Immunological Aspects of Cancer

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J. E. CASTRO

INTRODUCTION

An immunological approach to the treatment of cancer has many theoretical features to commend it. There should be specificity, so that tumour cells alone are destroyed whilst normal tissues are unaffected. Provided the tumour is uniform and all of the cells have appropriate antigens, every malignant cell should be destroyed and even distant metastases dealt with. So far these speculative advantages are unfulfilled and the initial optimism that surrounded immunotherapy has not been sustained. Acceptance of the precepts of tumour immunology continues but these disappointing observations had led to increasing scrutiny of certain aspects. The purpose of this chapter is to review the principles which underly tumour immunology and immunotherapy, so that the more detailed studies that follow can be considered in perspective.

TUMOUR ANTIGENS (Chapter 2)

For a tumour to initiate an immunological response, it must possess distinctive antigens. Much of the early work in tumour immunity was confused because it was not appreciated that tumours, like other tissues, exhibit transplantation antigens. Only when syngeneic tumours are used can tumour antigens alone be studied and it was the introduction of inbred mouse strains which allowed Foley in 1953[1] to produce the first evidence for specific antigenicity of experimental tumours. Demonstration of these antigens requires that pretreatment with syngeneic tumour will influence the growth of a subsequent challenge with the same neoplastic cells. If pretreatment alters growth of a different tumour, then cross-reactivity between the tumours used for pretreatment and challenge is evident.

Viral antigens

The initial view was that tumours induced by carcinogens had specific antigens, characteristic of the individual tumour, whereas virally-induced tumours exhibit cross-reactivity. The antigens induced by either DNA or RNA viruses are the same for all tumours induced by a single virus, irrespective of the histology of the tumour, but different from those induced by different viruses. Tumours induced by DNA viruses (polyoma, SV 40, adenoviruses 3, 7, 12, 18 and 31, etc.) have similar immunological findings to each other. They induce neoplastic cells both *in vivo* or *in vitro* which then fail to produce further infectious virus. RNA viruses (mouse leukaemia virus, chicken sarcoma virus, Bittner virus) differ considerably from DNA viruses both in structure and manner of replication.

Persistence of the viral genome in the tumour is indicated by serological demonstration of virus specific antibodies or recovery of infectious virus during hybridization of transformed non-productive permissive cells. From such

observations it appears that perpetuation of the malignant phenotype depends upon the presence of the viral genome.

Information on the transmission of viruses is important both for understanding the relationship between viruses and the cell and as a prelude to the control of infection. Vertical transmission occurs when the virus passes from one generation to the next and the ubiquity of many viruses has led to the suggestion that they are integrated and transmitted with the cellular genome. Horizontal transmission occurs when a virus passes from one individual to another; but vertical and horizontal transmission are not mutually exclusive.

Relationship between tumour and fetal antigens

Individually characteristic tumour antigens have been demonstrated for many chemically and physically induced experimental tumours. However, it is now clear that they also express cross-reacting antigens and there is increasing evidence that at least some of these are phase specific or embryonic antigens. Examples of such antigens are the thymus-leukaemia antigen (TL), Gix antigen and fetal antigens. TL antigens can be detected serologically on normal thymus cells from some mouse strains (TL positive), but not others (TL negative). Leukaemias developing in the negative mice frequently express the TL antigen and it is probable that there is a repressed structural gene coding for the TL determinant which is only derepressed by the malignant process.

Some chemically induced tumours have been shown to have fetal antigens on their surface but the significance of these observations, with regard to them acting as rejection antigens, demands a demonstration that normal immunologically competent adult animals are capable of mounting immune responses against such antigens. There is indirect evidence to support this view, for *in vitro* cytotoxic tests have shown that lymphocytes from tumour bearers are sensitized against fetal antigens. Furthermore, fetal tissues implanted into mice made deficient in cell-mediated immunity, grow considerably larger and with an increased spectrum of tissues than in normal mice. These findings, together with the observation that pretreatment with fetal tissues modifies the growth of a second fetal tissue implant, suggests that the tissues used for pretreatment provoke a transplantation reaction in normal mice.

Parmiani and Della Porta[2] pretreated adult mice with syngeneic adult tissues, sarcoma or fetal tissues or with allogeneic adult tissues and observed the effects on litter size, premature birth and viability of progeny. There was significant reduction in the frequency of pregnancies and in the litter sizes after sarcoma or fetal tissues but not after adult tissues. This suggested that these effects result from the induction of a specific immune cytotoxic action on embryo cells after sensitization to fetal antigens by pretreatment with embryonic or tumour tissues.

In 1906 Schöne showed a relationship between immunization with fetal tissues and growth of tumours. Recent workers, using genetically defined mice,

confirmed this relationship, but whereas most have found that pretreatment with fetal tissues protects against subsequent tumour challenge, others observed enhancement of tumour growth and occasionally no effect. The reason for these contradictory results is not known. It has also been shown that embryo-immune rats are capable of limiting tumour metastases.

Human tumour antigens

In humans tumour-specific antigens cannot be demonstrated by transplantation techniques, and methods rely upon the *in vitro* demonstration of cell-mediated or humoral immunity. Some tests involve the demonstration of tumour antibodies by membrane immunofluorescence, complement fixation or cytotoxicity. Tests for measurement of cell-mediated immunity to tumours involve a cytotoxic assay that measures the ability of lymphocytes to lyse target tumour cells or inhibit their growth. Other tests, for example inhibition of macrophage migration, involve the action of lymphokines (Chapter 10).

By these methods immunological responses to neuroblastoma, malignant melanoma and osteosarcoma have been demonstrated as well as to many other tumours including cancer of the bladder, hypernephroma, testicular tumours, Wilm's tumour, gliomas, meningiomas, leukaemias and carcinomas of the breast, lung endometrium and ovary.

There is the suggestion that some human cancers may be associated with a viral aetiology. For example, there is growing evidence that carcinoma of the nasopharynx results from infection with Epstein–Barr Virus (EBV). Sera from 30 patients with the disease showed an increase in antibodies to EBV, capsid antigen and to the O and R components of the virus. Titres of these antibodies were higher with increasing tumour load but lower in patients who survived a long while after the disease (3–29 years).

The geographical distribution of Burkitt's tumour suggested that it might result from infection spread by an arthropod vector. Cross-reactivity of antigens between different patients with the tumour and the isolation of EBV from cultured cell lines of Burkitt's tumour support this view, but many tumours contain viruses that may be contaminants, and it is therefore impossible to show that EBV is the aetiological agent in Burkitt's lymphoma.

There is also evidence that human tumours express phase-specific antigens (Chapter 4). In 1965 Gold and Freedman[3] reported studies concerned with the antigenic analysis of human adenocarcinoma of the colon. Antitumour serum was prepared in rabbits and rendered tumour specific by absorption. It was found that colon carcinomas were antigenic and a similar antigen was found in fetal gut, liver and pancreas during the first trimester. With the development of sensitive radioimmunoassays, similar antigens were found in the circulation of patients with colon cancers and it was hoped that this would be a useful diagnostic test. However, the test has been found to lack specificity, for, although it is found to be positive in 73% of patients with cancer of the colon

and rectum, 92% with pancreatic cancer and 60% with cancer of the liver, it is also detected in patients with non-malignant diseases, for example in 9% of patients with colonic polyps, 21% with colonic inflammation, 42% with cirrhosis and 53% with acute pancreatitis. The most useful aspect of measurements of carcinoembryonic antigen at present appears to be the early detection of recurrent tumour.

There are many other fetal antigens and alphafetoprotein is one which is probably more tumour specific. This protein is found in 31–78% of the sera of patients with hepatic carcinomas, 80% with testicular tumours, 22% with pancreatic carcinomas and 17% with gastric cancers. The level does not appear to be raised in patients with inflammatory diseases.

IMMUNOLOGICAL SURVEILLANCE (Chapter 9)

The mechanism whereby a host mounts an immunological response against the antigens expressed by a tumour as immunosurveillance. The concept of surveillance was initially proposed by Ehrlich and later substantially modified and developed by Burnet[4]. It suggests that a mutant cell, which has the potential to develop an overt tumour, has at least one antigen with a biochemical sequence different from that normally found in the host. A response is therefore mounted against this antigen and a clone of immunologically competent cells appears and eliminates the abnormal mutants.

The concept of surveillance is accepted by many oncologists although some serious challenges of this theory have recently been made. There is considerable evidence for some form of surveillance, particularly the observations that most tumours possess antigens which are capable of arousing a cytotoxic or cytostatic reaction in autochthonous hosts. Certainly most chemically induced tumours are immunogenic but the quantitative responses vary widely from one tumour to another and occasional tumours initiate no immunity at all. Such non-reactivity may result from serum inhibitory factors, although some tumours have been shown to lack surface antigens. The situation with spontaneous tumours is different. They commonly evoke only a very weak immunological response or none at all. These data are difficult to interpret.

The failure to demonstrate tumour antigens may be evidence against surveillance, but the lack of immunogenicity may be the very reason for the clinical occurrence of such tumours and indeed may be evidence in favour of the hypothesis. The difficulty with surveillance is that by their very nature the abnormal mutants are never present if they have been effectively dealt with. The situation in humans is not clear and whether most human tumours result from naturally occurring oncogenic agents is a matter for debate.

Other methods for investigating surveillance are to use measures which interfere with immunological reactivity, like newborn thymectomy, irradiation or immunosuppressive drugs and to observe the effects on susceptibility to neoplasia. The results are confusing. Nehlsen[5] gave mice long-term rabbit an-

timouse thymocyte serum (ATS). She found the incidence of tumours after this treatment was not increased, but mice given ATS and exposed to an oncogenic virus showed a marked increase of tumours. Similar results have been reported by Balner and Dersjant[6]; mice given ATS alone developed no more tumours than control untreated mice, whereas those given ATS and a chemical carcinogen developed more tumours, at an earlier time than mice given carcinogen alone. The finding that mice deprived of cell-mediated immunity and exposed to an oncogenic agent developed more tumours is evidence in favour of immunosurveillance. However, in the more realistic situation without these agents, the observation that immunosuppressed mice do not have more tumours than untreated mice is evidence against the theory. The only convincing evidence for surveillance in a spontaneous tumour system is the lung adenoma of mice where immunosuppression is associated with a marked increase of tumours. It may be that surveillance occurs more readily in the lung than at other sites.

In human patients taking immunosuppressive drugs after renal transplantation, there is an increased incidence of tumours. In such patients Penn[7] reported an overall corrected incidence of 5.6% compared with 0.058% for a normal age-matched population. However, transplanted patients were more closely observed than controls and many of the tumours were *in situ* cervical lesions or skin cancers which might not be observed in control patients. The histological types of these tumours are markedly different from the normal population, for nearly 50% of the tumours were mesenchymal in origin and many were reticulum cell sarcomas. This suggests that the mechanisms operating in immunosuppressed patients may be different from those involved in normal patients. The observations may be partially explained by the suggestion that immunosuppressive agents may themselves be oncogenic or that the antigen drive of an allogeneic organ graft in combination with immunosuppressive drugs may initiate tumours of the lymphoid system and account for the high incidence of such lesions. Alternatively opportunistic viral infections may be the aetiological agents.

In patients with alterations of immunity there is an increase of tumours, particularly diseases like Wiscott–Aldrich syndrome or ataxia telangiectasia, both of which affect cell mediated immunity and are associated with increased tumours. There is also an increased incidence of tumours at the extremes of life and a relative depression of immunity has been shown in ageing animals and humans.

In contrast, methods which stimulate immunity are associated with less tumours. Administration of BCG has an antitumour therapeutic effect and can lessen the incidence of neoplasia. The results are feeble and rather than demonstrating the efficiency of surveillance mechanisms show that they are naturally weak and can be improved by artificial means.

At a time when the only known function of T lymphocytes was to destroy foreign grafts, immunosurveillance gave a satisfying explanation. Today the

observations suggest that the function of T lymphocytes is to defend against viruses and parasites.

Recently Prehn[8] has suggested that stimulation of the immune response may encourage tumours to develop. Although specific immune reactivity may sometimes be adequate to control a neoplasm, lesser degrees of immune reactivity may promote growth of latent tumours.

Escape from surveillance

It is an all too common observation that tumours develop in animals and men and that they grow progressively and kill the host. There are three possible explanations for this. Firstly, Hewitt[9] has shown that there was no evidence of tumour immunogenicity in isotransplants of 27 different murine tumours of a strictly spontaneous origin. In the case of seven randomly selected tumours, prior *'immunization'* of recipients with autochthonous lethally-irradiated cells *increased* their tumour receptivity. Several experiments failed to give evidence that immunity could be non-specifically induced or that adoptive lymphocyte transfer from sensitized mice could inhibit tumour transplantation or growth *in vivo*. Most experimental studies use tumour systems which entail immunity associated with viral or chemical induction of the tumours. Hewitt[9] feels that spontaneously arising tumours are the only appropriate model for simulation of human cancer. Such a contention excludes the role of viruses or indeed the role of carcinogens in the induction of human cancers and there is considerable evidence that this is not so.

Secondly, the whole concept of surveillance may be incorrect and there is a body of evidence to suggest that this may be the case. Immunodeficient patients exhibit a restricted range of tumours and several diseases with pronounced immunosuppression like leprosy and sarcoidosis do not show an increased incidence of tumours. Naturally-immunodeficient mice (nude mice) or those made deficient by administration of ALS do not show an increased incidence of tumours, although they are more susceptible to infections and viral oncogenesis. Such observations, whilst arguing against a surveillance mechanism mediated by T lymphocytes do not exclude surveillance executed by a non-T cell population, perhaps by macrophages.

The third possibility is that tumour-associated antigens are expressed on the cell surface but they do not function as effective rejection antigens, even though they have the potential to elicit specific immune responses in the tumour bearing host. There are escape mechanisms by which tumours evade the control of immune responses, and several mechanisms of escape have been suggested. For example, when antigenic autochthonous tumours are exposed to immunological reactions that do not entirely eliminate them, immunoresistance may develop in the same way that bacteria develop resistance to chemotherapeutic agents. Other phenotypic changes that resemble immunoresistance may occur, for a persistent immunological reaction may cause

the tumour cell surface to alter or modulate so that it is no longer expressing a configuration which will be recognized by the sensitized lymphoid cells. Sneaking through of tumours is a concept based on the observations of Old *et al*[10]. They found that medium sized inocula of antigenic tumour cells were rejected whereas large or very small injections of tumour cells grow to an irreversible, clinically apparent tumour before an immune reaction is mounted. This may be a very important mechanism in the natural establishment of tumours and it may be that vascularization of the tumour is that event which prevents immunological attack of the tumour.

Whilst the tumour colony is small, proliferation from the host's vascular system begins and most tumours are vascularized by the time they reach 1–2 cm in diameter. In an established tumour, the host vascular endothelium is exposed to immunological attack. Because the tumour is vascularized by the ingrowth of host blood vessels, it is recognized as 'self' (Chapter 11).

The successful escape of tumours from surveillance may result from changes in the host. In animals the response to a variety of antigens, including those of tumours, has been shown to be controlled by genetic factors. Furthermore, patients with tumours show a non-specific depression of immune response or anergy. The central question in relation to this intrinsic immunosuppression and cancer is whether subjects who develop cancer are immunosuppressed or whether the growing tumour induces a state of immunosuppression. The mechanisms involved in this immunosuppression are not clearly understood. It has been suggested that suppressor cells may be involved. Alternatively, successful adaptation of tumours may be due to systemic factors which block the usual interaction of host defences and tumour cells. Excess soluble antigen, antigen/antibody complexes or antibody to either effector or target cells, have all been invoked (Chapter 7).

EFFECTOR MECHANISMS OF TUMOUR DESTRUCTION (Chapters 5 and 6)

The immunological mechanisms of tumour destruction are not known and there is disagreement about the relative roles of the separate components of the immunological apparatus in this process. Different mechanisms seem to dominate in varying situations. Both cell-mediated and humoral mechanisms may be involved. Cytotoxic effects exerted by patients' lymphocytes on tumour cells *in vitro* are assessed in a variety of ways and probably reflect a variety of mechanisms. In any given test the mechanisms involved could depend upon the tumour system, the assay system, the source of target cells, the source and means of purification of effector cells and other factors. Frequently, the data are insufficient for distinction between the possibilities.

The importance of T lymphocytes is clear, for specifically sensitized T lymphocytes are able to destroy tumour cells *in vitro* and *in vivo*. They can transfer immunity to non-immune animals and infusion of syngeneic or

allogeneic immune lymphocytes into tumour-bearing hosts sometimes has therapeutic effects. The T cell system is, however, more complex with sub-populations that include virgin cells, memory cells and killer cells (Chapter 5). Helper and suppressor T cells also have roles in a variety of immunological phenomena. Current studies suggest that T cell cytotoxicity can be divided into an activation and cytotoxic step, both with different antigenic requirements. In some situations T cell activation can lead to activation of macrophages with wide cytotoxic potential.

A particularly important distinction is between T cell and K (killer) cell cytotoxicity. K cell cytotoxicity is absolutely dependent upon IgG and consequently K cell activity is suggested by any diminution of cytotoxicity brought about by anti-Ig (Chapter 5).

Evidence has also been presented for 'N' (null) lymphoid cells exerting an antibody independent cytotoxic effect.

The role of phagocytes in tumour destruction is not clear but resistance of animals to tumours parallels reticulo-endothelial phagocytic activity (Chapter 6). During growth of transplanted, syngeneic tumours, there is an increased production and function of macrophages which results from changes in cellular factors rather than opsonins. Although stimulation of reticulo-endothelial function protects against some tumours, the biological mechanisms involved are not clear. It is well recognized that phagocytic cells are important for initiating, maintaining and regulating immune responses. Apart from processing antigens for lymphoid cells, phagocytic cells may be cytotoxic for tumours. Normal phagocytes may be 'armed' by exposing them to immune lymphoid cells. The arming process had been partially elucidated and it is due to a soluble supernatant factor produced by lymphoid cells. When an 'armed' phagocyte encounters an appropriate specific target cell it becomes hyperactive, the activity of such a cell becomes non-specific and may cause cytostasis of tumour cells *in vitro*. Phagocytic cells have other functions that may be important for control of tumours. They have been shown to be essential for the lymphoid cell response to phytohaemagglutinin. Furthermore, in many tumour situations interaction between cytotoxic lymphocytes and target tumour cells is inhibited by blocking factors; phagocytes, by removal of excess antigen or antigen–antibody complexes from the circulation, may play an important part in restoring a balance between cellular immunity and blocking.

Phagocytic cells may be important for affecting the distribution of metastases. When hamsters are grafted with a tumour which metastasizes widely, there is no phagocytic response in the draining lymph nodes. When a similar tumour, which only rarely metastasizes is used, there is marked hyperplasia in the regional nodes and cells from the tumour are phagocytized in the nodes.

Antibody has a recognized role in certain types of cell-mediated cytotoxicity. That associated with K cells has been described and theoretically T cells could have specificities dictated by Ig absorption on their surfaces. Other