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REVIEW

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A primer on cancer immunology and immunotherapy

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Abstract The role of immunity in cancer has been abundantly demonstrated in murine tumor models as well as in man. Induction of clinically effective antitumor immune responses, based on this information, in patients with cancer however, remains elusive. This is not because tumors lack recognizable antigens [in fact there is evidence that there are thousands of potential novel targets in each tumor cell] but rather due to the fact that the induction of responses is not adequate nor particularly well understood. Tumors seem to be rather effective at limiting immune responses. Many of the molecularly defined antigens that have been detected on tumor cells are derived from self-proteins and as such are subject to tolerizing mechanisms. Such tumors have also developed escape mechanisms capable of evading or suppressing immune responses. Understanding the role of dendritic cells during the effector phase of the immune response and the complex interactions of stromal, immune, and tumor cells in the tumor microenvironment represent the next challenges to be understood for tumor immunology.

Keywords Primer · Tumor immunology · Immunotherapy

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Introduction

The immune system is capable of mounting immune responses and this knowledge derives from recent findings demonstrating (1) the molecular identification of tumor antigens recognized by T cells and antibodies; (2) the identification of molecular changes associated with antigen and major histocompatibility complex molecule (both allelic and global) losses observed in many tumors; (3) the correlation of immune infiltrates with NK, DC, and T cells and their association with improved prognosis in most tumors; (4) the responsiveness of some patients with melanoma, renal cell cancer, and lymphoma to immunotherapy which either stimulates (IL-2, IFN-α) an immune response or inhibits suppressor or inhibitory pathways (CTLA-4 antibody, cyclooxygenase inhibition, etc.); and (5) the adoptive transfer of immune cells or antibodies mediating antitumor effects.

The ability to advance the field of tumor immunology and direct more successful therapies will require revising many of these precepts in the context of antigen discovery, antigen delivery to immune cells, cell therapy, and measurement of the immune response as well as the identification of suitable biomarkers and surrogates of tumor responses. The compendium of articles in this issue of *Cancer Immunology and Immunotherapy* was authored by lecturers who presented various topics at a summer course in Greece. The increase in interest in tumor immunology has propelled this field, coincident with a deeper understanding of molecularly defined targets, cytokines, and the receptors and counter-receptors engaged in cellular responses.

Characterization of tumor antigens

R. Rees (Nottingham Trent University) and **D. Jaeger** (Germany) reported on the serological analysis of tumor antigens by recombinant cDNA expression cloning (SEREX). Their groups have identified a number of genes overexpressed or specific to malignant tissues. One

of these, MTA1 (metastasis tumor associated antigen 1) encodes a 715 amino acid protein that is localized in the nucleus and is involved in chromatin remodeling associated with tumor cell invasion and metastasis particularly in advanced breast cancer. TACCA, T21, and T128 genes also identified by the same groups, encode nuclear proteins regulating the cell cycle. So far they have determined MTA1 sequences with HLA-A2-binding motifs, which were proven to be immunogenic in HLA-A2 transgenic mice generating specific CTLs.

F. Le Naour (Paris) reported on tumor antigens identified by proteomics using 2-D gel electrophoresis of proteins isolated from a tumor cell line from a patient with liver cancer. The spot profile from this cell line was compared with profiles from primary tumor cells from 37 patients with liver cancer. Thirteen spots were identical, defining several proteins including carveticulin, betaglobulin, hsp60, cytokeratins 8 and 18, creatine kinase-B, F1-ATD synthase, and Sm23. He also discussed mainly the role of the tetraspanin cluster of antigens in the metastatic potential of tumor cells, as well as the profile of antigens in the tumor tetraspanins, and postulated that high levels of tetraspanin expression is a good prognostic factor for breast, lung, colon, and pancreatic cancers. The tetraspanin families may also play a role in tumor antigen uptake and presentation.

C. Castelli (Milan) presented recent data on the functional role of heat shock proteins (HSPs) on tumor immunity. HSPs are a family of chaperone proteins assisting the correct folding of proteins after new synthesis or under stress conditions, and in tumor-free conditions they play a role in regulating homeostasis. There is increased production of HSPs in response to stress mediated by bacterial infections, heat, growth of tumor cells, etc. Castelli addressed the role of several HSPs, including HSP70 and GP96, in potentiating antitumor responses against transplanted tumors in animals and also referred to current clinical trials in patients with melanoma vaccinated with gp100 peptides and HSP as adjuvant. Patients developed T-cell immunity to gp100 but also to MART-1/Melan-A due to mechanisms underlying epitope spreading. Intriguing responses in patients with colorectal cancer were observed.

H.-G. Rammensee (Tübingen) described new technologies utilizing proteomics and genomics for defining tumor antigens to be used as cancer vaccines. In this process, MHC molecules are highly purified from the tumor cells (e.g., the renal cell carcinoma line RCC01) and peptides are eluted by mild acid treatment followed by run mass spectrometry. In this way, 70 different peptides may be identified from HLA-A2 molecules belonging to various proteins including the MET oncoprotein keratin 18, adipophilin ADFP, cyclin D1/PRAD1, LMP2, and NS1-BP. MET peptides were used for sensitizing T cells in vitro, which subsequently were shown to kill renal tumor cells. The same peptides are now being used in preclinical trials as preventive and protective vaccines.

D. Schadendorf (Mannheim) analyzed the various methodologies to define tumor antigens. These different approaches led to the identification of a still growing number of antigenic peptides providing the basis for the development of new active and passive immunotherapies and for the monitoring of spontaneous and vaccine-induced T-cell responses. Some of these antigens are now used in different clinical protocols.

Antitumor cytotoxic effector cells

C.N. Baxevanis (Athens) gave an overview on HER-2/*neu* biology and discussed recent advances in understanding how anti-HER-2/*neu* responses are developed in humans and how these contribute to the destruction of HER tumor cells overexpressing this oncoprotein. He placed emphasis on the role of both classical CTL-recognizing HER-2/*neu* MHC class I–restricted peptides and T_H sensitized to HLA-DR-restricted peptides synergistically inducing effective anti-HER-2/*neu* antibodies. In addition, he analyzed the important role of anti-HER-2/*neu* antibodies (Herceptin) in directly mediating antitumor effects or promoting NK and CTL responses against HER-2/*neu*–expressing tumor cell lines.

M. Papamichail (Athens) analyzed several crucial aspects of the biology of NK and NKT cells involving signaling mediating by KIR or activating receptors and targeting of tumor cells by these protein structures. He discussed novel differentiation pathways of CD14 stem cells under the influence of Flt3 and IL-15 that result in the generation of mature NK cells with remarkable cytotoxic activities. Several cytokines, including IL-12 and IL-18, are potent stimulators of NKT cells, which upon adoptive transfer were shown to confer protection on SCID mice against the growth of human tumor xenografts. Such cytokine-activated NKT cells were demonstrated to collaborate with host NK cells for generating efficient antitumor response. He addressed the role of NK cells or NK cell lines (NK92) in the cellular immunotherapy of solid tumors.

G. Forni (Turin) described the progress of mammary tumor development in HER-2/*neu* transgenic mice and new technologies for effective therapeutic vaccination (electromediated with HER-2/*neu* peptides). He pointed out the finding that mice cured from their tumors had increased levels of IFN-γ and HER-2/*neu*–specific antibodies in their sera. He concluded that immunotherapy combining both arms of immunity (i.e., cellular and humoral) supported by cytokines (i.e., IL-12, IFN-γ, or GM-CSF) might be of benefit to patients with HER-2/*neu*–overexpressing tumors.

C. Melief (Leiden) reviewed viral antigens that are associated with different types of cancer. He discussed recent views on the collaborative interactions of T_H cells with DCs for enhanced sensitization of cytotoxic effector T cells against viral tumor peptides. In this scenario T_H cells provide signals via CD40L/CD40 interaction to drive DCs to a mature stage that is phenotypically and

functionally distinct from immature DCs. Such mature DCs not only potentiate cytotoxic respones but also enhance CD8+ T-cell survival. Preclinical models demonstrated that local CD40 ligation leads to systemic, therapeutic antitumor immunity and cross-presentation of tumor antigens by host dendritic cells. Local administration of anti-CD40 antibody has shown significant antitumor effects in murine models with less toxicity.

P. Romero (Lausanne) presented data on monitoring tumor-antigen specific T-cell responses and T-cell frequencies in melanoma patients undergoing immunotherapy through vaccination with melanoma peptides. He mostly focused on the use of microproliferation and microcytotoxicity assays for functional assessment of T_H and CTL, respectively, and ELISpot assays for quantitating IFN-γ production. He also described the production of new technology tetramers for estimating frequencies of peptide-specific T cells in the blood before and after vaccination. Perhaps the most interesting observation was that the immune response assessed with tumor was associated with defects primarily in interferon gamma production.

Preclinical and clinical studies of cancer immunotherapy

J. Vakkila (Helsinki) reviewed the distinct biology of primary genomic instability leading to primarily pediatric tumors, in distinction from the secondary genomic instability associated with adult neoplasms. Thankfully rare, pediatric tumors are associated with substantial responsiveness and cure to aggressive combination chemotherapy regimens and an unusually sparse immune [NK, DC, T-cell] infiltrate, substantially separating them from the pathologic appearance of tumors arising in adults. Efforts to monitor signaling molecules in circulating cells in the peripheral blood were reviewed and presented focusing on the critical role of NFκB.

W. Wels (Frankfurt am Main) described the construction of an anti-HER-2/*neu*–recognizing single chain Fv (scFv) from a murine hybridoma. This scFv was first tested for staining HER-2/*neu*$^+$ tumor cell lines and then was linked to a signaling unit (i.e., the γ chain of the Fcγ receptor or the ζ chain of the TCR). The chimeric construct was subsequently transduced into the NK92 cell line, which specifically recognized and lysed HER-2/*neu*$^+$ tumor cells in vitro and in preclinical settings in animals that had undergone xenotransplantation with HER-2/*neu*$^+$ human cell lines. He also referred to phase I clinical studies that have been initiated with the transduced NK92 cells.

K.-M. Debatin (Ulm) analyzed mechanisms that lead to apoptosis and placed emphasis on key-elements that initiate proapoptotic or antiapoptotic mechanisms in tumor cells. He claimed that regulation of expression of such molecules may be of interest in suppressing tumor growth in preclinical settings. He overviewed mechanisms that result in the suppression of antiapoptotic enzymes by small DNA interference, antisense oligonucleotides and antibody inhibition. Finally, he presented data on the in vivo antitumor effects of proapoptotic molecules encapsulated into cationic liposomes.

M. Lotze (Pittsburgh) reviewed intriguing data suggesting that tumors were enhanced in their growth by the nuclear factor, HMGB1. This molecule is released from necrotic, but not apoptotic death and appears to potentiate inflammation in concert with other proinflammatory cytokines including IL-1 and IL-2. It, like uric acid, may be a unique measure of necrotic cell death promoting and enhancing immune reactivity in cancers. The use of systemic cytokines as approved therapies in melanoma [IL-2 and alpha interferon] as well as the non approved factors, IL-12 and IL-18 were reviewed in some detail as gene and cytokine therapies.

P. Coulie (Brussels) discussed the current status of peptide and protein vaccines targeting the tumor germline antigens that he and his group have identified in melanoma. Intriguingly, the robust response in a small subset of patients was associated with unique clonotyic T-cells reproducibly identified over time and from biopsied lesions, often to epitopes distinct from the immunizing peptide. Most provocatively, assessment of whole tumor reactivity was observed to exceed by several orders of magnitude that found to individual peptides and to most often precede vaccination. The notion that vaccines were helpful in modifying disease in only a small, $<20\%$ set of patients almost independent of the route or method of vaccination was discussed.

F. Farzenah (London) reviewed functional approaches to control the insertion of certain genes into tumor cells using viral vectors. He concentrated on the functional analysis of genes controlling important cellular processes such as cell survival and apoptosis. Also he discussed the development of novel strategies for the expression of multiple genes as fusion proteins that are subsequently processed into their biologically active constituents.

E. Angevin (Villejuif) discussed the composition of exosomes, their production and isolation, and their potential application in cancer immunotherapy. He also presented preclinical and clinical data on their use, especially in melanoma patients.

Regulation of antitumor immunity

T. Whiteside (USA) provided essential information on the biology of DCs. She analyzed phenotypically and functionally diverse subsets of DCs (i.e., plasmacytoid vs myeloid). The functional differences between Langerhans cells, intestinal DCs, follicular DCs, and plasmacytoid DCs in T-cell areas of lymph nodes and thymus were also discussed. The role of DCs in angiogenesis via the production of angiogenic factors induced by LPS and CD40L was associated with the functional role of DCs in tumor immunity. There was detailed analysis of the role of DCs as vehicles for peptide vaccination studies both in preclinical and clinical trials.

G. Pawelec (Tübingen) provided an overview of the mechanisms that may exist for the escape of tumors from immunosurveillance. These included failure to express MHC antigen, decrease of, and heterogeneity of expression of, tumor antigens, failure of the tumor cells and/or host DCs to express adhesion or accessory molecules required for T-cell interaction, secretion of immunosuppressive substances, induction of immune nonresponsiveness via anergy or cloncal deletion of responding cells, induction of suppressor cells, changes in T-cell signal transduction molecules, and prevention of tumor apoptosis by mechanisms blocking function of proapoptotic molecules. He also discussed the possibility of the existence of tumor cell progenitors among stem cells, limiting the effectiveness of therapies targeting the more distal and more numerous recognizable tumor non-stem cells.

Conclusions

Advances in the next third of the century will require integration of the fundamental precepts developed in the last two. An increasing focus on the tumor itself, in spite of the complexities and difficulties involved in its evaluation, will likely be required to advance beyond the molecular phenomenology of today's clinical and preclinical research. Understanding how tumors are "addicted to death" will be elucidated by taking a systems biology approach, integrating the full complexity of biologic mechanisms and cells involved in the process of tissue damage and repair. Predictions of the future are always problematic but it would seem to us that the three areas of major advance are likely to be (1) identifying the "functional unit" of effectors within the tumor microenvironment to include not only NK and T cells but also the epithelial and stromal cells within which they reside, (2) a deeper understanding of antigen-presenting cells in the effector phase of the immune response, and (3) understanding how tumors evolve during inflammation. We have already observed important inroads against cancer with anti-inflammatory agents including nonsteroidal agents and immunizations preventing viral oncogenesis. As is likely true for most advances in public health, prevention of cancer is likely to be the most effective and least expensive strategy. Until we are in a position to effect these changes, we will need to find alternative means to identify early neoplastic tests and to control them, perhaps with the biologic reagents described here.

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