EDITORIAL

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Cancer Immunology

—Past, present, and future—

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It is quite impossible to present within a reasonable space a comprehensive comment on all investigations concerning this theme. This article will present a wide-ranging overview of cancer immunology including history, current topics and future prospects. I also plan to mention recent topics in cancer immunotherapy.

In the study of the basic principles of cancer immunology, animal models have been critical research tools. The model was designed to induce a tumor, excise it, and reimplant it in the primary host. Immunogenicity was measured as a function of tumor transplant acceptance (low immunogenicity) or rejection (high immunogenicity).

The first outstanding discovery proving the existence of tumor antigens was the relationship between cancer antigens and MHC systems in animals. The breeding of artificially inbred mice commenced in 1909 and the strain was not fully inbred until many years later¹⁾. The fact that noninbred animals could frequently be made immune to transplantable tumors has been known since Little's experiments in 1914²⁾. Gorer's analysis of the H-2 complex of mice led to the rapid growth of knowledge about histocompatible antigens in animals and humans³⁾. On the basis of this research, it was concluded that rejection shown in the experiments on the detection of TSTA was not due to the cancerous nature of the graft but to the strangeness of MHC. The vast number of experiments performed with noninbred animals before this discovery had to be abandoned, which was a very painful process.

After this painful process, the reports of Gross⁴⁾, Foley⁵⁾, and Prehn and Main⁶⁾ that inbred mice could be immunized against transplants of chemically induced sarcomas arising in animals of the same strain initiated the modern era of cancer immunology in the 1950's.

However, overoptimistic interpretations of Gross's work which was performed on chemically induced-tumors have been perpetuated until recently. The conviction had spread in the 1960's that tumor antigens were not artifacts but might even be found in all tumors if we looked hard enough. This hypothesis caused confusion in the development of immunotherapy. How can we understand the following comparative failure of clinical trials on cancer immunotherapy after this hypothesis? These failures paved the way for yet another swing of opinion.

The second important advance came from our greatly improved understanding of cancer immunogenicity and the process of carcinogenesis. If we checked the origin of cancers carefully in these papers already mentioned above, tumor specific antigens were usually defined by transplantation experiments in syngenic mice using tumors induced by chemical carcinogens, virus or ultraviolet radiation, but not using spontaneously occurring tumors.

Later, several clinical failures of tumor immunotherapy motivated Hewitt⁷⁾ to publish a challenging paper in 1976. He pointed out that spontaneous regression was never observed using spontaneous tumors in the home strain, and he went on to question the current assumptions of tumor immunologists. After considerable debate, Hewitt's views on the immunogenicity of spontaneous tumors and the validity of animal tumors have been quite widely accepted. Currently it is widely accepted that tumor specific antigens may not exist or may be very weak in many human cancers.

It may also be very important and even-handed to criticize Hewitt's paper. They regarded the results of the 7 experiments they performed as negative, but they could have been observing the phenomenon of immunological enhancement. Therefore, their paper can

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not be regarded as providing conclusive evidence for lack of immunogenicity of spontaneous tumors.

In addition, the relative immunogenicity of the tumors in question is of primary importance and, as a rule, the higher the immunogenicity the greater the prophylactic or therapeutic effect. For this reason, various attempts, such as Kobayashi's xenozenization⁸⁾, Boon's mutagenesis⁹⁾, and Hamaoka's hapten treatment¹⁰⁾, have been made to increase the immunogenicity of a number of weakly immunogenic tumor lines. However, after mutagen treatment, one of the methods of antigen elevation, specific immune protection against even the original non-immunogenic tumor is conferred. This indicates that spontaneous tumors also express antigens as target structures for immune response and for tumor rejection under certain conditions.

This is the reason why we should not give up searching for specific immunotherapy even against spontaneous tumors.

The third major scientific advance is the clarification of structural or genetic changes in tumors. Until recently, attempts to investigate tumor-specific antigens at a structural level had not met with much success. Two extensively investigated approaches for this purpose are the use of monoclonal antibodies and the classical biochemical approach. It is technically easier for us to identify antigens recognized by antibodies than to use biochemical purification and amino acid analysis. Using antibodies, antigens shared by tumor cells and normal cell counterparts have been identified on some human tumors. The major antigens which were defined on human tumors are oncofetal antigens, products of which are expressed on normal cells in the early stage of embryonal development and at a very low level on some adult normal cells¹¹⁾. Some of them are now known to function as key adhesion molecules¹²⁾, but usually these are not strictly tumor-specific.

On the other hand, a small number of such molecules have been biochemically defined^{13 ~ 15)}. Some of these are viral antigens expressed in tumor cells¹⁶⁾, while the relationship of some others to viral antigens is unclear.

A surprising majority of nonviral tumor antigens have been found to be homologous in structure with stress-induced proteins in chemically induced mouse sarcomas¹³⁾. Administration of each of these antigen preparations from the tumor rendered the animal immune to challenge with live cells of the tumor. It is suggested that these stress-induced proteins may not be tumor antigens per se, but may be carriers of immunogenic moieties such as short peptides¹⁷⁾.

When we discuss tumor immunogenicity, we should

not forget MHC restriction in tumor antigens. Zinkernagel first demonstrated that T cell recognition of target cells is restricted by target cell expression of the appropriate MHC class I antigen¹⁸⁾. At present, this phenomenon is applied to tumor immunology and the following explanations are given. In general, tumor cells with low levels of expression of MHC molecules are poorly immunogenic in experimental models. Experiments show that transfecting MHC genes into tumor cells with low MHC expression can markedly increase tumor immunogenicity¹⁹⁾. These results indicate the potential importance of the level of MHC expression in tumor recognition by the immune system. Although some experiments indicate that levels of MHC expression may be important for immunogenicity in some systems, this is not true for all tumors. There is also an opinion that tumor cell expression of MHC class I molecules is probably necessary for rejection, but it may not be sufficient for rejection by an autologous host²⁰⁾.

The development of new methods aimed at defining antigen epitopes of tumor associated antigens will enable us to better define ways to increase tumor immunogenicity and develop new therapeutic strategies. One possibility is interferon. It has been known for some time that the interferons modulate the expression of class I antigens²¹⁾. The effect of interferon appears to be at the level of gene transcription. Other cytokines such as TNF have also been shown to induce MHC gene expression²²⁾.

Immunotherapy has become an integral part of the field of tumor immunology. Clinical studies on complex microbials, such as bacillus Calmette Guérin (BCG)²³⁾, in the two previous decades suggested the potential value of immunotherapy in humans. After this success in very few kinds of cancers, the logical sequence in new cancer drug development, which had evolved over the previous 30 years, has been disappointing and immunotherapeutic approaches based on nonspecific stimulation of the body's immune system have, for the most part, been abandoned. However, our understanding of the interaction between malignant cells and cellular effectors of the immune system, cytokines and growth factors has greatly expanded over the past decade, providing the basis for developing specific, effective treatment. Recent advances in molecular biology, hybridoma technology, and genetic engineering have led to an increased appreciation and clarification of immune mechanisms and have sustained interest in modulating the immune response to promote tumor recognition and eradication. Here, I would like to describe recent topics in immunotherapy, especially monoclonal antibodies, cytokines and adoptive immunotherapy.

Serotherapy for cancer using conventional antibodies

was attempted over 50 years ago, but most sera have poor specificity and low titers against putative antigens. Monoclonal antibodies, which were established by the hybridoma technique, can overcome some of these problems, and add the advantage of consistent production. At present, adequate clinical trials of monoclonal antibodies alone have been carried out in a variety of cancers. With antibodies alone, the results have been very disappointing. Responses have in each case been mainly partial and of short duration²⁴. In contrast, impressive results have come from the use of anti-idiotypic antibodies for B cell lymphoma²⁵. We cannot forget conjugated monoclonal antibodies with toxins, cancer chemotherapeutics²⁶ and radioisotopes, or chimeric antibodies composed of mouse immunoglobulin-variable regions ligand to human constant regions²⁷. Indeed, further studies and new techniques are required to apply them for human clinical use.

The exponential expansion in recombinant DNA technology has facilitated the production of large quantities of highly purified biological substances for use in clinical trials. These molecules are human proteins that modulate immune function and recognition and response to neoplastic cells. Cytokines are produced in very small amounts in the body, and their anticipated antitumor action has made them promising for cancer treatment.

The first group of cytokines to be used clinically was the interferons. Although many clinical trials have now been carried out, their only important activity is against hairy cell leukemia²⁸, low grade non-Hodgkin's lymphoma, chronic myeloid leukemia, untreated multiple myeloma and HIV-related Kaposi's sarcoma, as well as renal cell cancer and malignant melanoma. The other cytokine to undergo extensive clinical trials is interleukin-2. Originally described as a T cell growth factor causing proliferation of activated cells²⁹, it has subsequently been found to have a wide range of immunological activity including the activation of natural killer cells, cytotoxic T cells and lymphokine activated killer cells.

The use of the tumor necrosis factor (TNF) has recently been permitted in clinical trials. In phase 1 trials, the maximum tolerated doses, pharmacokinetics, and principal side effects have been determined; however, no significant tumor regression has been demonstrated in human trials³⁰.

Adoptive immunotherapy is the passive transfer of effector cells to a tumor-bearing host. The ability to proliferate lymphocytes *in vitro* with interleukin-2 has given rise to studies on this cell therapy. Clinical trials of interleukin-2 in combination with lymphokine

activated killer cells (LAK) soon showed effects in renal cell carcinoma and melanoma³¹. Later tumor infiltrating lymphocytes (TIL) have been extracted from tumors and used to produce novel cytotoxic populations³². Previous studies have shown that TILs are 50 to 100 times more efficient in killing tumor targets than are lymphokine-activated killer cells.

When transfected into tumor cells, cytokines are capable of preventing growth of subcutaneously implanted murine tumors, hence preventing tumor-induced animal deaths. These experiments pave the way for expression of genes encoding proteins with antitumor activity, such as the tumor necrosis factor. As a method of examining the distribution of TILs infused into cancer patients, a selectable gene was introduced into TILs by retrovirus-mediated gene transfer before infusion into patients. These observations have led to the first approved clinical study of foreign gene transfer in humans³³. In the near future, the benefits of tumor cell-targeted cytokine gene therapy will be more widely recognized.

How can we apply some of the concepts I have outlined here to advance cancer therapy? First, an enormous amount of animal work remains to be done, but animal models, however elaborate, will provide answers only for the species involved. It should now be stressed that our final goal is the complete cure of human cancer by immunotherapy. In order to reach this goal, results obtained using animal models should be more carefully analyzed. I now believe that studies which evaluate effects of immunotherapy without considering tumor immunogenicity, are almost meaningless as clinical trials. Secondly, positive bias in the literature should be carefully considered ---positive results are more likely to get published than negative studies. Caution should be applied to exaggerated claims of success which are apt to appear owing to the overenthusiasm of the biotechnology industry. If these points are carefully considered, and steady efforts are made, we can expect a rosy future in this field. With the advances that have been made and the powerful new tools available, there are many potential permutations for the application of biological therapy for cancer. Even though clinical data are still quite premature, it is reasonable to assume that in the future several clinical trials in progress will be successfully concluded.

References

- 1) Bailey DW : How pure are inbred strains of mice? *Immunol Today*, 3 : 210-214, 1982.
- 2) Little CC, Tyzzer EE : Further experimental studies on the inheritance of susceptibility to a transplantable tumor, carcinoma (J.W.A.) of the Japanese waltzing mouse. *J Med Res*, 33 (New ser.

- 28) : 393-453, 1915-1916.
- 3) Gorer PA : The antigenic basis of tumor transplantation. *J Pathol Bacteriol*, 47 : 231-252, 1938.
- 4) Gross L : Intradermal immunization of C3H mice against a sarcoma that originated in an animal of the same strain. *Cancer Res*, 3 : 326-333, 1943.
- 5) Foley E J : Antigenic properties of methylcholanthrene-induced tumors in mice of the strain of origin. *Cancer Res*, 13 : 835-837, 1953.
- 6) Prehn RT, Main JM : Immunity to methylcholanthrene-induced sarcomas. *J Natl Cancer Inst*, 18 : 769-778, 1957.
- 7) Hewitt HB, Blake ER, Walder ASA : A critique of the evidence for active host defense against cancer, based on personal studies of 27 murine tumors of spontaneous origin. *Br J Cancer*, 33 : 241-259, 1976.
- 8) Kobayasi H, Kodama T, Gotohda E : Xenogenization of Tumor Cells. *Hokkaido Univ Med Library Series*, 9, Sapporo, 1977, p1-124.
- 9) Boon T : Antigenic tumor cells variants obtained with mutagens. *Adv Cancer Res*, 39 : 121-151, 1983.
- 10) Hamaoka T, Takatsu K, Okuno K et al. : Functional characterization of the killer-helper factor responsible for the induction of cytotoxic T lymphocytes from thymocytes, and evidence for the nature of this factor as distinct from T cell-replacing factor (TRF) in regards to B cell triggering. *J Immunol*, 126 : 659-665, 1981.
- 11) Gold P, Freeman SS : Specific carcinoembryonic antigens of human digestive system. *J Exp Med*, 122 : 467-475, 1956.
- 12) Hakomori S : Possible function of tumor-associated carbohydrate antigens. *Current Opinion in Immunology*, 3 : 646-653, 1991.
- 13) Ullrich SJ, Robinson EA, Law LW et al. : A mouse tumor-specific transplantation antigen is a heat shock-related protein. *Proc Natl Acad Sci USA*, 83 : 3121-3125, 1986.
- 14) Srivastava PK, Deleo AB, Old I J : Tumor rejection anti-gens of chemically induced sarcoma of inbred mice. *Proc Natl Acad Sci USA*, 83 : 3407-3411, 1986.
- 15) Sato N, Yagihashi A, Okubo M et al. : Characterization of tumor rejection antigen molecules of chemically induced murine colon tumor C-C26. *Cancer Res*, 47 : 3147-3151, 1987.
- 16) Rogers MJ, Galetto G, Hearing VJ et al. : Purification of glycoprotein bearing tumor transplantation antigen specific for Friend, Moloney, and Rauscher MuLV-induced tumors. *J Immunol*, 132 : 3211-3217, 1984.
- 17) Srivastava PK, Maki RG : Stress-induced proteins in immune response to cancer. *Current Topics in Microbiol Immunol*, 167 : 109-123, 1991.
- 18) Zinkernagel RM, Doherty PC : Restriction of *in vitro* T cell-mediated cytotoxicity in lymphocytic choriomeningitis within a syngeneic or allogeneic system. *Nature*, 248 : 701-702, 1974.
- 19) Porgador A, Feldman M, Eisenbach L : H-2K^b transfection of B16 melanoma cells results in reduced tumorigenicity and metastatic competence. *J Immunogenet*, 16 : 291-303, 1989.
- 20) Naftzger C, Houghton AN : Tumor immunology. *Current Opinion in Oncol*, 3 : 93-99, 1991.
- 21) Wallach D, Fellous M, Revel M : Preferential effect of γ -interferon on the synthesis of HLA antigens and their mRNAs in human cells. *Nature*, 299 : 833-836, 1982.
- 22) Chang RJ, Lee SH : Effect of interferon-gamma and tumor necrosis factor-alpha on the expression of an Ia antigen on a murine macrophage cell line. *J Immunol*, 137 : 2853-2856, 1986.
- 23) Mathe G, Amiel JL, Schwartzberg A et al : Active immunotherapy for acute lymphoblastic leukemia. *Lancet* 697-699, 1969.
- 24) Dillman RO : Monoclonal antibodies for treating cancer. *Ann Intern Med*, 111 : 592-607, 1989.
- 25) Meeker TC, Lowder J, Maloney DG et al. : A clinical trial of anti-idiotypic theory for B cell malignancy. *Blood*, 65 : 1349-1363, 1985.
- 26) Oldham RK, Lewis M, Orr DM et al. : Adriamycin custom tailored immunoconjugates in the treatment of human malignancies. *Mol Biother*, 1 : 103-113, 1988.
- 27) Morison SL : Transfections provide novel chimeric antibodies. *Science*, 229 : 1202-1207, 1985.
- 28) Goldstein D, Laszlo J : Interferon therapy in cancer : From imagination to interferon. *Cancer Res*, 46 : 4315-4329, 1986.
- 29) Morgan DA, Rusceffi FW : Selective *in vitro* growth of T lymphocytes from normal bone marrows. *Science*, 193 : 1007-1008, 1976.
- 30) Blick M, Sherwin SA, Rosenblum M et al. : Phase I study of recombinant tumor necrosis factor in cancer patients. *Cancer Res*, 47 : 2986-2989, 1987.
- 31) Rosenberg SA, Lotze MT, Muul LM et al. : A progress report on the treatment of 157 patients with advanced cancer using lymphokine activated killer cells and interleukin-2 or high dose interleukin-2 alone. *N Engl J Med*, 316 : 889-905, 1987.
- 32) Rosenberg SA, Spiess P, Lafreniere R : A new approach to the adoptive immunotherapy of cancer with tumor infiltrating lymphocytes. *Science* 233 : 1318-1321, 1986.
- 33) Rosenberg SA. : The immunotherapy and gene therapy of cancer. *J Clin Oncol*, 10 : 180-199, 1992.

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