Surgical Review 3

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PREFACE

Surgical Review 3 presents, as its predecessors, the personal views of experts invited to write on subjects in which, in many cases, they have themselves made a substantial contribution. The range of subjects chosen is wide and reflects the fact that surgical management has been much improved by advances in other fields. Thus, for example, one can read of the potentials of the interferons, digest a pragmatic guide to nutrition of the surgical patient and learn of the progress in post operative pain management, in addition to reviews of more conventional surgical subjects such as rectal carcinoma and amputation. We have, as before, attempted to keep editing to a minimum hoping that the freshness of approach is unimpaired.

All of the credit for the book's speedy production lies with the publisher's team.

J S P Lumley
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IMMUNOTHERAPY FOR CANCER: THE IMPOSSIBLE DREAM?

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This chapter is an attempt at summarising the complex, confusing and frequently controversial subject of tumour immunotherapy. It is divided into three sections: the first reviews the scientific background, the second considers the results from clinical studies reported to date and the third is a personal view of the present status of immunotherapy for cancer and the prospects for its future development.

SCIENTIFIC BACKGROUND*

The search for human tumour antigens

The first major stimulus to twentieth century tumour immunology came in 1906 from Paul Ehrlich's observation that when a growth was transplanted from one animal to another of the same species, it failed to progress and was apparently destroyed by the new host. This was taken as clear evidence that immune mechanisms could lead to tumour rejection. Subsequently however, it was observed that a similar rejection was seen when normal, nonmalignant, tissues were transplanted. In all these studies the animals used were not inbred and so were genetically dissimilar (allogeneic), thus what Ehrlich and others had in fact observed was the general phenomenon of transplant rejection rather than a specific antitumour effect. Later work defined the basis for transplant rejection by identifying that all cells carry histocompatibility antigens, which are genetically determined, and which provoke a state of immunity in allogeneic hosts, leading to their destruction. Further studies on tissue transplantation over the last decade or so have defined a range of human histocompatibility antigens, known collectively as HL-A antigens, which are carried on the cell surface and are genetically determined by the major histocompatibility complex (MHC) at the HL-A locus on the C-6 chromosome pair.

* Detailed references have not been given in this section but the interested reader should consult References 1–14 for recent reviews covering various aspects of this topic.

Hopes that specific antitumour immunity might still be demonstrated were resurrected by the work of Ludwik Gross in 1943. He used inbred C3H mice which were genetically identical (syngeneic) and showed that a methylcholanthrene induced tumour raised in one mouse was rejected when transplanted in a syngeneic recipient suggesting that the tumour possessed antigens which were not present on normal tissues. This was confirmed by subsequent studies in which both normal and malignant tissue were transplanted. In these experiments the normal tissue grafts took whereas the tumours were rejected, so although the mice were identical with respect to histocompatibility antigens the cancer was recognised as foreign by the recipient's immune system. Thus the tumour must have possessed specific antigens making it immunologically distinct from normal tissue; these have been termed tumour specific transplantation antigens (TSTA). Such antigens have now been identified on a number of chemically or virally induced animal tumours. Hopes that similar TSTAs might be present in man were raised by the discovery of the oncofetal antigens.

In 1965 Gold and Freedman used immunological assays to identify a glycoprotein that was released from human colon carcinoma but was not present in normal large bowel tissue [15]. The same material was also found in fetal gut cells and hence was termed carcinoembryonic antigen (CEA). Early hopes that CEA represented a tumour specific marker were shown to be unfounded when the protein was detected in the serum of a proportion of patients with various nonmalignant conditions including Crohn's disease, ulcerative colitis, alcoholic cirrhosis and pancreatitis. So, although serial estimations of CEA levels have been helpful in monitoring the progress of patients with large bowel cancer, CEA assay has not provided a definitive diagnostic test for this condition. Even more disappointing, in general terms, was the realisation that no cell mediated immune response could be demonstrated to CEA either in vitro or in vivo and thus it would not provoke a rejection reaction. Although a number of other human tumour related antigens have been described they all share the very weak immunogenicity of CEA and have been collectively termed as tumour associated antigens (TAA) in order to distinguish them from potentially more significant TSTAs.

The failure to identify TSTAs in any common human tumour has led some authorities to suggest that the whole concept of tumour immunotherapy has no foundation – if tumours are not antigenic how can the immune system be expected to identify them as foreign and destroy them? This is a sweeping, and possibly unjust, condemnation, but a valid corollary is that much of the laboratory work carried out in support of tumour immunology has involved induced animal tumours with very active TSTAs and that these probably represent a totally inappropriate model for the human situation and have been, at best, irrelevant and possibly dangerously misleading.

If strongly immunogenic TSTAs cannot be demonstrated on human tumours why should one expect immunotherapy to play a part in cancer management?

The case for continuing rests on at least two separate arguments. Firstly a methodological point: the easiest ways of demonstrating TSTAs rely on transplantation experiments in syngeneic populations, such studies in man are both impractical and unethical and consequently progress towards defining human TSTAs has been delayed. Equally well, human TSTAs may provoke blocking antibodies which coat the antigenic site and inhibit their detection. In both instances the conclusion is the same: TSTAs may well exist but we have failed to devise adequate techniques for demonstrating their presence.

The second argument brings together a number of items of circumstantial evidence based on clinical observations which accumulated during the 1960s and pointed to immunological controls in cancer, some of these may be summarised as follows:

a) Immunological deficiency increases the risk of cancer – in children with primary immune deficiency disease the risk of malignancy is 100 times greater than in a normal population and in those undergoing iatrogenic immunosuppression following organ transplantation, tumours are from 10 to 100 times more frequent than in age matched controls.

b) The body has the capability to control or destroy relatively small numbers of tumour cells – evidence for tumour control comes from post mortem studies in conditions such as neuroblastoma and thyroid and prostate carcinomas where the incidence of occult tumours exceeds clinically detectable disease by as much as 40:1, similarly the long dormant periods seen between primary treatment and the appearance of metastases in some cases of melanoma and breast cancer have been cited to support this concept. Evidence for tumour cell destruction is provided by the observation that, for a number of cancers the proportion of patients who have circulating tumour cells at the time of their primary treatment is far greater than the number who eventually develop metastases and is further supported by the occasional reports of spontaneous regression of metastases following excision of a primary lesion.

c) The immune status of cancer patients at the time of presentation correlates with their prognosis – intact immune function carries a better outlook than impaired immunity. A view substantiated by the finding that tumours with a lymphocytic infiltration carry a better prognosis than those with no such reaction.

As these clinical data were accumulating, understanding of the effector limb of the immune response was also progressing. The overall division into humoral (antibody) and cellular arms had long been recognised and in the 1960s specific subpopulations of lymphocytes were defined which appeared to relate to these activities. Initially three classes of lymphocyte were identified: T cells, processed by the thymus gland, B cells (initially described in birds where they were derived

from the bursa of Fabricus, in man they are probably bone marrow derived), and null cells which were neither B or T cells. B cells were shown to play a key role in humoral immunity as they transformed to become the plasma cells which were responsible for antibody production. T cells were considered to be primarily involved in cell mediated immune responses such as delayed hypersensitivity and were identified as the effector cells in transplant rejection.

Immune surveillance

These discoveries coupled with the clinical observations outlined in the previous paragraph were used by Burnet, to elaborate on an idea hinted at by Ehrlich, when he proposed the concept of immunological surveillance [16]. The basis of his hypothesis was that the prime purpose of the MHC/HL-A antigen system was to work in conjunction with cell mediated immune responses to distinguish self from nonself as a defence against spontaneous tumours. In other words, when a somatic mutation converted a normal cell to its malignant counterpart, changes in the HL-A antigen characteristics of the cell surface would distinguish it from neighbouring cells and lead to its destruction by T lymphocytes. All the above clinical observations appear to accord with this theory, which rapidly gained wide acceptance although based entirely on circumstantial evidence. The first doubt to be raised was the question 'If surveillance exists, how do some tumours survive to become clinically apparent?' This was rapidly answered and a number of possible explanations for tumours escaping from surveillance were proposed, these included: insufficient antigenicity (some tumours would have only weakly immunogenic TSTAs and would sneak through the defence system unrecognised); antigenic modulation (with successive divisions the neoantigens of the cells may be lost or diminished); immunosuppression (some form of local or general defect in the immune system might allow the tumour to grow). Recently, however, the theory of immune surveillance has come under more severe attack as a result of both a deeper understanding of some of the clinical data and increased knowledge of the immune system itself. On the clinical side, for example, it has been pointed out that the theory fails to explain why lymphomata are so much commoner than other tumours in congenitally immunodeficient children, or why the incidence of cancer is not increased in such immunosuppressive illnesses as leprosy, sarcoidosis and chronic renal failure. Also it has been suggested that much of the clinical data presented in support of the theory could be explained on the basis of hormonal or other nonimmune mechanisms. At the same time understanding of the T cell system has increased considerably and at least three major subsets of cells have been identified: T helper and suppressor cells (T_h, T_s), which interact with B cells to regulate antibody production (and are themselves in turn controlled by the Ir genes on chromosome pair C-6) and cytotoxic T cells (Tc) which destroy foreign cells. But further studies of the function of the T cell system have indicated that its major activity seems to be directed against viral infection rather than

detection and destruction of autologous tumour. This realisation has led to doubts about the role of T cells in immune surveillance and these were reinforced by the recent observation that both immunosuppressed mice and nude (congenitally athymic) mice, which have no T cells, fail to show an increased incidence of spontaneous tumours. At first sight, this observation seemed to conclusively disprove the concept of immunosurveillance and once again bring into question the whole concept of immune defence against cancer. An alternative response, however, was to suggest that some cell other than the T lymphocyte represented the effector arm of the surveillance mechanism. Killer (K) and natural killer (NK) cells are possible candidates for this role. K cells are non-B, non-T lymphocytes which in the presence of antibody can cause tumour cell lysis in a reaction which has been termed antibody dependent cellular cytotoxicity (ADCC). NK cells have many of the morphological characteristics of T lymphocytes but they are not found in lymph (they reside in lymph nodes, the spleen and blood) although they are present in large numbers in both thymectomised and nude mice, and have also been demonstrated in man. NK cells have an apparently nonspecific direct cytotoxic action against a wide spectrum of tumour cells although the precise details of the regulation, and mechanisms, of their activity remain unclear.

The possibility that the K or NK cell might substitute for the T cell as the active agent of immune surveillance has not completely restored faith in the theory which is still open to considerable criticism and, at best, requires radical modification. The neatness of the overall concept, however, remains appealing and many investigators will require stronger evidence than can be presented at the moment before abandoning it completely.

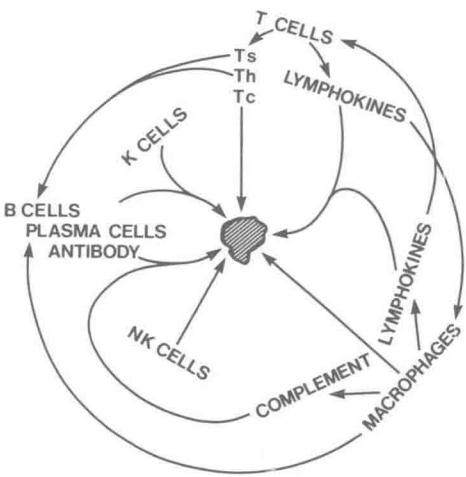

Figure 1. Summary diagram showing some of the components of the immune response and their interrelationships

Other factors in host-tumour responses

In recent years it has been realised that macrophages, and their monocyte precursors, are another group of cells involved in immune responses against tumours. It had been appreciated for some time that macrophages were essential for processing antigens in order to stimulate the appropriate responses in B and T lymphocytes but it is now also recognised that a wide range of stimuli render macrophages directly cytotoxic to tumour cells. Depending on the nature of the stimulus the cells may be primed either to attack a broad spectrum of malignant cells (nonspecific cytotoxicity) or to distinguish a single type of tumour for destruction (specific cytotoxicity). At present increasing importance is being attached to the macrophage-monocyte system in host-tumour reactions and clinical observations such as the correlation of sinus histiocytosis (evidence of macrophage activity) in tumour draining nodes with an improved prognosis and impaired macrophage function in patients with advanced disease have been used to support the experimental data. It has also been realised that laboratory preparations of lymphocytes have often been contaminated by macrophages and that activities previously attributed to T or B cells might actually be mediated by the monocyte-macrophage series.

Even this simplistic summary provides at least five possible effector mechanisms for immunological destruction of tumour cells: humoral antibody, T_c lymphocytes, the macrophage-monocyte system, K and NK cells (Figure 1). It is increasingly clear that these different systems are interdependent and that no one mechanism is entirely autonomous. Two things remain very unclear: how are these various functions regulated and how do they relate to the control of human malignancy? The first point has been partially explained by the demonstration that both macrophages and lymphocytes are secretory cells giving rise to a large number of nonantibody mediator substances which have been generically termed lymphokines. More than fifty macrophage derived lymphokines have been described ranging from well defined entities such as the components of the complement system to more speculative substances such as lymphocyte activating and colony stimulating factors. Similarly more and more lymphocyte products are being identified including interferons and macrophage arming and inhibiting factors. The presumption is that these substances are an integral part of immune response regulation but just where and how they fit into the overall pattern is still a matter of research and debate. It is also probable that some of these lymphokines may have a direct cytotoxic effect on tumour cells adding a further complexity to the host-tumour interaction. As to which effector mechanism(s) may be significant in human cancer defences, there is very little hard data on which to base an assessment. At present, interest is centred on the macrophage system, K and NK cells with T_c cells, humoral antibody and lymphokines relegated to a minor role but the emphasis is continually shifting and any definitive statements at this time would be premature.

TABLE I. In vivo tests of overall immune function

	ANTIGEN	
	Cellular immunity (delayed hypersensitivity reaction)	Humoral immunity (antibody production)
Primary response	Dinitrochlorobenzene (DNCB) BCG	Vi antigen of *E. coli* Keyhole-limpet haemocyanin (KLH)
Recall response	Mantoux (PPD) Streptokinase Idornase (Varidase) Mumps antigen	Tetanus toxoid Diptheria toxoid

These tests rely on challenge with various antigens. Depending on the choice of antigen one may assess either cell mediated or humoral responses. For each system, one may use antigens which the subject has previously encountered, testing only the effector pathway (recall response) or a new antigen which will test both afferent and efferent limbs of the response (primary response).

TABLE II. Measurement of individual components of the immune response

	Quantative	Qualitative
Lymphocytes B cells T cells Null cells	Total lymphocyte count EAC Rosette test E Rosette test By subtraction of B and T cell count from total	Stimulation with various mitogens to transform lymphocytes to lymphoblasts. Agents used include: phytohaemagglutinin (PHA), concanavalin-A (Con A) – primarily stimulating T cells and pokeweed mitogen (PWM) primarily stimulating B cells
K cells NK cells	No test in routine use No test in routine use	Tests rely on showing cytotoxicity to susceptible cell lines, either alone (NK) or coated with antibody (K), of cell suspensions from which B cells, T cells and monocytes have been removed and comparing the degree of cytotoxicity with normal controls
Monocytes/macrophages (in man this is usually confined to tests on peripheral blood monocytes)	Monocyte count (mononuclear cells are separated on Fycoll-Hypaque gradient and then transferred to glass where the nonadherent lymphocytes are removed by washing)	Demonstration of increased motility, adherence or phagocytosis following nonspecific stimulation with agents such as *C parvum,* BCG or endotoxin
Antibody	Immunoglobulin measurement by immunodiffusion methods	Challenge with antigens (see Table I)
Complement	Measurement of components of the complement system by immunodiffusion methods they bind to washed RBCs,	Haemolytic assays (ability of serum to lyse antibody coated RBCs) or immune adherence (testing ability of serum to coat cells with C3 so they bind to washed RBCs, which have C3 receptors

TABLE III. Summary of approaches to clinical cancer immunotherapy

1. Passive immunotherapy
   - Transfer of antibody in blood or serum
   - Monoclonal antibodies

2. Adoptive immunotherapy
   - Transfer of 'immune' lymphocytes
   - Lymphocyte products
     - Transfer factor
     - Immune RNA
     - Lymphokines
       - Interferon

3. Active specific immunotherapy
   - Immunisation with tumour cells or tumour cell extracts

4. Local immunotherapy

5. Systemic nonspecific active immunotherapy
   - BCG, mer-BCG
   - *C parvum*
   - Levamisole
   - Thymosin

To complete the picture of tumour-host interactions it should be remembered that in cancer patients one is not dealing with normal individuals and both the disease and its treatment may alter their immunological integrity. In some conditions specific immunological abnormalities have been identified; for example, Hodgkin's disease is associated with defective T cell function, myeloma patients show impaired antibody synthesis and in chronic lymphatic leukaemia immunologically competent B and T lymphocytes are suppressed by their malignant counterparts (although, surprisingly, in acute lymphatic leukaemia humoral and cellular immunity remain initially intact). In the majority of tumours, however, immune function appears normal during the early stages of disease with progressive failure of both humoral and cell mediated reactions as the tumour progresses. This failure may be exaggerated by the effects of treatment: surgery, radiotherapy and cytotoxic chemotherapy all depress immune function and whilst this effect is usually transient, radiation induced T cell inhibition may persist for a year or more after the completion of treatment. Tables I to III summarise the tests which are currently available for assessing the immune function of cancer patients.

Comment

No one would deny that malignant cells are biologically distinct from normal cells, cancer immunotherapy is based on the belief that they also differ immunologically.

Although a considerable volume of indirect evidence exists to support this belief until strongly immunogenic TSTAs are identified on human tumours the final proof needed to convert hypothesis to fact is lacking. The failure to demonstrate such TSTAs should not, however, be viewed as an absolute barrier to clinical investigation of this area. Many major therapeutic advances have resulted from empirical approaches with elucidation of underlying biological mechanisms coming years after the treatment has been accepted as established practice. The failure of conventional approaches to cure more than 50 per cent of cancers justifies the pursuit of clinical studies in immunotherapy. There is no doubt, however, that the scientific rationale for such trials is less certain than many enthusiasts have suggested and frequently one is left feeling that the complexity of the immune response has been used to obscure the fundamental fact that its relevance to human defence against neoplasia has not been conclusively proven.

RESULTS FROM CLINICAL TRIALS

Over the last 100 years there have been numerous clinical experiments with immunotherapy in cancer. These may be classified in five broad categories: passive immunotherapy, adoptive immunotherapy, active specific immunotherapy, local immunotherapy and systemic nonspecific active immunotherapy.

Passive immunotherapy

This involves the transfer of serum components, usually antibodies, thought to have antitumour activity. A number of case reports of regression in malignant melanoma following transfusion of blood from donors whose disease was in remission have been offered as clinical evidence to support this approach [17, 18], but a subsequent randomised study based on these observations failed to show any benefit [19]. Increasing doubts about the role of circulating antibody in the control of tumours and the possibility that systemically administered antibody might serve merely to mask tumour antigens protecting them from host defences and thereby enhancing, rather than restricting, their growth has led to a loss of enthusiasm for this technique. This situation is rapidly changing, however, following the development of methods for preparing monoclonal antibodies. In 1976 Kohler and Milstein described a technique using mouse myeloma cells to provide antibodies to specific antigens. This involved the fusion of spleen cells from a mouse immunised against the given antigen with a murine myeloma line [20]. The resulting hybridomas, then produce a single antibody determined by the immunised cells that took part in the fusion. The spleen cell provides the blueprint for the antibody; the continuously replicating myeloma cell provides the factory for its production (Figure 2).

The possible applications of this new technology to tumour immunology and immunotherapy are only just beginning to be explored but there are several ways

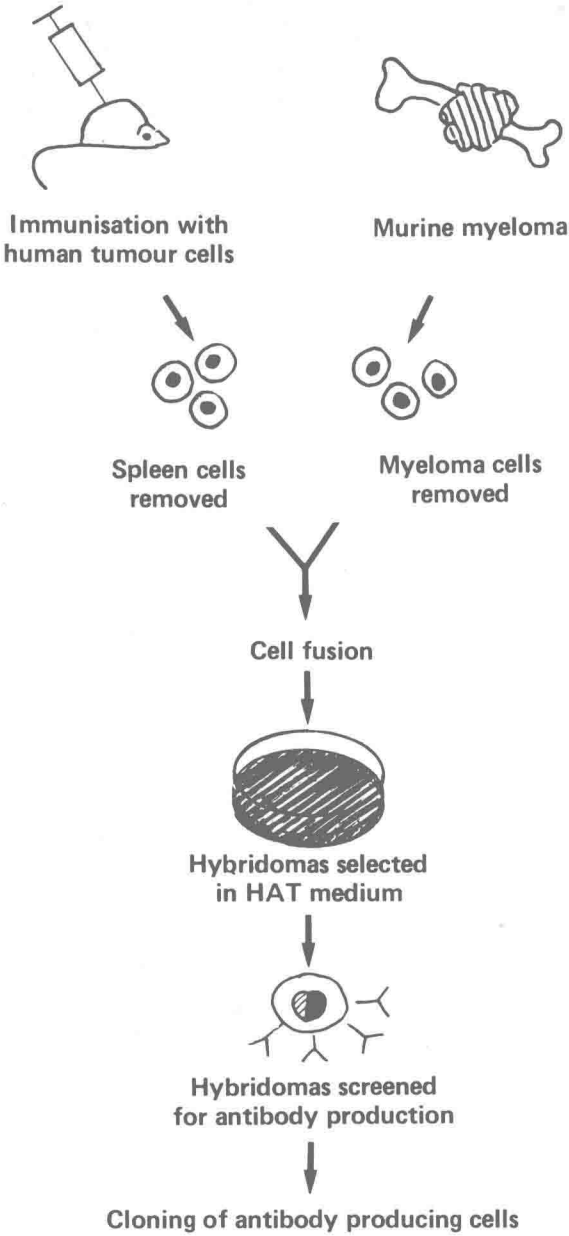

Figure 2. Production of monoclonal antibodies. (HAT medium contains hypoxanthine, aminopterin and thymidine and only hybridoma cells carry the necessary enzymes to survive in this medium)