International Meeting on Cancer Vaccines: How Can We Enhance Efficacy of Therapeutic Vaccines?

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Abstract

The major aims of the International Meeting on Cancer Vaccines were to review the state-of-the-art research on cancer vaccines, to compare different experimental approaches of therapeutic vaccination and to discuss critical issues and perspectives. The results from recent clinical trials in patients treated with different types of cancer vaccines were presented. Reasons for the limited response and possible modalities for enhancing efficacy of therapeutic vaccines were subjects of major discussion. A consensus was achieved on the need of combining cancer vaccines with other anticancer treatments. Of note, evidence stemming from studies in animal models pointed out new rationales for a selective combination of cancer vaccines with chemotherapy. In addition, some main presentations focused on new adjuvants (CpG oligonucleotides) and on the role of cytokines (i.e., type I IFN, interleukin 12, and interleukin 15) in promoting an antitumor immune response to vaccines. A considerable attention was given to regulatory T cells and to strategies for suppressing their function, thus enhancing vaccine efficacy. An entire session was devoted to the use of dendritic cells for the development of cancer vaccines. The results of clinical studies and the advantages of using new modalities for preparing dendritic cell-based vaccines were discussed.

Therapeutic Vaccines against Cancer: Lessons Learned from Clinical Trials

The large majority of clinical studies on cancer vaccines have been performed in patients with advanced disease. Giorgio Parmiani pointed out that in the phase I–II trials of vaccination of stage IV melanoma patients with peptide-based vaccines, only a minority (12%) of the treated patients experienced a clinical response; the more recent use of autologous dendritic cells (DCs) loaded with peptides or tumor lysates, although increasing the frequency of patients developing a vaccine-specific T-cell response as compared with peptide-based vaccination, has not yet led to a significant improvement in clinical response. Thus far, the only two phase III trials showing a statistically significant clinical benefit of vaccination were based on the use of autologous tumor cells or lysates. This might suggest the advantage of cancer vaccine formulations in which the full antigenic repertoire of the tumor is represented and, particularly, unique tumor antigens. Parmiani referred to the use of heat shock proteins derived from the autologous tumor; he reported that the activation and expansion of tumor-reactive CD8+ T cells was observed in ~50% of melanoma or colon carcinoma patients vaccinated with autologous tumor-derived heat shock protein gp96 and that this response was associated with a favorable disease course after vaccination. However, the rate of clinical response (18%) was not better than that generally achieved with peptide anticancer vaccination. Interestingly, the heat shock protein gp96 vaccine induced in vivo both an expansion and a significant increase of activity of natural killer cells (Licia Rivoltini). Similarly, an expansion and enhanced effector function of natural killer cells was observed in melanoma patients vaccinated with autologous DC-derived exosomes pulsed with MAGE-3 peptides (Laurence Zitvogel). As discussed by Alexander Knuth, the therapeutic efficacy of cancer vaccines may depend on their capability of stimulating integrated immune responses involving CD4+ and CD8+ T-cell and B-cell responses. He reported that the vaccination with recombinant vaccinia- and fowlpox-NY-ESO-1 constructs strongly promoted NY-ESO-1-specific CD8+ T-cell responses that, in some patients, were accompanied by the induction of antibodies, as well as of CD4+ T-cell responses to NY-ESO-1. Whether these integrated responses to NY-ESO-1 can translate into major clinical responses will be tested in future trials.

A series of lectures addressed the reasons of the two paradoxical findings observed in patients subjected to anticancer vaccination: (1) the occurrence of clinical responses in patients with low frequencies of antivaccine T cells; and (2) the lack of clinical response despite a good induction of tumor-specific T cells. A possible explanation of how a low frequency of antivaccine CTLs can correlate with a clinical response was provided by Pierre G. Coulié. The results of a detailed analysis of the CTL responses in melanoma patients vaccinated against the MAGE-3 antigens suggest that antivaccine CTLs exert their main effect by triggering, as a consequence of tumor destruction, the stimulation of other antitumor CTL precursors that undergo a massive activation at the tumor site because of antigen abundance. A number of presentations stressed the importance of the direct ex vivo analysis of the phenotypic and functional properties of T cells in the tumor microenvironment to understand the reasons of the discrepancy between the presence in the circulation of tumor-specific T cells and the lack of tumor regression, a most frequent finding in patients subjected to anticancer vaccination. Defects in the effector function and/or differentiation of tumor-specific T cells in tumor-invaded lymph nodes or at the tumor site were reported by several speakers. Francesco M. Marincola reported the results of the functional analysis and genomic profiling, indicating that vaccine-induced T cells are characterized by a quiescent cytotoxic T-cell phenotype not capable of effector function unless a secondary stimulation is provided. Interestingly, the functional profiling in tumors of the effect of systemic interleukin (IL)-2 therapy added to active immunization suggested that this cytokine induces or enhances the effector function of vaccination-induced T cells by causing an acute inflammatory process at the tumor site.

How can we obtain a major improvement of the clinical response to cancer vaccines? The large majority of contributions presented at the meeting were focused on strategies for enhancing efficacy of therapeutic vaccines, as illustrated in the following sections. Nonetheless, the issue of preventive vaccination against cancer was also addressed during the meeting. Prevention of pathogen-associated cancers, representing ~15% of human tumors, is becoming a realistic perspective. Vaccination against hepatitis B virus is considered as a valuable...
Enrico Garaci, who reviewed early studies in tumor-bearing mice therapies. emphasizing the perspective of their use in combination with standard phase I studies in patients treated with CEA-TRICOM vectors, enhance the efficacy of cancer vaccines. Of interest, Fox therapeutic regimen. Both Bernard A. Fox and Leisha A. Emens rejection in the majority of the animals. The group has now started a suppression) resulted in an impressive response, leading to tumor cytokines are induced during the rebound after drug-induced myelosuppression. Garaci also underlined the importance of the enhancing effects of IFN and thymosin—doses. This suggests the possibility of defining IL-12 doses capable of exerting immune adjuvant activity in the absence of toxicity.

The most remarkable clinical results on adjuvants have been presented by Arthur M. Krieg, who reviewed the efficacy of CpG oligonucleotides. The results of a clinical study have shown that CpG, used as adjuvant in vaccination strategies against hepatitis B virus, markedly enhances the immunogenicity of the hepatitis B vaccine, inducing an early seroconversion in the majority of subjects after only one vaccination. Krieg also presented the results of the immune monitoring, carried out by Romero’s group, of melanoma patients vaccinated with a Melan-A/Mart-1 peptide given in Montanide with or without CpG. Of note, 8 of 8 patients vaccinated with the peptide in Montanide together with CpG showed clear-cut evidence of generation of Melan-A-specific CD8+ T cells, whereas only 7 of 12 of patients vaccinated with the peptide plus Montanide alone developed some CD8+ T-cell response. Similar to the results of the hepatitis B
virus vaccine trial, the immune response observed in subjects vaccinated with the peptides together with CpG occurred earlier than in control patients.

**Learning How to Overcome the Suppressive Activity of Regulatory T Cells**

Cornelis J. M. Melief discussed the role of CD4⁺ CD25⁺ T cells in the regulation of the CTL response to self versus non-self antigens. By using the pml-1 model of T-cell receptor-transgenic mice, whose CD8⁺ T cells recognize an epitope derived from the self-melanoma antigen gp100, it was shown that CD4⁺CD25⁺ T-cell depletion did not affect the priming of a CTL response against the self-gp100 epitope or a non-self adenosine-derived antigen. However, upon boosting, the presence of CD4⁺CD25⁺ T cells completely suppressed the secondary expansion of the gp100-specific CTLs, whereas the non self antigen-specific secondary CTL response was not affected. These results additionally strengthen the importance of developing strategies of cancer immunotherapy capable of overcoming the inhibitory effects exerted by regulatory T cells.

Paul F. Robbins reviewed the studies of adoptive transfer of tumor-infiltrating lymphocytes after administration of a nonmyeloablative regimen in patients with advanced melanoma and reported a correlation between antitumor response and persistence of T cells expressing CD82, CD27, and the IL-7-R. In a study of nonmyeloablative irradiation strongly enhanced the therapeutic efficacy of the adoptive transfer of gp100-specific CD8⁺ T cells combined with human gp100-fowlpox vaccination and IL-2 administration. Of interest, studies in RAG-1 knockout or CD4 knockout mice indicated that elimination of CD4⁺CD25⁺ T regulatory cells and help in the form of IL-2 or CD4⁺CD25⁺ T cells were required for optimal immunotherapy of established B16 tumors. Collectively, the results indicate that lymphodepletion enhances cancer regression and autoimmunity by eliminating CD4⁺CD25⁺ T regulatory cells and by improving access of adoptively transferred T cells to activating cytokines. Moreover, the data suggest the advantage of exogenous administration of IL-15 as supportive cytokine with respect to IL-2, which in contrast to IL-15, is required for the expansion and functional activity of CD4⁺CD25⁺ T regulatory cells. Clear evidence that tumor progression itself can negatively affect the function of tumor antigen-specific T cells was provided by Hyam I. Levitsky. He showed that in mice bearing the A20HA B-cell lymphoma, expressing the influenza hemagglutinin (HA), only a minority of adoptively transferred HA-specific CD4⁺ T cells could enter the cell cycle, despite the increasing availability of the nominal antigen during tumor progression. These divided, antigen-experienced, HA-specific CD4⁺ T cells exhibited markedly impaired responses to HA peptide in vitro and were impaired in their ability to differentiate into IFN-γ-producing Th1 cells and to up-regulate CD40L in vivo. The divided HA-specific CD4⁺ T cells acted as negative regulatory cells in vitro by suppressing, through cell contact and in an antigen-specific fashion, the functional responses of HA-specific naïve or CD4⁺ effector T cells and, in vivo, by diminishing the clonal expansion of naïve HA-specific T cells and blocking their differentiation into IFN-γ-producing Th1 cells.

**DCs and Cancer Vaccines**

The overall evaluation of the results of clinical trials shows that DC-based vaccines are superior in inducing an immune response against the tumor, although the general clinical response observed thus far is not significantly different from that achieved with other cancer vaccines (Parmiani). If we consider the rapid progress of knowledge on DC biology and the early phase of clinical experimentation, we might be optimistic on the chances of implementing the efficacy of DC-based vaccines. In most of the clinical studies with DC-based vaccines, the generation of DCs was based on the classical method of preparing immature DCs after a 7-day treatment of monocytes with GM-CSF and IL-4, followed by an additional incubation step with maturation signals. In this regard, there is a consensus on the concept that DC maturation correlates with a higher capability to induce an antitumor immune response in patients. This conclusion stems, for instance, from the results presented by Gosses J. Adema, who compared the activity of immature versus mature monocyte-derived DCs in a trial with melanoma patients vaccinated with DCs loaded with keyhole limpet hemocyanin protein and gp100 and tyrosinase peptides. The advantages of using monocyte-derived DCs induced toward maturation by a mixture containing IL-1β, IL-6, tumor necrosis factor α and prostaglandin E₂ have been discussed by Gerold Schuler, who presented the results of two clinical trials in melanoma patients vaccinated with the following: (1) Mage-3 peptide-loaded DCs; and (2) DCs loaded with multiple MHC class I and class II peptides. He also emphasized the importance of standardizing the parameters for defining DC maturation and batch release criteria. Many uncertainties still exist on which DCs have optimal characteristics for their use in the preparation of cancer vaccines, how they should be loaded with antigens, how many cells should be injected, and which is the best route of immunization. We have recently begun to understand the biological significance of DC heterogeneity, and the classical two-step method of DC generation based on the initial IL-4 treatment has been somehow challenged by findings showing a possible advantage of using monocyte-derived DCs generated in the presence of other cytokines. Jacques Banchereau discussed that exposure of DC precursors to different types of cytokines may result in opposite outcomes such as in the induction of either highly active or tolerogenic DCs. He showed that monocyte-derived DCs generated in the presence of cytokines such as IL-4, tumor necrosis factor α, IL-15, or IFN-α exhibited a different gene expression profile (signature), which may predict their behavior. Of interest, the signature of the antigen-specific T cells generated after coculture with antigen-pulsed DCs generated with IFN-α was consistent with their high CTL activity. Consistently, Maria Ferrantini reviewed the results of in vitro and in vivo studies carried out at the Istituto Superiore di Sanità, showing that monocyte-derived DCs generated after a 3-day treatment with type I IFN exhibit a clear-cut advantage for the development of a potentially protective immune response with respect to the conventional immature DCs.

**Summary**

How can we turn the tumor-specific immune response into a therapeutic response? After 2 days of intensive discussion on various strategies, it became apparent that there was a consensus on the need of combining cancer vaccines with other interventions thought to be necessary for achieving a good therapeutic response. We now have new knowledge for suggesting optimal modalities in using cancer vaccines in combination with other treatments, including chemotherapy. Results from some ongoing clinical trials based on these new rationales are expected to provide information on the clinical perspectives of combining cancer vaccines with chemotherapy. Another topic of major discussion has been the identification and optimal use of new adjuvants, which are essential for breaking tolerance toward self antigens and counteracting tumor-induced immune suppression. A lot of discussion has been focused on T regulatory cells and how to overcome their activity by different intervention strategies, including chemotherapy. In view of the increasing knowledge on DC biology, we now have new opportunities to perform comparative preclinical
and clinical studies aimed at defining optimal modalities for generation, antigen loading, and administration of DCs. Lastly, we are beginning to dissect the quality and the magnitude of the antitumor immune response and to learn how to correlate this with the clinical response. With the introduction of new technologies for the tracking of the antitumor immune response, we can now design more selected clinical studies, which will certainly lead to a better definition of the immune correlates of the antitumor response. In view of all this, now that we have also challenged the dogma on the incompatibility of a strict association between vaccination and more conventional treatments such as chemotherapy or radiation, we may envisage that the following years will see a considerable advance in the development of cancer vaccines and their use in the management of at least some human malignancies.

Appendix

The meeting was held April 19–20, 2004, at the Istituto Superiore di Sanità, Rome, Italy. It was co-organized by the Istituto Superiore di Sanità and the United States National Cancer Institute with the aim of discussing the state of art and perspectives of the research on cancer vaccines. In addition to the organizers (F. Belardelli, M. Ferrantini, G. Parmiani, and J. Schlom), the speakers included Drs. G. J. Adema (University Medical Center, Nijmegen, the Netherlands), A. Anichini (National Tumor Institute, Milan, Italy), P. Antony (National Cancer Institute, Bethesda, MD), J. Banchereau (Baylor Institute for Immunology Research, Dallas, TX), M. P. Colombo (National Tumor Institute), P. Correale (Siena University School of Medicine, Siena, Italy), P. G. Coulie (University of Louvain, Brussels, Belgium), A. Donda (University of Lausanne, Epalinges, Switzerland), L. Emens (The Johns Hopkins University School of Medicine, Baltimore, MD), E. Ferriès (Immuno Designed Molecules, Paris, France), B. A. Fox (Earle A. Chiles Research Institute, Portland, OR), T. F. Gajewski (University of Chicago, Chicago, IL), P. Gallo (I.R.B.M. P. Angeletti, Pomezia, Italy), F. Guadagni (Regina Elena Cancer Institute, Rome, Italy), A. Knuth (University Hospital Zürich, Zürich, Switzerland), J. W. Hadden (IRx Therapeutics, Inc., New York, NY), H. J. Levitski (The Johns Hopkins University, Baltimore, MD), P. L. Lollini (University of Bologna, Bologna, Italy), A. M. Krieg (Coley Pharmaceutical Group, Wellesley, MA), J. Harford (National Cancer Institute, NIH, Bethesda, MD), F. M. Marincola (Department of Transfusion Medicine, NIH), C. J. M. Melief (Leiden University Medical Center, Leiden, the Netherlands), P. Nistico (Regina Elena Cancer Institute), E. Proietti (Istituto Superiore di Sanità), G. Rasi (Italian National Research Council, Rome, Italy), L. Rivoltini (National Tumor Institute, Milan, Italy), P. F. Robbins (National Cancer Institute, NIH), P. Romero (Ludwig Institute for Cancer Research, Lausanne, Switzerland), A. Santin (University of Arkansas for Medical Sciences, Little Rock, AR), G. Schuler (University Hospital of Erlangen, Erlangen, Germany), B. Suligoi (Istituto Superiore di Sanità), E. Wang (Department of Transfusion Medicine, NIH), P. Zajac (University Hospital Basel, Basel, Switzerland), and L. Zitvogel (Institut Gustave Roussy, Villejuif Cedex, France).

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