TUMOUR-SPECIFIC ANTIGENS: THEIR POSSIBLE SIGNIFICANCE IN THE ETIOLOGY AND TREATMENT OF MALIGNANT DISEASE

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In the past decade appreciable evidence has accumulated from clinical observation to support the concept that host factors may influence the development and progression of human cancer. While some tumours grow without apparent restraint, others such as carcinoma of the breast and prostate may be inhibited by appropriate endocrine modification (Huggins 1967). Further evidence suggestive of the importance of host factors in human neoplasia derives from the following phenomena: the spontaneous regression of tumours (Everson 1964); the regression of metastases following treatment of the primary tumour and conversely by the sudden appearance of tumours after long latent periods, sometimes coinciding with immunosuppressive therapy (Woodruff 1964, Wissler 1964); the failure of circulating tumour cells and autotransplants to form metastases (Grace 1964, Nadler and Moore 1965, Southam 1964, 1965); the favourable response of certain tumours (for example, placental choriocarcinoma and Burkitt lymphoma) to chemotherapy (Mathé, Dausset, Hervet, Amiel, Colombani and Brule 1964; Fass, Herberman and Ziegler 1970); the histological pattern of reaction of lymph nodes draining the tumour (Black and Speer 1958, Hamlin 1968); and the association of some tumours with abnormal immunity mechanisms (Fairley 1969).

Presently available evidence implicates the immune response as a regulatory factor in these types of neoplasms. This response is effected by lymphoid cells in two ways: indirectly by the production of classical antibody (humoral immunity), or directly without antibody as intermediary (cell-mediated immunity); and its induction depends on the presence of distinctive tumour antigens not present in, or foreign to, normal host tissues. These antigens are the logical target for immunotherapeutic attack. The ultimate aim of research in tumour immunology is thus the development of clinical methods for immunisation. It is already apparent, however, that the study of the immunological characteristics of animal and human tumours will also contribute substantially to knowledge of the causation of malignant disease—particularly with respect to the role of viruses—and will determine the validity of the concept that cell-mediated immunity provides a natural anti-cancer surveillance system.

In this paper the evidence for the existence of tumour-specific antigens in animal and human tumours is discussed; in addition, possible etiological and therapeutic implications arising from experimental studies are reviewed with particular reference to osteosarcoma.

TUMOUR-SPECIFIC TRANSPLANTATION ANTIGENS (TSTA)

Although the rejection of transplanted tumours has long been the subject of investigation, it was not until the availability of inbred strains of animals eliminated histocompatibility differences between individuals that the antigenic status of tumours could be elucidated. In such systems the degree to which an immunised animal is rendered resistant to a challenge inoculum of viable tumour cells is a measure of the immunogenicity of the tumour.

In general, immunisation against experimentally-induced tumours may be accomplished by one of the following procedures: 1) total excision of tumour mass or impairment of the blood supply to the tumour by ligation; 2) multiple implantations of "live" tumour prevented
from progressive growth by exposure to x-irradiation or gamma-irradiation or cytotoxic drugs; 3) inoculation of subthreshold numbers of viable tumour cells.

An additional method of inducing resistance to tumours of viral origin involves pretreatment of adult hosts with oncogenic virus or with homografts of other tumours induced by the same virus. For example, infection of adult mice with polyoma virus does not lead to tumour formation, but as a result of this treatment the mice develop a resistance to transplants of tumours which arise following inoculation of the virus into neonatal mice of the same inbred strain.

Antigenic strength — The strength of tumour antigenicity is usually determined in transplantation tests from the maximum number of tumour cells rejected by immunised but accepted by non-immunised hosts or controls pretreated with irradiated normal tissues. This property varies widely from tumour to tumour, depending on the species, method of tumour induction and the tissue in which the tumour arises (Green, Anthony, Baldwin and Westrop 1967). For example, sarcomas and hepatomas induced by classical carcinogens are among the most antigenic experimental tumours studied, whereas weak antigenicity is in general a property of those tumours which arise spontaneously (that is, without deliberate inducement).

In many instances, immune responses have been demonstrated in animals with primary tumours, and the existence of tumour-specific antigens in a wide variety of experimentally-induced neoplasms has been proved beyond doubt (Klein 1968).

Antigen cross-reactivity — Studies on tumour-specific antigens using transplantation procedures soon revealed an important finding — namely, that tumours induced by chemical carcinogens have individual antigens. It has so far proved impossible to immunise a host with one tumour so as to confer protection against a second tumour of identical morphology and arising in the same inbred strain. It has even been shown that independently induced primary tumours in the same original host possess unique non-cross-reacting antigens.

By contrast, virus-induced tumours carry a virus-determined antigen which is common for all neoplasms induced by the same virus regardless of morphology or strain or even species of origin. Cross-immunisation is thus feasible among tumours induced by the same virus, but not among tumours induced by different viruses.

The phenomenon of antigenic cross-reactivity among tumours induced by the same virus has particularly important implications for etiological studies of tumours of unknown origin. It is difficult to see how virus could induce the same antigenic specificity in cells of different tissues and different species unless it was directly coding for it.

Nature of the antigens — Considerably more is known about virus-determined tumour antigens than of those tumours induced by chemical or physical methods. The cell surface antigen induced by DNA viruses is not identical with the antigens of the virion. This cellular antigen persists even when infectious virus production by the cell has long ceased. As the antigen is specific for the inducing virus, its persistence provided the first evidence that the virus left part of its genetic information in the cell after malignant transformation.

In neoplasms induced by RNA viruses the cell surface antigen might be shared with that of the virion. These viruses mature at the cell membrane by budding, and during this process the virus particles (which are continuously released by the tumour cells) receive an outer coating derived from the cell membrane which can be observed with the electron microscope.

Role in tumour resistance — There is abundant experimental evidence that the tumour rejection phenomenon when studied in highly inbred laboratory animals is induced by tumour-specific antigens, and that these antigens, like normal histocompatibility antigens, are localised on the cell surface where they evoke immunological recognition and initiate the mechanism of rejection (Klein 1970). Their classification as tumour-specific transplantation antigens distinguishes them from other types of tumour antigen which are present in embryonic tissue and occasionally in normal adult tissues, but whose role in tumour rejection has yet to be determined.
THE MECHANISM OF TUMOUR CELL REJECTION

As stated earlier, the immune response against tumour-specific antigens may be mediated either by sensitised lymphoid cells or by humoral antibodies.

Role of lymphoid cells—It has been demonstrated in both in vivo and in vitro experiments that lymph node cells or macrophages from animals immunised against tumour cell antigens specifically inhibit the growth of the corresponding target tumour cells, thus implicating a direct cytotoxic action of the lymphocyte upon the tumour.

Role of humoral antibodies—Circulating antibodies reacting with cell surface antigens are also detectable in some tumour systems. Immune serum in the presence of complement is cytotoxic but not invariably so, since a threshold antigen concentration at the cell surface must be exceeded for a cytotoxic reaction to take place. Even when a cytotoxic reaction does not occur, antibodies may still be present and demonstrable by immunofluorescence. Thus, immunity to virus-induced tumours, especially murine leukaemias, may be passively transferred with specific antiserum as well as lymphoid cells, indicating that humoral cytotoxic antibody is the probable effective mediator of resistance. In contrast, there is no convincing evidence that immunity to chemically-induced tumours may be passively transferred by humoral antibody. In some systems immune serum has produced the completely opposite effect of enhancement of tumour growth (Möller 1964), to which reference is made below.

Immunological surveillance—Burnet (1967) has repeatedly postulated that the raison d’etre of cellular immunity is as a surveillance system against neoplastic mutations occurring in the body and continually suppressed by lymphocytic action. One of the premises of the surveillance theory is that an intact immunological system is an essential prerequisite for the expression of cell-mediated immunity. This hypothesis is supported by a significant incidence of malignant diseases in patients with impaired immunity mechanisms and among those on immunosuppressive agents following organ transplantation. Experimental support for Burnet’s theory derives from the fact that manipulations known to inhibit the immune response such as total body irradiation, neonatal thymectomy or treatment with antilymphocyte serum increase the incidence and hasten the appearance of certain tumours, leaving little doubt that immunological surveillance normally eliminates potentially neoplastic cells in some cases. It is therefore reasonable to suppose that during carcinogenesis, strongly antigenic cells are destroyed due to a satisfactory sequence of immunological recognition and response.

Factors permitting tumour growth—An unsolved problem is why this surveillance mechanism functions satisfactorily in some tumour/host situations but not others. As already implied, this failure may be related to the immune status of the tumour-bearing host which is known to vary for different systems. At one extreme is immunological tolerance occurring with some RNA viruses transmitted vertically from parent to offspring before or shortly after birth: in this situation immune surveillance does not seem to operate. At the other extreme, the signs of an active immune response can be detected in man and animals bearing tumours, although it has seemingly little effect.

Why tumours should grow progressively in immunologically normal hosts is a central problem in tumour immunology. There are several possible explanations which are not necessarily mutually exclusive:

1) It could be a matter of simple cellular kinetics wherein the tumour proliferates more rapidly than lymphocytes can be recruited for immunisation and division. There is appreciable experimental evidence to suggest that the killing of a single tumour cell requires at least a one-to-one confrontation with an immune lymphocyte. Thus if the rate of production of immune lymphocytes at any time lags behind the rate of tumour cell replication, the tumour will have a survival advantage.

2) It has been shown experimentally (Old, Boyse, Clarke and Carswell 1962) that a small embolus of cancer cells does not release antigen into the circulation and consequently does
not effect immunisation of the host. Cells may only be shed in significant numbers when tumour has reached a size beyond the control of the host reaction.

3) There is evidence that certain experimental tumours lose tumour-specific antigen on transplantation within the strain of origin. It is reasonable to suppose that loss of specific antigen by immunoselection may occur in tumours which are not transplanted but which remain sufficiently long in the host in which they arise (Woodruff 1969), a conclusion supported by the fact that many weakly antigenic tumours have long latent induction periods (Baldwin, Barker, Embleton and Moore 1970). Of significance in this context is the fact that many sarcomas have mixed cell populations. Not uncommonly, chondrosarcoma evolves to fibrosarcoma and giant-cell tumour to fibrosarcoma or osteosarcoma. The possibility that elimination of a particular cell type may result in concomitant loss of tumour antigen is clearly feasible.

4) Experimental studies have established that circulating antibodies which are not cytotoxic may coat malignant cells and so prevent their destruction by immune lymphocytes. Such a reaction could conceivably occur in vivo and this could explain why tumour antibodies sometimes cause enhancement (Kaliss 1965).

It is clear that for an effective immune response leading to rejection of antigenic tumour cells, several conditions have to be fulfilled. The host/tumour relationship represents a dynamic situation involving simultaneous development of humoral and cell-mediated responses and growth of the tumour. The existence of physiological or immunological factors advantageous to tumour growth may allow the tumour to reach a critical size beyond the control of the defences of the host.

TUMOUR-SPECIFIC ANTIGENS OF HUMAN TUMOURS

While tumour-cell rejection is the most unequivocal sign of an efficient host response, such experiments in man, although undertaken (Southam 1965), are beset with obvious difficulties. The demonstration of antibodies which react specifically with tumour cells and the pattern of such reactivity among tumours of similar histological type, together with the demonstration of lymphocytes capable of damaging tumour cells in vitro are thus the approaches most intensively pursued. Such reactions have now been demonstrated for Burkitt lymphoma (Klein, Clifford, Klein and Stjernswärd 1966), nasopharyngeal carcinoma (Chu, Stjernswärd, Clifford and Klein 1967), malignant melanoma (Morton, Malmgren, Holmes and Ketcham 1968), neuroblastoma (Hellström, Hellström, Pierce and Bill 1968), adenocarcinoma of colon (Hellström, Hellström, Pierce and Yang 1968), carcinoma of bladder (Bubenik, Perllmann, Helsestein and Moberger 1970) and sarcomas of osteogenic and soft-tissue origin (Morton and Malmgren 1968, Eilber and Morton 1970a, Moore and Price 1970).

In some cases the extent of malignant disease has been correlated with the immunological reactivity of the patient to his own tumour. In patients with Burkitt lymphoma (Fass, Herberman and Ziegler 1970), and malignant melanoma (Lewis, Ikonopisov, Nairn, Phillips, Fairley, Bodenham and Alexander 1969; Fass, Herberman, Ziegler and Kirbyabwire 1970), immune responses to tumour antigens were detected when the disease was localised but generally diminished when the disease was advanced and disseminated.

In patients with skeletal and soft-tissue sarcomas there was a relationship between persistence of a high titre of antisarcoma antibody and a favourable clinical course (Eilber and Morton 1970b). Furthermore, declining antibody titres were seen in all patients who developed recurrence either after resection of the primary sarcoma, or pulmonary metastases. This correlation between the antisarcoma titres and progression of sarcomas may in due course provide useful information in the management of patients with these tumours.

Implications for tumour etiology—To some extent the existence of tumour-specific antigens in some human neoplasms might have been anticipated from the fact that the occurrence of tumour-specific transplantation antigens (TSTA) in animal tumour systems is the rule rather
TUMOUR-SPECIFIC ANTIGENS

than the exception. However, one of the most interesting features to emerge from studies on human tumour antigens is the cross-reactivity which exists for tumours of similar histological type. Antigenic cross-reactivity among animal tumours is a property of those induced by the same virus. The occurrence of cross-reacting antigens in human neoplasms of similar histological type may therefore be indicative of a common virus etiology, but due regard must be given to the possibility that virus may be present in tumour cells only as a passenger and not as a causal agent. It is known that virus-determined antigens can be made to appear in established neoplastic cells by superinfection with a virus known to be oncogenic but bearing no responsibility for the causation of that particular tumour. Such antigens are similar in all respects to the antigens present on and in those cells known to have been induced by the same virus. This phenomenon is known as antigenic conversion.

Etiology of osteosarcoma—An important extension of the work of Morton and Malmgren (1968), who first reported the presence of antibody specific for osteosarcoma cells in the sera of patients with this tumour, has been the demonstration that sera of such patients also react with intracellular antigen in cells of fibrosarcoma, chondrosarcoma, and giant-cell tumour, indicating the presence of common cross-reacting antigens in these histological variants of sarcoma (Moore and Price 1970). In accordance with the principles and limitations outlined above, this phenomenon is suggestive of a common virus etiology for bone sarcomas. More direct evidence for an associated infectious agent derives from the epidemiological studies of Morton and Malmgren (1968), who revealed a high incidence of antibodies to osteosarcoma not only in the sera of patients with this disease, but also in the sera of their relatives and contacts which reacted specifically with common antigens in osteosarcoma. The occurrence of specific antibodies in the sera of normal individuals shows, however, that disease is not an invariable consequence of infection.

Further immunological evidence for an associated infectious agent in osteosarcoma has received support from 1) ultrastructural and tissue-culture studies, and 2) from an investigation into the pathological effects of extracts of osteosarcomas in laboratory animals.

The existence in these neoplasms of viral particles morphologically similar to the avian and murine sarcoma viruses has been reported (Morton, Malmgren, Hall and Schidlovsky 1969; Morton, Hall and Malmgren 1969). Moreover, the passage of an infectious agent from a culture derived from a sarcoma to a culture of normal human embryo cells has been demonstrated. A viral etiology is also suggested by the recent isolation of murine osteosarcoma virus (FB1 virus) that induces osteosarcomas in mice which have many similarities to the human disease (Finkel, Biskis and Jinkins 1966).

Extracts from human osteosarcomas containing virus-like particles produced localized, calcium-containing lesions within a few days of injection into neonatal hamsters and mice (Finkel, Biskis and Farrell 1967). Many of the hamsters developed multiple bone fractures within a few weeks. Viral particles were also identified in the lesions arising at the site of injection of the human material. Most significant, however, is the observation that these extracts induced osteosarcomas when inoculated in newborn Syrian hamsters (Finkel, Biskis and Farrell 1968). Although low, the incidence of tumours was significantly greater than would naturally have been expected in this species.

At present, unresolved questions include the problem as to why there are characteristic patterns of age, sex and site of high incidence for osteosarcoma, and other primary bone tumours, including fibrosarcoma, chondrosarcoma and giant-cell tumours; and secondly, why some infected individuals develop tumours and others do not. Morton and Malmgren (1968) also reported the presence of low antibody titres in the sera of one-quarter of normal blood bank donors to osteosarcoma cells, suggesting that infections with this agent or an antigenically related agent are common. Comparable epidemiological studies in normal animals have frequently revealed antibodies to naturally occurring oncogenic viruses in the chicken (Duran-Reynals 1940) and the mouse (Rowe 1961).
There are a number of clinical observations suggestive of a viral etiology for osteosarcoma: 1) it has a predilection for young people (Price 1955); 2) the disease may occur, albeit rarely, at multiple sites in the same patient simultaneously (Halpert, Russo and Hackney 1949; Moseley and Bass 1956; Price and Truscott 1957), a condition paralleled in dogs (Owen 1969); 3) the disease has occasionally appeared simultaneously in as many as three members of a single family (Roberts and Roberts 1935; Epstein, Bixler and Bennett 1970); 4) other reports of a familial occurrence of sarcomas suggest a genetic origin (Pohle, Stovall and Boyer 1936; Harmon and Morton 1966; Robbins 1967; Schajowicz and Bessone 1967), which is consistent with the vertical transmission of a virus.

Whereas recent epidemiological studies on bone cancer in children (Glass and Fraumeni 1970) and persons of all ages (Boyd, Doll, Hill and Sissons 1969) have not disclosed any time-space variations to implicate environmental factors such as infectious agents, the possible accessory role of these agents in the etiology of osteosarcoma is not necessarily excluded.

Implications for tumour therapy—The possibilities for immunotherapy of human malignant disease in general (Fairley 1969) and of osteosarcoma in particular (Woodruff 1969) have been recently reviewed in the light of current concepts of tumour immunology. The majority of these are still at the stage of basic research; a few are at the stage of experimental therapeutic research, and some have reached the stage of clinical trials. It cannot be overemphasised that all the animal tumour experiments indicate that immunological procedures are inherently incapable of destroying large amounts of tumour. Immunotherapy may therefore take the form of passive, adoptive or active treatment, in conjunction with conventional methods of treatment, such as surgery, chemotherapy and radiotherapy.

Passive immunotherapy consists in the administration of antibody. The most serious difficulty with this form of treatment is the risk of enhancement of tumour growth. A number of clinical trials have been reported which have not provided convincing evidence of any beneficial effect (Southam 1961, Mathé 1969). However, modifications such as the use of serum antibodies as carriers of therapeutic agents which may be either cytotoxic drugs or radioactive substances merit investigation (Ghose, Cerini, Carter and Nairn 1967).

Adoptive immunotherapy consists in the transfusion or grafting of immunologically competent cells. When a short-term effect is required this may be achieved by transfusing lymphocytes; for a long-term effect, stem cells (bone marrow) may be grafted.

In experimental systems adoptive immunity may be transferred with lymphoid cells from animals sensitised with tumour, whether they be uniform with the strain of origin of the tumour (syngeneic), or a different strain of the same species (allogeneic) or a different species altogether (xenogeneic). Thus Alexander (1968) and colleagues induced the regression of primary rat sarcomas by treatment with thoracic duct lymphocytes from sheep specifically immunised against tumour biopsies.

Relatively few clinical trials on adoptive immunotherapy have been undertaken, but there are indications that it may be of benefit in certain circumstances (Woodruff and Nolan 1963, Nadler and Moore 1966, Mathé 1969). Transfused allogeneic lymphoid cells may react against normal tissue antigens of the host which are shared by the tumour, and the success of this type of treatment depends, among other factors, on minimising this reaction.

Active immunotherapy consists in the potentiation of the patient’s own immune defences either non-specifically, by the use of stimuliants of the reticulo-endothelial system, or specifically, with inactivated tumour biopsy or antigenic fractions thereof, or a combination of the two forms of therapy.

The basis for the first of these two approaches are the experimental observations that reticulo-endothelial stimulants such as B.C.G. and C. Parvum retard the growth of chemically-induced tumours and induce resistance to murine sarcomas and mammary carcinomas. This approach has been used by Mathé (1969) and colleagues who treated patients with acute leukaemia with a variety of drugs to reduce the number of leukaemic cells to a minimum and,
when the patients were in complete remission, they were treated with regular immunisation with B.C.G. Seven patients are still in remission more than one year after cessation of chemotherapy: in four the period of remission has exceeded two years; and in one, more than three years.

One preliminary experimental report suggests that combination of chemotherapy and non-specific immunotherapy may also be effective against some established sarcomas, although the order and time of administration of the various therapeutic agents is likely to be of critical importance for each tumour (Currie and Bagshawe 1970).

These and other clinical and experimental observations indicate that non-specific immunotherapy is amenable to much wider clinical trials, especially in patients with solid tumours that have been treated by radiotherapy or surgery.

The potential therapeutic value of autoimmunisation was demonstrated by Haddow and Alexander (1964), who showed that administration of irradiated tumour cell vaccines derived by partial surgical excision from rats with chemically-induced primary fibrosarcomas increased the radiocurability of the remaining tumour.

On the basis of this observation, Anderson, de Sousa, Halnan, Kelly and Hannah (1970) attempted autoimmunisation in one colonic and eight mammary carcinomas by inoculating the patients with suspensions of irradiated (14,000r) cells from their own tumours and examined the lymph nodes draining the sites of inoculation for evidence of activation. The presence of immunologically reactive cells (immunoblasts) in the nodes was taken as evidence of the induction of cell-mediated immunity (de Sousa and Anderson 1970), and a controlled clinical trial of this method by this group is currently under way.

Studies in patients with malignant melanoma have shown that autoimmunisation with irradiated tumour cells leads to the appearance of cytotoxic antitumour antibody (Ikonopisov, Lewis, Hunter-Craig, Bodenham, Phillips, Cooling, Proctor, Fairley and Alexander 1970), an observation which could have important implications for the elimination of blood-borne malignant cells destined to form metastases (Wissler 1964).

In this context the results of the clinical immunotherapeutic studies in osteogenic sarcoma being currently undertaken by Southam (1970) and colleagues are awaited with considerable interest. In this trial patients under twenty-one years of age with osteosarcoma primary in a long bone and who had a potentially curative amputation have been treated with ultra violet-irradiated whole cell suspensions of their own tumours. The evaluation of immunotherapy is based primarily on the time from amputation to the appearance of pulmonary metastases, and there are some indications that this period in patients subjected to immunotherapy may be significantly longer than in controls who underwent amputation only.

**SUMMARY AND CONCLUSIONS**

1. That viruses may be involved in the causation of human tumours has long been suspected but not yet proved. The discovery that osteogenic sarcoma can be induced by viral agents in mice and hamsters makes the proposition that human sarcomas may also have a viral origin basically tenable on presently available evidence. In order to distinguish between passengers and causative agents it will probably be necessary to demonstrate antigenic cross-reactivity in tumours of similar type collected from different geographical areas, and the oncogenicity in subhuman primates of extracts containing virus from human tumours. Such information is likely to become available in the next few years.

2. The demonstration of tumour-specific immune reactions in an increasing number of patients with various forms of neoplasm, including skeletal sarcomas, and the correlation of these reactions with the clinical status of the disease sustains the hope that eventually immunotherapy may contribute to the control of cancer in man.

3. Animal experiments have revealed that the potentiation of immune responses may lead to the elimination of small foci of neoplastic cells. The role of immunotherapy in the treatment
of cancer may therefore be as an adjunct to surgery, radiotherapy and chemotherapy (Alexander 1968). Once the primary tumour has been removed it may be possible to employ immunotherapeutic measures to destroy the relatively few remaining cells that give rise to late metastases; this is particularly apposite to juvenile osteosarcoma.

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TUMOUR-SPECIFIC ANTIGENS


