Tumour immunology: a review[1]

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The study of the immunology of tumours has a long, if not totally respectable, history. The early successes of immunology in the prophylaxis and even in the immunotherapy of infectious disease led to hopes that a similar approach would be successful against malignant disease. Although it soon enough became clear that in its more naive forms this proposition was false, the general idea underlying it was strongly reinforced in the 1950s when it was proposed that the essential function of the cellular immune system – whose only other known function at that time was its clearly non-physiological involvement in tissue graft rejection – was indeed to police the *milieu interieure* for malignant cells. Although the available evidence on the incidence of tumours in T-cell deficiency states in man and animals lent only the most qualified support to any such idea, this concept of 'immune surveillance' provoked a great deal of work and raised considerable expectations of the potential for immune control of malignant disease. These expectations have not so far been met and interest in tumour immunology has waned at perhaps just the moment when advances in the molecular biology of malignant transformation may lead to some genuine advances in the basis of the subject.

The essential question to which tumour immunologists still need to address themselves is whether tumour cells, and particularly malignant tumour cells, do show differences from their normal cellular counterparts that the immune system can recognize. If the answer to this question is affirmative then one would further wish to know: (1) whether the tumour-associated antigens are specific for the process of malignant transformation or whether they represent a marker for the differentiation stage of the cells forming the tumour; (2) whether the antigenic changes are recognized by the host's own immune system or whether they need to be recognized by an allogeneic or xenogeneic immune system; (3) whether the immune response against the tumour-associated antigens could give rise to the death of the tumour cell. This is, in general, a property of antigens on cell membranes.

Tumour-specific transplantation antigens (TSTA) of chemically-induced murine sarcomas

A striking discovery which seemed to give an answer to all these questions was made originally by Gross (1943) and subsequently by Foley (1953), who showed that if syngeneic mice were immunized with methylcholanthrene-induced sarcoma cells they became immune to subsequent challenge with the same tumour. Such immunity could be generated even in the animal in which the tumour had been raised, and it could be transmitted by T-lymphocytes to unimmunized animals. Most strikingly of all, the specificity of the immunity was to the individual tumour concerned and not to other sarcomas, even those raised by the same carcinogen in the same mouse. For a quarter of a century following Foley's work this remained the canonical model of immunity to non-virally-induced tumours. Not only was it a clean and reproducible system to work with but the problem of how a mouse could generate an apparently unlimited variety of distinct transplantation antigens in response to a chemical carcinogen fascinated immunologists. Furthermore, since many human cancers are believed to be due to chemical carcinogens, it was felt that this model might be a valid one for approaching the immunology of human tumours. Unfortunately it now seems virtually certain that this last assumption was erroneous. After many failures to raise antibodies with the same individual specificity shown by the rejection assay, one such monoclonal antibody was finally raised by Lennox and his colleagues (1981). Using this antibody to analyse the

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antigen involved, these workers came to the conclusion that it was a recombinant retrovirus envelope protein and that the TSTA of the particular tumour studied would cross-react with the retrovirus of the AKR mouse. The explanation that the individual tumour-specific antigens are recombinant retrovirus proteins is intellectually satisfying since it explains how a wide variety of different antigens can be generated in a single animal. However, it also leads to the conclusion that this system is likely to be restricted to species such as mice where there is widespread infection with retroviruses and where the possibility of recombination therefore exists. This is not the case in man where, although rare horizontally-transmitted retroviruses have recently been discovered as the cause of adult T-cell leukaemia and of AIDS, there is no widespread endogenous virus and the conditions for forming recombinant retrovirus proteins are probably absent. This is in accord with the fact that similar individual tumour-specific transplantation antigens have never been satisfactorily described in man, although the lack of inbred populations makes the experimentation very difficult. It seems likely, therefore, that the study of chemically-induced murine sarcomas as a model for human cancer should, from the immunological point of view, be treated with the greatest reserve.

Virally-coded antigens on tumours

There are species of mammals other than man with well characterized tumours which are due to malignant transformation by oncogenic viruses. These include both RNA viruses (e.g. retroviruses) and DNA viruses (e.g. herpes viruses). Perhaps the best example, and the greatest triumph so far of tumour immunology, is that of Marek's disease in chickens (Biggs 1975). The herpes virus which causes Marek's disease goes through its productive cycle and produces new infectious virus only in the cells of the feather follicle epithelium. Infection of lymphocytes may be semiproductive where viral antigens but no infectious virus are produced and the cells die; or it may transform the lymphocytes in which case the viral genome is integrated and only the early proteins of the replicative cycle are produced. One of these is a protein that is found on the membrane of the transformed lymphocyte and is here given the acronym 'MATSA' (membrane-associated tumour-specific antigen). All tumours due to Marek's disease carry MATSA, and MATSA is immunogenic in chickens. Birds who develop the lymphoma tend to have no detectable antibody to MATSA, presumably because they are in antigenic excess and because the immune response cannot cope with the extent of tumour growth. However, immunization of animals prophylactically either against the virus (using the herpes virus of turkeys, which is antigenically closely similar but not pathogenic for chickens) or against MATSA (using transformed lymphocytes) is capable of preventing the disease. Widespread immunization of chicken flocks has led to the elimination in them of Marek's disease.

The human disease for which it is hoped that Marek's disease may be a good model are the tumours associated with the Epstein-Barr virus (EBV), namely Burkitt's lymphoma and the nasopharyngeal carcinoma occurring in China. EBV is not normally oncogenic but gives rise to infectious mononucleosis. Although the virus can be made to replicate in certain B-cell lines, it does not do so in most B-cells and the site of its replication in man *in vivo* is not clearly known. It is possible that the oropharyngeal epithelial cells are to EBV what the feather follicle cells of the chicken are to the virus of Marek's disease (Rickinson 1984), and it is in these cells that replication occurs *in vivo* while the lymphocytes infected with the virus are transformed but do not support complete viral replication. Transformation by EBV is associated with the appearance in the cell of a characteristic nuclear antigen called 'EBNA' (Epstein-Barr nuclear antigen) and attempts to find a membrane-associated transformation antigen using antibodies have proved unsuccessful in most hands in spite of very considerable efforts so to do. The existence of a membrane-associated transformation antigen which is given the acronym 'LYDMA' (lymphocyte-dependent membrane antigen) has, however, been shown using a specific lymphocytotoxicity directed against it rather than antibodies (Moss *et al.* 1981), and there is good evidence that there is specific T-cell immunity in seropositive subjects against EBV-transformed lymphocytes. Efforts are being made to

produce a vaccine against EBV aimed largely at preventing the associated malignancies which, particularly in China, are a serious problem. The possibility of immunizing against the transformation antigen using LYDMA-positive cells has not so far been attempted in man. Such a trial would be formidably difficult to do and it is clearly not a harmless procedure to immunize people against allogeneic lymphocytes, thereby compromising the possibility of their being given successful organ grafts.

A good example of a retrovirus-induced tumour is the lymphoma associated with feline leukaemia virus (*see* Rojko & Olsen 1984). The virus produces a variety of different clinical consequences in its host, and leucopenia and immunodeficiency are in fact commoner than the lymphoma. It has been shown that antibodies to the viral transformation antigen 'FOCMA' (the transformation antigens of all virally-induced tumours are given elaborate acronyms – a characteristic property by which they may be recognized!) is associated with resistance to leukaemogenesis. In man it is now established that an endemic form of adult T-cell leukaemia found in South Western Japan is due to a retrovirus (ATLV or HTLV-1) and that the same or closely-related viruses cause sporadic and uncommon T-cell leukaemias in other populations (*see* Karpas 1984). Since this virus appears to be horizontally transmitted, the prospects for vaccination against it appear promising.

Differentiation antigens

Most of the antigens that have been described on human tumours that distinguish them from the normal cells from which they are believed to derive are specific to the histogenetic type of the tumour rather than to the individual tumour or to the process of malignant transformation. Thus there have been described tumour-associated antigens that are common to melanomas, to neuroblastomas, to cancers of the colon and to many others. In an extremely detailed study of the antigens of human leukaemic cells (*see* review by Greaves 1981) it has been concluded that such antigens reflect essentially a particular differentiation stage at which the tumour cells are arrested, i.e. that they represent, to use Greaves' phrase, a 'frozen phenotype'. Although this has not been rigorously established for most other tumours, it seems probable that it will be a more or less general truth and that the tissue-specific tumour antigens are likely to represent differentiation antigens of the various stages of the cell lineage in which the tumours arise. This means that they will not be entirely specific to the tumours involved but that there will be populations of normal cells that carry similar antigens. It follows that if a successful immune attack against such differentiation antigens could be mounted it would carry the danger of damaging the development sequence of the corresponding normal cell type. This might or might not be clinically significant. The ideal TSTA that can be regarded as falling into this group is the immunoglobulin idiotype that occurs on the surface of B-cell lymphomas. This is clearly not an antigen related to malignant transformation or to a particular causative agent: it is a marker for a particular clone of B-cells. If all B-cells carrying this idiotype were to be destroyed it would do the host no harm and would presumably eliminate the tumour. There is, however, a major difficulty in mounting such an immune response if the corresponding immunoglobulin is secreted in large amounts into the serum, but tumours occur which do not secrete and these have been used as targets for immunological tumour elimination. The elegant studies of Stevenson and his colleagues (1982), using a guinea-pig B-cell lymphoma, have shown that successful immune attack on the idiotype of this tumour can be mounted but that it requires the use of effectively monovalent antibodies, since bivalent antibodies cause capping and modulation of the immunoglobulin from the tumour cell so rapidly that complement-mediated cell destruction does not occur.

Oncofetal antigens

Tumour cells can sometimes 'switch on' genes which are more usually associated with much earlier stages (and sometimes only fetal) development. An interesting experimental example is the recent demonstration by Brickell *et al.* (1983) that particular antigens in the major histocompatibility complex (MHC) which are not normally expressed in adult cells may be

transcribed and expressed in malignantly-transformed lines. There is also work by Medawar & Hunt (1978) showing that immunization with fetal tissue can protect mice from the subsequent induction of tumours by methylcholanthrene. These authors would like to believe that this is the same phenomenon that underlies the epidemiological finding that women who become pregnant early in life suffer from a lower incidence of breast cancer than women who never become pregnant at all.

There is also an extensive group of secreted oncofetal antigens of which alphafetoprotein, the fetal equivalent of albumin, produced by teratomas and hepatomas, is the best known example. Such secreted antigens do not, however, form suitable targets for tumour rejection.

Finally, there is a group of antigens that are probably best described as the product of disturbed metabolism. If a cell is growing unusually rapidly or has an unusual chromosome constitution, it is likely that the carefully orchestrated enzyme formation which is required for the proper metabolism of its various components may become unbalanced and that products are synthesized which are not made in a normally-growing cell. Such a process is known to affect carbohydrates, and improperly glycosylated glycoproteins are a feature of malignant cell growth – and probably of other varieties of rapid and disorganized growth. Such abnormal glycosylation appears to be the basis of the group of antigens known as carcinoembryonic antigen; and, perhaps more specifically, may be the cause of the observation that tumours may occasionally not represent accurately the host's genetic blood group make-up in that the final steps of glycosylation may be either absent or incorrectly performed. Although there are rare examples where antibodies to blood group antigens present on a tumour but absent in the host are capable of causing tumour rejection, such products of improper metabolism have been more widely used as diagnostic markers for tumours than as a target for tumour rejection.

Antigenic markers for malignancy in general

The desire to have a single test for identifying any malignant disease in man has led over a long period to attempts to define serological reactions which can be used to diagnose cancer as such. This field of endeavour, has, however, a dismal track record and is admirably reviewed by Currie (1982). Such tests as the Makari skin test, the macrophage electrophoretic mobility test (MEM) and the structuredness of the cytoplasmic matrix (SCM) test have all failed to live up to the claims that were made for them. These tests have used unconventional immunological techniques which have not been validated in well understood immunological systems.

New candidates for antigens present on all or nearly all tumour cells continue to be reported, however. One carefully studied new candidate is the Ca antigen of Harris (1984) which again is carbohydrate in nature. Carbohydrate antigens present on the erythrocytes of only tumour-bearing subjects have also been reported (Metcalfe *et al.* 1984). From the published data so far it seems unlikely that these will turn out to be universal tumour markers, although the extent to which they will be clinically useful remains to be evaluated.

Immune response to tumour-associated antigens

Where tumour-associated antigens have been shown to exist, either or both the formation of specific antibodies and the generation of T-cell reactivity have been demonstrated. Where the antigens are present on the cell membrane (for example, the virally-coded transformation antigens on virally-induced tumours, or the TSTA of chemically-induced murine sarcomas) it has been shown that such cells can often be lysed *in vitro* by the serum of immunized animals in the presence of complement. The lysis of tumour cells by antibody and complement is, however, more difficult to bring about than is the lysis of erythrocytes or even of non-growing nucleated cells such as lymphocytes, and there is experimental evidence that this relative resistance to complement-mediated lysis is due to membrane repair mechanisms. It has been shown that poisoning cells in such a way as to impair membrane synthesis allows them to be lysed much more readily by the complement system (Schlager *et al.* 1978). It is perhaps therefore not surprising that experiments on tumour immunity

in vivo generally show that the immunity can be transferred not with serum but only with T-cells.

In vitro testing by the microcytotoxicity assay frequently demonstrates killing of tumour cells by the cytotoxic T-cells of tumour-bearing animals. However, *in vivo* experiments in mice tend to show that it is the Lyt 1+2− cell that transfers tumour immunity and suggests that it is not the cytotoxic T-cell but the mediator-secreting T-cell which is most effective in transferring antitumour activity, and this suggests that the final effector cell is likely to be a macrophage or an NK-cell stimulated by the mediators produced by the T-cell; and indeed in many systems this can be shown to be the case.

It is of interest that the mediator systems of the immune response – the complement system, the activated macrophage, and the NK-cell – all seem to show some degree of preferential activity against tumour cells compared to their activity against, for example, normal lymphocytes. This apparent capacity of the effector systems of the immune response to recognize the malignant phenotype has given rise to a revised version of the idea of immune surveillance, namely that it is not the T-cell system that controls the body's response against the emergent tumour cells, but that it is the effector mechanisms themselves that have this capacity and are capable without specific immune recognition of reacting against such cells. The evidence in favour of this view is highly circumstantial. The best is the evidence of Karre *et al.* (1980) that the resistance to leukaemia in mice is associated with the high NK-cell phenotype and that the grafting of NK-high bone marrow to NK-low marrow confers an increased resistance to leukaemogenesis upon them. The 'beige' mouse has a genetic defect affecting NK-cells (Roder & Duwe 1979), which is believed to be the equivalent of Chediak-Higashi syndrome in man, and this is associated with some increase in tumour incidence but the effect is not striking. Furthermore, the genetic deficiency which involves the failure of lysosomal fusion and the formation of characteristic giant granules in macrophages and polymorphs is by no means restricted to the NK-cell.

Macrophage defects in man generally lead to infections with intracellular parasites rather than a particularly high incidence of tumours, and complement deficiencies also give rise to problems with infections and with immune complex disease rather than with tumours. The idea may again, therefore, be treated with some reserve.

Mechanisms of tumour escape

A further question of considerable significance is why it is that even when specific immunity against tumours can be demonstrated by suitable assays, the *in vivo* consequence is usually not tumour rejection but tumour growth. The answer here again is not likely to be single but to vary from circumstance to circumstance.

One possibility that is canvassed from time to time is that some of the immune reactions which can be demonstrated *in vitro* are in fact artefactual and do not represent real phenomena. There is indeed reason to believe that some of the phenomena that have been studied over the years are artefactual. A particular cause for confusion arose from the growing of tumour cells *in vitro* in xenogeneic sera (Golstein *et al.* 1979). It is now well recognized that under these conditions cells, and particularly rapidly-growing tumour cells, pick up from the xenogeneic sera antigens presumably glycolipid in nature that become firmly bound to the tumour cell membrane and there elicit immune responses which may give spurious evidence of tumour specificity. Now this phenomenon has been recognized, however, it is clear that it does not account for all the antitumour reactions discovered.

Secondly, it is self evident that if an immune response is to cause tumour regression it must destroy cells more rapidly than new cells are generated by tumour growth. Since the immune reactions are often quite weak and since the immune mechanisms may have adequate access only to the outside of the growing tumour (if the inside does not have adequate vasculature to allow either the humoral or the cellular components of the immune response free access), it is quite likely that in many cases immune destruction is indeed too slow to do more than to slow down the rate of tumour growth. This, indeed, is the most usual consequence of immunizing experimental animals against chemically-induced tumours.

Immunity can be demonstrated by a greatly-slowed growth rate, but it frequently cannot be demonstrated by the regression of established tumours. It may be argued that since the duplication time of a human tumour is often in the region of 40 days compared to the 48 hours that is seen in murine chemically-induced tumours, this particular problem may be less important in man.

It may also be the case that weak immune responses may stimulate the growth of tumours rather than suppress it. This is a point of view put forward by Prehn (1972) and may explain the phenomenon of 'sneaking through' where very small inocula of tumour cells grow better than somewhat larger ones. Although there are examples of an immune response stimulating tissue growth (e.g. the effect of the long-acting thyroid stimulator on thyroid growth), the mechanism of growth enhancement by antitumour immunity is not established and there are few adequate test systems on which to investigate it.

The possibility that tumours produce immunosuppressive agents that suppress the immune response *in vivo* is also real. Alphafetoprotein is a well known tumour product that can be demonstrated to have immunosuppressive activity. It is, however, to be doubted whether such immunosuppression would be important early in tumour development.

The major mechanism which for many years has attracted the attention of immunologists is the possibility that the potentially rejecting T-cell response is inhibited by the presence in the plasma of blocking factors. This phenomenon can readily be demonstrated *in vitro* where the microcytotoxicity assay is used to show tumour killing. It is typically found that a tumour-bearing animal's lymphocytes demonstrate positive cytotoxicity which can be blocked by its serum. The nature of the blocking factors has been investigated over many years and they have been found on occasions to be antibodies, antigen-antibody complexes and free tumour antigens. Immune complexes as blocking factors were given particular attention (*see* review by Hellstrom & Hellstrom 1974) and this gave rise to attempted therapeutic interventions by plasma exchange (and sometimes more elaborate methods) to remove immune complexes. The experiments by Terman *et al.* (1980) using staphylococcal protein A columns to remove immune complexes were perhaps those with the most dramatic effects, producing – in some instances – tumour necrosis very rapidly after extracorporeal circulation through such columns. It is, however, no longer believed that this tumour necrosis is due to the removal of immune complexes, and it is rather suspected that factors eluting from the columns will produce tumour necrosis by mechanisms analogous to Coleys' toxin or tumour necrosis factor.

The liberation of free tumour antigen is a theoretical problem in all attempts at tumour therapy because it is difficult to envisage situations where the initial immune attack upon a tumour, when successful, will not generate a good deal of soluble antigen or membrane-bound fragments of antigen which would act as decoys for lymphocytes or antibody.

Prospects for tumour immunology

The data reviewed so far do not lead to the conclusion that there are immediate prospects for the immunological control of malignant disease. It would, however, be wrong to paint too pessimistic a picture. It is in the first place clear that where tumours carry major viral transformation antigens, the possibility of preventing such tumours by immunization is very good. The two major oncogenic human tumour viruses, hepatitis B and the EB virus, as well as the more recently recognized human T-cell leukaemia viruses, are transmitted as virions and not genomically which suggests that adequate immunization programmes should be able to eliminate these viruses from the population. Thus there is every reason to believe that the hepatitis B vaccines already available or just becoming available will, if used on a sufficient scale, be able to eliminate hepatomas in those parts of the world where they are so common. Vaccines against the EB virus and the human T-cell leukaemia viruses are not so far available, but it is likely that they will fairly rapidly become so. In the case of EB virus, universal vaccination may well prove worthwhile. With the human T-cell viruses where the disease, except in the endemic area of South West Japan, is so rare, the benefits may be relatively much less. In the remainder of human tumours, where there is really no evidence

for a viral cause, the prospects are much less good. Nevertheless, there are situations such as non-secreting B-cell lymphomas where tumour-specific transplantation antigens exist, and here the problems are those of achieving adequate immunization either actively or passively. The therapy of such tumours with monoclonal antibodies is already being attempted either using the antibodies *per se* and relying on the human complement system to produce lysis, or by using immunotoxins and coupling the antibodies to ricin or similar toxic agents. Here the problems of antigenic modulation, of selecting lines of tumour cells that do not bear the antigens and of achieving an adequate kill are still to be overcome, but it is likely that such technical problems will be solvable. Similarly, it may be possible to use passive immunotherapy with monoclonal antibodies against tumours which show differentiation antigens on their membranes where such differentiation antigens are not essential for the survival of the normal cells. It is quite possible that melanomas and neuroblastomas and other tumours may become treatable in this way, even though the antigens concerned are not truly tumour-specific.

It is also to be anticipated that the techniques for the active immunization of patients against such tumours will be markedly improved. There has been considerable experimentation in methods of enhancing the immunogenicity of tumour antigens in the autochthonous host, and in mice some success has been achieved. One such system involves the coupling of tuberculin to tumour cells and then using these coupled tumour cells to immunize animals which have delayed hypersensitivity to tuberculin (Vyakarnam *et al.* 1981). This has proved to be a potent method of enhancing immunogenicity and is analogous to the well known hapten-carrier effect in antibody formation. It has been found to be effective in mice not only in enhancing tumour immunity to methylcholanthrene-induced tumours but in showing up antigenicity in an epithelial tumour where conventional immunization was found to be entirely non-immunogenic.

It would therefore be wrong to believe that no progress in tumour immunology has been made, and if the expectations of the 1980s are lower than those of the 1960s it is likely that they will not be equally disappointed.

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