatment of B-cell lymphomas. CD20 is expressed on >95% of B-cell lymphomas. In patients with newly diagnosed B-cell lymphoma, 63% responded to I^131 anti-CD20 therapy with a complete response, while 34% responded with a partial response. A total of 34% of patients with relapse disease had a complete response and 37% had a partial response. Patients with refractory disease responded less well, with 17% achieving a complete response and 48% a partial response.

### Summary

The field of tumor immunology is as diverse as the malignancies themselves. Currently, immunological strategies will likely have to be tailored to the type of malignancy, as well as the characteristics of the patient, until a universal treatment or vaccination method is established. Is telomerase the answer? Cancer vaccines will likely be more effective for the prevention of relapse or possibly even the de novo development of new tumors. Strategies for effective prophylactic vaccination are emerging and include peptide-, protein- and DNA-based vaccines targeting tumor-associated antigens.

Although it is now understood that cancer patients demonstrate immunosuppression, this may be overcome by using vaccine with the appropriate cytokines, such as GM-CSF and IL-4 (ie, combination approaches). For existing malignancies, a wealth of cellular- and antibody-based strategies exists which can be individually tailored to a specific tumor. An important example of this is the use of I^131 anti-CD20 antibody to target newly diagnosed B-lymphomas. Active cellular-based strategies, rather than passive antibody-based strategies, such as adoptive T-cell or DC therapy may likely be more effective for solid tumors, unless the tumor burden is substantially reduced. The use of cytokines to modulate and sustain the immune response, as well as correct immune defects in cancer patients, is becoming an increasingly important issue.

Finally, the evolution of antigen-loss variants, following effective vaccination or therapy, points to the need of polyantigen vaccines and therapies. ImClone’s development of a chimeric multi-antigen protein vaccine to target melanoma provides an excellent example. The continued identification and characterization of tumor-associated antigens paves the way for development of poly-antigen strategies.