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Perspectives and Commentaries

Immunologic Diagnosis of Cancer

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CHEMICAL CARCINOGEN- AND VIRUS-INDUCED TUMOR ANTIGENS

THE EXQUISITE specificity of immunological reactions offers, at least on a theoretical basis, a potential means of managing patients with cancer. In order for the immune system to initiate an appropriate response to tumor tissue, the tumor must possess distinctive antigens. Much of the early work in tumor immunology was inconclusive, largely because it was not appreciated that tumors, like other tissues, exhibit transplantation antigens. However, with the development of inbred mouse strains and the availability of syngeneic transplantable tumors, the classic experiment by Foley provided the first evidence for the presence of specific antigenicity of methylcholanthrene-induced experimental tumors [1]. In order to demonstrate rejection of transplanted tumor cells between syngeneic animals, pretreatment of the tumor transplant recipient animal with tumor tissue must be undertaken in some fashion, to show that the subsequent tumor transplant will be rejected. The necessity for priming the genetically identical recipient animal in order to demonstrate tumor cell rejection indicates that a relatively low level of immunogenicity is a feature of chemically induced tumor antigens. Another characteristic feature of these chemically induced tumor-specific transplantation antigens (TSTA) is the extreme polymorphism of antigenic structures induced during malignant transformation by a given chemical carcinogen. The genetic basis for such polymorphism is as yet unclear. Although TSTA share many biological similarities with H-2 antigens, present data indicate that the genes of the major histocompatibility complex (MHC) are not involved in the generation of diversity of TSTA [2].

Subsequent studies in experimental animals demonstrated that virally induced tumors exhibit immunologic cross-reactivity. Tumor antigens produced by either DNA or RNA viruses are similar for all tumors induced by a single virus, irrespective of the histology of the tumor, yet different from those induced by different viruses.

Despite the fact that the aforementioned studies indicate the development of an autochtonous anti-tumor immune response in the experimental animal, tumors develop, grow progressively and eventually prove lethal to the host. Studies by Hewitt and colleagues have failed to find any evidence for immunogenicity in 27 different murine tumors of spontaneous origin, in contrast to the above studies involving tumors of chemically and virally induced origin which demonstrate immunogenicity *in vivo* [3]. A potential explanation for this dichotomy is found in the studies of Prehn [4], who demonstrated that tumor frequency and immunogenicity diminishes, and the latent period increases, as the dose of chemical carcinogen is reduced. Thus both chemical and so-called spontaneous tumors may be considered to represent a continuous spectrum, the extreme ends of the scale being represented by chemically induced tumors at one end and 'apparently' spontaneous tumors at the other.

HUMAN TUMOR ANTIGENS

Ethical considerations preclude the use of the tumor transplantation technique in man as a method for demonstrating tumor antigenicity.

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Instead, the observed autologous host anti-tumor immunity *in vitro* is probably the clearest evidence that tumor antigens do indeed exist in man [1]. Much of the pertinent work in this area has been done initially using the immune response in patients bearing melanomas as the experimental model. In this, and subsequently in other solid tumor systems, numerous studies have demonstrated the presence of a common set of antigens in tumors of a given histopathologic type and tissue origin. One of the particular difficulties in studying anti-tumor immunity in man is the fact that most tumors induce primarily a cellular, as opposed to an antibody, response to their own tumor. Difficulty in developing quantifiable, simple and readily reproducible *in vitro* anti-tumor assays has led to many contradictory results, but is generally agreed that anti-tumor immune responses do occur in man and, to a degree, can be useful in predicting the clinical course of a tumor-bearing patient [5, 6].

INDENTIFICATION OF HUMAN ANTIGENS

Several approaches have been applied to identify and isolate human tumor antigens. One of the earliest approaches involved the immunization of heterologous species with a view to producing anti-tumor antibody. Subsequent absorption of the antisera so produced with normal human tissue was used to render the antisera tumor-specific. The carcinoembryonic antigen (CEA) and alpha-fetoprotein (AFP) are probably the two best examples of tumor-associated antigens that have been identified using this approach. In the majority of other human solid tumors this approach has not proved successful in the attempt to raise tumor-specific antisera. Nevetheless, our experience with both CEA and AFP has shown that, although these antigens are not tumor-specific, assays in which these parameters are measured serially in the cancer patient are useful in patient management [7].

The failure to produce antisera that recognize tumor antigens by heteroimmunization with intact tumor tissue or tumor tissue extracts is due to the production of antibodies to normal tissue components that usually constitute the greatest fraction of the antibody population in such antisera; the difficulties of quantitatively removing the anti-normal tissue antibody by absorption with normal tissue; the presence of low titer antibody that recognizes tumor-specific or associated components in the polyspecific antisera; and the presence of cross-reactive antibody. The mouse monoclonal antibody (hybridoma) technique of Kohler and Milstein overcomes many of the above difficulties, as it allows for the selection of putative anti-tumor antibody that may constitute a minor fraction of the total antibody pool. However, although cross-reactions are less of a problem, they are not entirely eliminated by this technique. Over the past several years this method has been widely employed in efforts to produce specific anti-tumor antibody. Cross-reacting antibody can be observed, which makes it imperative that extensive screening on normal and tumor tissue be undertaken [8, 9]. Monoclonal antibody systems show promise in the immunodiagnosis of cancer [10]. It is likely that by using murine hybridoma techniques, antibodies of sufficiently narrow specificity may not be forthcoming, despite the selection procedures employed. The use of regional lymphoid tissue draining the sites of tumor or peripheral lymphocytes from cancer patients, when fused to an appropriate *human* myeloma cell line, should, theoretically, after appropriate selection, generate monoclonal antibody with the requisite narrow anti-tumor specificity. The establishment of *human* hybridoma technology has been hampered by the non-availability of human myeloma cell lines to support the production of large quantities of human monoclonal antibodies *in vitro*. Autoreactive human monoclonal antibody is more likely to detect specific tumor antigens and prove more useful than murine antibody for diagnostic imaging.

TUMOR MARKERS IDENTIFIED BY ANTI-TUMOR IMMUNE RESPONSES

Another approach to the identification of tumor antigen involves the use of the tumor bearing host's cellular anti-tumor immune reaction to monitor the purification of specific tumor markers. The article by H. Kr. Kotlar, appearing in this journal is a variation of such an experimental approach [11]. Dr Kotlar has found that the serum from a cancer patient bearing a particular tumor contains a factor that reacts with an antigen derived from that same tumor type. This serum factor–tumor complex inhibits the ability of trypsinized normal leukocyte indicator cells to adhere to glass. This modified leukocyte inhibition assay (H-LAI) was employed to monitor the purification of the antigen-specific serum factor involved in the above reaction. Multiple physiochemical procedures were employed to isolate the factor, and in each case the relevant fractions were tested for H-LAI activity, a slow, tedious and difficult procedure. Limited amounts of purified and semi-purified factor at each stage precluded the performance of detailed dose–response curves. In the end, an antigen-

specific serum glycoprotein factor consisting of 43K and 28K subunits was purified 1120-fold. Trace contamination of the final product with albumin and another serum protein molecule was noted. Heteroantisera raised to the factor, although impure, were used via Rocket immunoelectrophoresis to show that increased concentrations of the factor were found in the serum of lung cancer patients as opposed to normal sera and sera from breast cancer patients. However, only small numbers of sera were studied.

It must be stressed at this time that, although these observations are interesting, they must be extended using purified antigen and antibody, as well as many well-defined serum samples from a number of normal individuals and patients affected by a variety of inflammatory, benign and malignant disorders. The author has emphasized that, although H-LAI factor itself is putatively antigen-specific in measuring antitumor immunity *in vitro*, heteroantisera to the purified factor show reactions with substances in normal and breast cancer sera. The heteroantisera raised may contain two distinct specificities—an antibody that reacts with a common antigenic site shared by all H-LAI serum factors and another antibody that reacts with a unique site: the tumor antigen recognition site. Monoclonal antibody may thus be most useful in the further characterization of this molecule. An interesting question concerns the origin and nature of this tumor antigen reognition factor. The possibility that the molecule may be composed of constant and variable (recognition) segments and consists of subunits of 43K and 28K suggests that this molecule may represent the recently described T cell receptor [12–14]. Studies of the reaction of the antibody to the H-LAI factor with peripheral blood lymphocytes and regional lymph nodes draining lung tumor tissue might prove of interest in this regard.

FUTURE PERSPECTIVES

To date, no truly specific tumor antigen has been described, and there is controversy as to whether such a molecule exists. Nevertheless, a number of tumor markers or tumor-associated antigens have had an impact on the management of cancer patients. Dual markers, the β subunit of HCG and AFP, in combination with effective chemotherapy, have contributed to our therapeutic success in managing germ cell tumors of the testes. In the case of CEA, future development of effective chemotherapy will permit optimal use of this useful tumor-associated antigen in patient management. Although the field of tumor markers has had its peaks and valleys, the development of monoclonal antibody of requisite specificity should overcome some of the earlier difficulties encountered and provide a readily available, reproducible supply of well-characterized antibody reagents for use in immunoassays, diagnostic imaging and specific targeting of chemotherapeutic drugs to tumor tissue.

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