Some Immunological Considerations Relevant to the Study of Human Bladder Cancer

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Summary

The likelihood that immunosurveillance, concomitant immunity, and immunodepression play a role in the development and spread of neoplasms of the urinary bladder is discussed. The circumstantial evidence for the existence of concomitant immunity to bladder cancer-associated antigens is briefly reviewed, and the implications of the hypothesis of Zinkernagel and Dougherty of a genetic restriction to the cytotoxicity of T-cells for virally determined target cell antigens and of the concept of immunoregulatory cells for our understanding of the immunology of bladder carcinoma are discussed.

Introduction

This paper is meant to provide a brief introduction to some classical and modern immunological concepts that may be relevant to our study and understanding of the biology of bladder cancer in humans. Therefore, the items in the "References" were selected primarily to provide the reader with a few general and current references to each of the areas of study rather than to reference the original works.

The considerable current efforts directed toward the study and understanding of immune reactions to tumors in humans are, in great part, a result of the clear-cut demonstration of TSTA in animal tumor models (24, 30, 34). With the development in the early 1950's of highly inbred strains of mice and thus a uniform genetic background (syngeneic) which eliminated reactions due to histocompatibility antigens, it was clearly demonstrated that chemically induced tumors in mice contain individually specific TSTA. That is, each tumor apparently had a different TSTA. Immunization of mice with a syngeneic chemically induced tumor conferred immunity to a subsequent inoculum of that same tumor, whereas an inoculum of an unrelated syngeneic chemically induced tumor would grow and kill the animals. Also, in the reciprocal experiment where the mice were immunized with the 2nd tumor and then challenged with both tumors, only the 2nd tumor was rejected, while the 1st one would grow. It soon became clear that the chemically induced tumors, although having individually specific tumor-associated antigens, were different from virally induced tumors that shared tumor-associated transplantation antigens (29). Immunization of mice with virally induced syngeneic tumor conferred cross-reactive immunity to other tumors induced by the same virus in the same strain of mice.

Studies on the mechanism of this tumor transplantation immunity to both types of tumor antigens showed that it was mediated by the cellular immune system (thymus dependent). Also, immunity could be readily transferred to syngeneic animals with lymphoid cells but could not be transferred with serum or antibody. This evidence, along with the parallel evidence that the thymus-dependent immune system was responsible for rejection reactions across histocompatibility antigens, provided the impetus for the intensive study of cell-mediated immune responses, since humoral immune responses were thought to be less important in tumor immunity.

Since humans are not inbred, and tumor transplantation experiments are impractical as well as nearly impossible to do, considerable emphasis was placed on developing in vitro correlates of cell-mediated immunity. With animal models, assays of cytotoxicity by lymphoid cells for tumor cells were developed and then applied to the study of immunity to tumors in humans. Hellström and Hellström (19) were among the first to report that patients with tumors quite often had blood lymphocytes that were cytotoxic in vitro for their own tumor cells or cells from tumors of a similar histological type from other patients but were not toxic for cells from tumors of dissimilar histological types (e.g., leukocytes from patients with breast cancer kill breast cancer target cells but not melanoma cells). These findings, although now somewhat controversial (2, 40), had 2 very significant implications. One of these implications was that human tumors indeed had antigens that could provoke a host immune response (a sine qua non for immunotherapy), and the other was that, since tumors of a given histological type have cross-reacting antigens, this was circumstantial evidence for a viral etiology of tumors in humans. Again, this circumstantial evidence had obvious implications for immunotherapy, immunodiagnoses, and prophylaxis.

Since it was somewhat paradoxical that an animal or person should have cytotoxic lymphocytes for tumor cells in culture while the tumor continued to grow in the body, Hellström et al. (17, 18) were prompted to examine the effects of serum on this in vitro CMC. They found that serum from tumor-bearing animals or patients would abrogate the in vitro CMC in a rather specific way. Sera from patients...
bearing melanoma blocked the cytotoxicity of blood leukocytes from melanoma patients for melanoma target cells but did not interfere with the cytotoxicity of blood leukocytes from patients with breast carcinoma for breast carcinoma target cells. These findings led to the concept of humoral immune responses="blocking" and to the widely held belief that cellular immune responses to TSTA are beneficial and humoral immune responses are antagonistic or harmful. This very simplified background, then, provides a basis for which the following discussion attempts to focus on the current status and state of knowledge, as it relates to several concepts and phenomena that may be of relevance to the study of immune reactions to human tumors in general and of particular relevance here in terms of immune reactions to bladder carcinoma.

Immunosurveillance

Although Paul Ehrlich had discussed the idea in the early 1900′s, Burnett was the first to propose formally that the purpose of the cellular immune system, which operates so efficiently in terms of organ graft rejection, was to enable the host to reject clones of tumor cells that must frequently spontaneously arise (33, 39). This has been quite a popular concept, despite a lack of hard data to support it and in spite of abundant data to discredit it. The main arguments for the existence of immune surveillance are circumstantial. Immunodeficiency diseases (especially those with a genetic defect in cell-mediated immunity) have an associated increased incidence of neoplasms. An increased incidence of neoplasia is also seen in patients on immunosuppressive regimens (e.g., for prolongation of kidney graft survival). The problem with this evidence is that almost all of the increase in neoplasia in these cases is in the lymphoreticular system, which is the target of the genetic defect or the immunosuppressive therapy. There is little or no increase in incidence of solid tumors in these patients. The very young and the very old have been said to have an increased incidence of neoplasms; at these ages the immune system is thought not to be at full strength. Again, this argument does not stand close scrutiny since the increases of cancer in the young and old are of select types and one can think of several cancers peaking in incidence during middle life, a time when the immune system is at its best. Additional circumstantial evidence supporting immunosurveillance is provided by the finding of impaired immune responses in cancer patients and the significantly increased incidence of tumors among those who already have cancer. But these same data can be used to support the viewpoints that cancer causes immunosuppression and that susceptibility to cancer is genetically linked. For a more detailed discussion and rebuttal of these general arguments for immunosurveillance, refer to the review by Stutman (39).

However, there are several facets of the immunosurveillance hypothesis that deserve serious consideration here. Human bladder carcinoma is rare in youth, is at peak incidence through the ages of 50 to 70, and then declines in incidence in older age groups. This happens in spite of the progressively declining immune function associated with aging. If immune surveillance were really operative, one might expect a progressive increase with each decade of age. But even if the incidence of bladder cancer increased with each decade, those concerned with environmental carcinogenesis would not have to evoke an immunological mechanism to explain the association of increasing incidence of cancer with increasing age. They could point to the fact that the length of exposure and the total dosage of carcinogen influences development of neoplasia. The geneticist might have yet another viewpoint. For bladder carcinoma, the peaking in incidence with a subsequent decline could be due to removal or elimination of those persons in the population susceptible to whatever the oncogenic agent is. Support for this point comes from the highly inbred (syngeneic) mouse strains which have varied incidences of neoplasia when exposed to different standard oncogens. One must select a susceptible strain in order to induce mammary carcinoma or sarcomas with virus or to induce sarcomas with chemicals; otherwise one obtains too few tumors for study (39). Since the genetic polymorphism of humans is so large, we probably have a wide spectrum of relative resistance or susceptibility for any given agent.

Since some bladder carcinomas are known to be due to chemical carcinogens, studies such as the one by Stutman (39) are particularly relevant. He has clearly shown that the incidence of tumors in immunoincompetent nude (thymus-less) mice exposed to methylcholanthrene is not increased compared with the incidence of tumors in immunocompetent heterozygotes carrying this gene. This is a very strong argument against immunosurveillance playing a role versus chemical carcinogenesis. The story is a bit more complex in the case of virus-induced tumors in mice since the presence of the thymus also influences immunity to virus. Thus, absence of the thymus allows replication and spread of the virus from cell to cell and thus increases the dose of the oncogenic agent.

If immune surveillance is not a concept of importance to bladder neoplasia in man, what then is the possible relationship of the immune response to this disease?

Concomitant Immunity

The term “concomitant immunity” was given to the state in which animals with a growing tumor would paradoxically reject a 2nd inoculum or challenge of the same tumor. This term was coined by Bashford in 1908 after he observed the phenomenon with an allogeneic tumor graft model (10, 27, 43). With the development of highly inbred strains of mice and the demonstration of TSTA, interest in this phenomenon resurfaces, although it was seen as somewhat of a curious paradox that was difficult to understand and of unknown importance. When an animal with a progressively growing syngeneic tumor graft is challenged early in the course of its disease with a small inoculum of the same tumor, this 2nd inoculum of tumor is rejected. However, if at the same point in time, the animal is challenged with a non-cross-reacting antigenic tumor, this unrelated tumor will take and grow. Later in the course of growth of the 1st tumor, a secondary challenge with the same tumor cells will readily take and progressively grow. At that point in time, immunity to other unrelated antigens
is usually not affected. From these studies, it is quite clear that concomitant immunity is specific and is manifest early and for only a finite period of time. With further progression of the original inoculum of tumor cells, immunity to antigens unrelated to the tumor becomes depressed and skin allograft survival becomes prolonged, suggesting that the animal is now nonspecifically immunodepressed. LeFrancois et al. (25) have serially studied the immune reactivity in vitro of lymphocytes from mice throughout the course of growth of transplanted antigenic mammary tumors and found that there is development of systemic CMC to these tumor cells that goes through a period of evolution and then decline, termed an “eclipse,” as the tumor grows large. Thus, in animals bearing progressively growing antigenic tumors, we have early development of systemic cell-mediated immunity to the specific tumor antigens, which eventually disappears or becomes undetectable prior to the development of a generalized immune unresponsiveness.

These findings lead to several considerations of importance to us in the context of human bladder carcinoma. One of these considerations is that, if the tumor bears no antigens, then of course we would not expect the development of concomitant immunity. Another consideration is that, even if the tumor is antigenic, it may be that the individual in whom the tumor is growing is unresponsive to that antigen, depending upon his inheritance of immune response genes or his immunological status at the time that the tumor is first developing. For instance, the individual could be an immunosuppressed kidney transplant patient or could be a cancer patient already burdened by another tumor and in an immunologically depressed state. If the tumor is antigenic and the host responds to these antigens, then might not this concomitant immunity be one of the body’s mechanisms for elimination of systemic tumor cells and prevention of the development of metastasis?

What, then, if any, is the evidence for concomitant immunity in man? The 1st and most obvious piece of circumstantial evidence would be the case of spontaneous regression of tumor in man (13, 28). These spontaneous regressions virtually always involve metastatic foci and not the primary tumor that usually has been surgically removed. This phenomenon then is different from what would be predicted by the theory of immunosurveillance, where it would be the primary tumor that is rejected before it is clinically detectable. Another piece of evidence is provided by Southam (37), who demonstrated concomitant immunity to tumor homografts in cancer patients. He found that a homograft of tumor cells was accepted and progressively grew locally in a patient with cancer. When the patient was given a 2nd inoculum of the same tumor at a different site, it was rejected while the 1st inoculum continued to grow. A 3rd line of evidence comes from the studies of cellular immune responses of patients to cancer cells in vitro (19). Studies of patients with bladder cancer by Bubenik et al. (5), O’Toole et al. (32), Bean et al. (3), and others (4, 15) suggest that some patients with bladder carcinoma have blood leukocytes that are cytotoxic to bladder tumor cells in vitro. Most interestingly, this cytotoxic effect appeared to be related to the presence or absence of tumor in the studies by O’Toole et al. (32). That is, this reactivity occurred when tumor was present, disappeared after adequate surgical removal of tumor, disappeared during radiotherapy, quite often reappeared after cessation of radiotherapy, and occurred infrequently in patients with extensive disease. Because this was cross-reactive immunity (seen on allogeneic target cells), it could be taken as evidence consistent with a viral etiology for human bladder tumors. Studies of humoral immune responses in patients with bladder cancer have also shown some cross-reactivity (14). Additional suggestive evidence of sensitization to cross-reactive, bladder-tumor-associated antigens in humans is provided by the data derived by limited skin testing of cancer patients with extracts of tumor cells. These data support cross-reactivity but are not definitive evidence of concomitant immunity, since the observed reactions may be measuring memory or “recall” analogous to that of tuberculin skin test reactivity in patients within active tuberculosis.

An alternative explanation to these immune reactions being directed to a common viral antigen is that these tests could simply be measuring autoimmunity to bladder epithelial-associated antigens (tissue-specific antigens). Nevertheless, the resultant immunity would still fall within the definition of concomitant immunity. For example, it might be therapeutic for the patient with thyroid cancer to develop autoimmune thyroiditis.

Immunodepression and Human Bladder Carcinoma

Indirect evidence that the immune system is related to the biology of human bladder carcinoma is provided by the now numerous reports on altered immune status in patients with bladder carcinoma (6, 9, 16, 20, 26, 31, 35, 36). The tests used to measure the immune status are of several types. Skin tests to recall antigens, namely, tuberculin, dermatophytn, streptokinase, streptodornase, mumps, etc., are based on the premise that the population at large has had prior exposure to these agents and most “normal” people are capable of mounting a recall response to at least 1 of these antigens. In addition, patients have been skin tested with chemicals that form haptons with their skin proteins, forming antigens to which they have never before been exposed. Two of these chemicals, DNCB and dinitrofluorobenzene, have now been fairly extensively used in testing the immune competence of patients with neoplasms of all kinds and in testing patients with bladder carcinoma. A similar, but different, approach has been to use a naturally occurring antigen for sensitization that most of the population has not previously encountered, such as keyhole limpet hemocyanin. Keyhole limpet hemocyanin, DNCB, and dinitrofluorobenzene skin tests differ from the recall antigen skin tests in that they measure multiple facets of the immune system such as antigen recognition, development of memory, ability to recall, and ability to mount an inflammatory response, and they are applied in relatively high doses to sensitize the subject; then, 14 to 20 days later, the subject is “challenged” with a smaller dose. The findings of such studies in general have been that patients with cancer have a decreased immune responsiveness to both recall and new antigens, and this decrease in immune responsiveness increases with advancing stage of disease and therefore correlates in a rough way with biological aggressiveness of the...
tumor. In vitro assays have also shown altered responses of these patients. That the immunodepression was not precedent to the development of tumor is suggested by the findings of a recurrence of skin test reactivity and/or in vitro activity to normal after successful removal of tumor in many types of cancer patients, which again argues against the theory of immunosurveillance (12, 20) which would propose that the immune deficit be precedent to the cancer.

Histological studies of bladder tumors suggest that infiltration of the tumor and adjacent bladder by lymphoid cells is associated with a more favorable prognosis (35). Although many would see this as circumstantial evidence for the presence of a tumor-directed immune response, an alternative explanation is possible. It may be that the infiltrate is due to bacterial or other stimuli (e.g., instrumentation) since the integrity of the urothelium has been altered by the neoplastic process. The infiltrate may be simply a measure of the host immune responsiveness much like a DNCB skin test. This same type of argument holds as a possible explanation for the favorable prognosis associated with histologically immunoreactive lymph nodes regional to the bladder at cystectomy (21). The lymphoid infiltrate in the bladder and the reactive regional lymph nodes may simply be clues as to which patients are immunocompetent or, alternatively, they may be indicators of which patients have tumors that are antigenic and thus provoke an immune response in the patient.

Another way of studying these patients is to look at their immune responsiveness in the test tube to allogeneic lymphocytes, antigens, and mitogens (6, 20, 26). Again, the consensus of these studies is that some patients with tumor have a decreased responsiveness in vitro and that this responsiveness may return to normal with removal of tumor. This evidence would also tend not to support the concept of immunosurveillance. The exact mechanisms of how tumors suppress the immune response are not yet clearly known, but there is suggestive evidence that the tumor cells may block or suppress by shedding excess antigen into the circulation, stimulate the production of blocking antibody or antigen-antibody complexes, produce toxic materials or immunoregulatory peptides, or stimulate the production of suppressor cells.

A major concern about the depression of specific immunity, especially that of concomitant immunity, is that it might be caused iatrogenically (7, 44). Evidence is accumulating that surgery, anesthesia, and radiotherapy depress immunological responses in humans. This leads to the worry that perhaps one is inadvertently allowing the shed tumor cells to set up metastatic foci when major surgery is performed to remove the primary tumor. Although there is evidence that these therapeutic regimens depress general or non-tumor-specific immunological reactions, there is as yet no conclusive evidence that they inhibit specific tumor-directed immune reactions in man. However, in the animal models of concomitant immunity, it has been a frequent finding that a challenge inoculum of tumor given at the time of surgical excision of the initial tumor will result in growth rather than rejection of the challenge inoculum, suggesting that the concomitant immunity has been depressed (43).

These findings should lead us not to stop removing primary tumors which are the source of cells that set up the metastatic foci but to pay due consideration to the possibility that our techniques are immunosuppressive. Serious consideration and study of this problem would then allow us to focus on experiments designed to either prove or disprove this point and, if true, lead us to studies of ways to counteract treatment-induced immunosuppression.

There was initially great hope that measurement of immune parameters in cancer patients would provide data of predictive value for the individual patient. To date, this is not the case, although it may soon be possible since our understanding of the basic immunological mechanisms is rapidly progressing. Nevertheless, there is feeling among some of disappointment and disenchantment with such studies. Just a few years ago one would not be able to get an immunotherapy trial approved if one were not monitoring in vitro immune responses. Currently, it would be difficult to get it approved with any significant emphasis on measuring in vitro responses unless the tests were especially novel. It should be apparent that either extreme should be avoided because this is a relatively young field of study which shows areas of promise. Also, there will be tremendous activity and new approaches as the new discoveries made in basic immunobiology with animal models and in vitro provide insight and raise questions we would have never thought of asking about the host-tumor interaction in man.

Contemporary Developments in Immunobiology

Several new developments in immunology have marked implications for our understanding of the host-tumor relationship. One of these implications was referred to briefly before, that of the concept of immune response genes that are located within the major histocompatibility complex in man and animals (1, 11). It is now clear that immune responsiveness to various antigens is under genetic control the way susceptibility is to some diseases. Some strains of inbred mice can respond to certain synthetic antigens that other strains of mice do not. Also, some will respond in different ways to a standard antigen. The implication of this for tumor immunology is that the level of immune responsiveness and, possibly, the antigens to which one is capable of immunological response may be controlled by these means. Therefore, even a highly antigenic tumor may by definition be nonimmunogenic in certain hosts if it will not be recognized or elicit a response. Or, alternatively, there may be different levels of strength or types of immune response to such a putative antigen. This of course would have obvious implications on the ability of the host to respond and prevent the dissemination of tumor cells throughout the body.

Another area of rapid development and potential relevance is the study of immunoregulatory cells (8, 22, 23). It has now become clear that feedback mechanisms mediated by cell-cell interactions exist and that under certain conditions stimulation with antigen in certain dosages or routes can induce “suppressor cells” that actually inhibit the development of an effective immune response. The immunoregulatory cells may play an important role in what once was thought to be a “classic tolerance” or “immune paralysis.” It is conceivable that the eclipse in concomitant immunity due to prolonged antigenic stimulation by a tumor...
may be in part due to stimulation of suppressor cells. Suppressor-like cells have been described in the blood of cancer patients in man and appear to come and go with recurrence and remission of Hodgkin’s disease (41). We (H. Herr, M. A. Bean, and W. F. Whitmore, Jr., unpublished data) have observed that in the lymph nodes of some patients with D1 bladder carcinoma, there are cells that have the ability to abrogate the function of the patient’s own blood leukocytes when interacting with other cells. It is too soon to tell whether or not immunoregulatory cells play a role in the biology of bladder cancer, but it is obviously a subject for further study.

Finally, another area is rapidly developing of tremendous importance to our understanding of tumor immune reactions. The recent work of Zinkemagel (46) and Zinkernagel (46) suggests that, after infection with virus, the cytotoxic T-cells that are formed recognize altered histocompatibility antigens. That is, the viral infection produces an alteration in the normally occurring histocompatibility antigen of the host such that the T-cell recognizes and responds to that altered self-antigen, which would be an antigen (or TSTA) unique to the particular histocompatibility antigens of the host. Or, in the case of a human, the tumor antigen would be unique to the individual’s own HLA antigens and thus would not often be cross-reacting, since humans express a wide variety of HLA types. The implications of this finding are enormous. It suggests that, even though one virus can induce similar tumors in different strains of animals, the associated tumor antigen will be unique for each of those strains of animals, and the cytotoxic T-cells generated in one strain will not cross-react on tumor cells from a different strain unless they share histocompatibility antigens. This has now been shown to hold true for SV40- and Rous sarcoma virus-induced tumors in animal models (42, 45). Since immune cross-reactions within human tumors would have been taken as evidence indicative of viral etiology and individually specific reactions would be indicative of chemically induced tumors, it becomes apparent that these concepts will no longer hold if the putative human tumor virus(s) interacts with the HLA gene region and produces altered self antigen(s). Considering the polymorphic genetic background of humans and the multitude of transplantation antigens, it is quite possible that tumors manifesting what appear to be individually specific antigens could be induced by viruses after all, in spite of our prior bias that individually distinct antigens are the exclusive property of chemically induced tumors. It is quite clear that major conceptual changes are now going on in the fields of immunogenetics and immunobiology, and only a few were touched on here. It is impossible at this time to tell exactly what impact they will have on the field of tumor immunology and, specifically, human tumor immunological studies. However, it should be quite apparent from the foregoing discussion that these findings have major implications in terms of development of immunodiagnostic and immunotherapeutic measures in man. For instance, vaccination with antigenic allogeneic tumor cells of a histological type similar to that of the tumor of the patient may produce no beneficial immune response if those tumor cells contain a unique antigen(s), because they would not evoke an immune response to the unique antigen(s) present on the tumor of the patient who is vaccinated. Another example is in the area of immunodiagnosis. If tumors have individually distinct antigens, it may be impossible to find a single common bladder tumor-associated antigen for immunodiagnosis. On the other hand, it may well be that current developments will give us the approaches and tools necessary to clearly investigate and understand the immunobiology of human bladder carcinoma in man.

References


Immunological Considerations

AUGUST 1977 2883

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