Gut, 1975, **16**, 490-499

Progress report

Tumour immunology and the gut

Malignant tumours of the gastrointestinal tract account for 20% of all neoplasms and are responsible for 26% of the mortality related to cancer. Conventional treatment, in addition to primary surgery, includes pre-operative radiotherapy[1] adjuvant chemotherapy[2], and combination chemotherapy in advanced disease[3,4]. Although such methods have increased survival rates to some extent, progress has been slow. The purpose of this review is to discuss the role of tumour immunology, including immunotherapy, in the investigation, management, and treatment of gastrointestinal tumours.

Tumour immunology has had a long and varied history[5-11]. The basic principles, however, are that tumours possess antigens not present in normal tissues; these antigens are capable of stimulating an immune response in the host and it is implied that this response influences the course of the disease. But some evidence has been presented which indicates that a weak immune response may be necessary for the initiation of tumour growth[12].

Such a simplistic view of tumour immunology hides the many complexities of the subject. Thus, while there is good evidence in experimental tumours for distinct antigenic change, the evidence in the human situation is not so clear[13]. Although it is considered that it is the cell-mediated immune response which is most important in the reaction against tumours, there are at least three cell systems involved: (a) T-cell mediated cytotoxicity, (b) K-cells or T-independent cells, and (c) macrophages[14]. Serum factors are also important. Cytotoxic antibody may inhibit tumour growth. Other factors in serum—'blocking' factors, antigen-antibody complexes, or soluble antigen—may increase or enhance tumour growth by inhibiting the action of cytotoxic lymphocytes. Lastly, while in animal systems the immune response may be harnessed or augmented to produce an effective immunotherapeutic weapon, in human tumours only a limited number have been shown to be clearly influenced by immunotherapy[9-11].

In tumours of the gastrointestinal tract a number of reports have now appeared which enable us to attempt to answer the following questions.

1 Do patients with gastrointestinal tumours have altered immune responses, either cell-mediated or humoral, to non-specific stimuli?

2 Do gastrointestinal tumours contain antigens which can elicit an immune response?

3 Can specific, tumour-directed immune responses be detected in patients with neoplasms of the gut?

4 Is there enough evidence to suggest that immunotherapy might be beneficial in patients with gastrointestinal tumours?

5 Is there an immunological reason for the rarity of small bowel tumours?

While this review will be concerned with human tumours, it is pertinent first to review certain animal data.

Animal Models of Gastrointestinal Neoplasm

It is now possible to induce, in small animals, tumours of the gastrointestinal tract. Such models have proved useful in investigating the potential of the immune response and the antigenic properties of such tumours. Although a great deal of work has been done on the induction of gastric tumours[15], it is with colonic tumours that most immunological work has been performed. Using dimethylhydrazine, for example, a high incidence of colonic tumours can be induced in rats and mice[16–17]. It has been shown that such tumours release antigenic products which can be quantitated and measured in the serum. They seem analogous to the carcinoembryonic antigen type of substance, although antigenically distinct[18]. Using this model it has been possible to assess the effect of treatment on the tumour using the colonic tumour antigen to monitor therapy. These induced tumours are cross-reactive indicating that there may be a common antigen[19]. Such tumour models provide a basis for the study of tumour-specific immune responses and make available a potential system for assessing immunotherapeutic techniques[20].

A second, broad area of experimental work relates to the growth of human tumours in immunosuppressed animals or in immunologically privileged sites. An excellent summary of such models is reported in a recent workshop report[21]. The hamster cheek pouch, for example is an immunologically privileged site and has been used to grow colonic tumours and transplantable lines established[22,23]. These tumours do produce carcinoembryonic-antigen-related products[23].

Immunologically deprived mice[24] produced by thymectomy, whole-body irradiation, and bone marrow grafting have also been used in this situation. This model seems to be particularly useful in growing colonic tumours[25,26] and the cell kinetics are similar to these of the tumour originally removed from the patient with colonic cancer[27]. Nude mice (nu/nu mice) show several abnormalities, but in immunological terms are congenitally deficient in thymus-derived cells (T-cells)[28]. As such they accept allogeneic and xenogeneic grafts, and, once again, human colonic tumours seem to grow particularly well. These can be shown to contain carcinoembryonic type antigen and may thus be used to monitor treatment schedules, and external scintillation counting using tagged antibody has been used to detect tumours *in vivo*[29]. Because of the very abnormal immunological environments of these tumours, they do not make good models for immunotherapy though they may be useful in the assessment of the chemotherapy of gastrointestinal neoplasms.

One gut tumour which has received particular attention in experimental animals is the amino-azo dye-induced hepatoma in the rat[30]. Using this tumour system it is possible to dissect the immune response, both cell-mediated and humoral, in great detail[31]. Such a model provides essential background information for the study of human tumour immunology.

Altered Immune Responses in Patients with Gastrointestinal Cancer

It has been known for many years that patients with cancer have depression of both cell-mediated and humoral immunity, especially in the advanced stages of the disease. Several such studies have included patients with gastrointestinal tumours.

SKIN TESTING IN PATIENTS WITH GASTROINTESTINAL NEOPLASMS

A relatively simple method of assessing cell-mediated immunity is to test the ability of the patient to mount a delayed hypersensitivity response to antigenic stimulation presented onto the skin surface or intradermally. The antigen may be one to which the patient has not previously been exposed or the antigen may be one against which the patient has previously been sensitized.

The most commonly used new antigen is dinitrochlorobenzene (DNCB). The skin is first painted with a sensitizing dose of DNCB and two weeks later the patient is challenged by a smaller dose. The reaction is assessed at 48 hours, and, depending on size, erythema, and induration of the response, the patient is scored as reactive or non-reactive. Several studies have been performed in patients with gastrointestinal neoplasms and these have consistently shown a lower percentage of positive reactions compared with normal age- and sex-matched controls[32,33,34]. The difference becomes greater as the extent of the disease increases. This depression of the cell-mediated immune response has been used in a prognostic way, patients with decreased responses having a poorer prognosis. It is clear, however, that in the individual patient the test may not have such a definite significance.

It should be pointed out that the test also depends on the ability of the patient to mount an inflammatory response and this may also be reduced in cancer patients[35]. The interpretation of the results then becomes more difficult.

Other antigens, including mumps, dermatophyton, and purified protein derivative, have been used to test previous exposure to antigens, the assumption being that as most patients have previously come into contact with these substances, a negative response indicates depressed delayed hypersensitivity. Such a depression has been found in gut tumour patients[33,36,37,38]. The general conclusion from these tests is that in patients with gastrointestinal cancers cell-mediated immunity is depressed.

Tests of lymphocyte function

One of the simplest ways of assessing this is to perform total lymphocyte counts. It has been reported[39,40] that patients with colonic tumours have lower than normal lymphocyte counts and that this depression is greater with advanced disease.

Another way of studying lymphocyte function is to stimulate lymphocytes in culture with a series of mitogens. Phytohaemagglutinin (PHA) stimulates T-cells predominantly and, as such, is a rough estimate of cell-mediated immunity. Several reports have shown that patients with gut neoplasms have lower than normal transformation indices[41–45]. Such studies have also suggested that the lower the transformation index, the poorer the prognosis. In addition, following resection of large intestinal tumours the transformation of lymphocytes may return to normal[43]. Using lymph node cells from patients with gastrointestinal tumours, similar results have been obtained[41].

Using the PHA transformation system the effect of serum from patients with gastrointestinal cancer on lymphocyte reactivity can be assessed. Evidence that serum factors may depress such transformation has been presented[46,47]. These results suggest that serum factors, perhaps produced by the tumour, may depress the immune response.

ANTIBODY RESPONSES IN PATIENTS WITH GASTROINTESTINAL NEOPLASMS

In addition to an alteration of cell-mediated immunity, there is evidence that the humoral immune response may also be depressed. Using heterophile antibody responses to red blood cells, it has been shown that in patients with large bowel cancers humoral immunity is decreased[48].

In a separate study it was shown that the response to tetanus toxoid was also depressed[49]. It is of great interest that the incidence of autoantibodies in patients with a variety of solid tumours was found to be similar to that in a control group[50].

ROLE OF NUTRITION IN THE DEPRESSION OF THE IMMUNE RESPONSE

There is a considerable amount of evidence in the literature to suggest that the nutritional status affects both cell-mediated and humoral immunity[51–54]. Although this may not account for the depressed immune responses in patients with gastrointestinal tumours, there is no doubt that such patients may well be malnourished. This should be taken into consideration when assessing results.

POSTOPERATIVE DEPRESSION OF THE IMMUNE RESPONSE

Non-specific and tumour-directed immune responses are depressed following surgical operations[55–59]. This makes it clear that immunological testing must be performed preoperatively. It has been suggested that the depressed immune response, occurring at a time when there may have been active dissemination of tumour cells, may be detrimental to the patient by facilitating seeding of metastases.

Antigens Produced by Gastrointestinal Tumours

It is a basic proposition of tumour immunology that unless the tumour produces or contains new antigens, then a specific tumour-directed immune response is not possible. The feasibility of immunotherapy depends on this factor.

There is considerable evidence that gastrointestinal tumours do produce new, or in some cases fetal, antigens. The best known of these is carcinoembryonic antigen (CEA) first described by Gold and Freedman[60–61]. Although this antigen is now not thought to be useful as a screening test for colonic cancer[62–65], the fact that it is present in the serum of patients with gastric, pancreatic, and colonic tumours makes it useful in the monitoring of disease spread and in assessing response to therapy[66–68]. It is possible, as with animal experiments, that radiolabelled anti-CEA antibody or anti-CEA antibody coupled with a cytotoxic agent may be useful in therapy, and several human results point optimistically in this direction[69–70].

The second well known antigen is α-fetoprotein. Although this is associated with hepatomas[71–72] it is also present in patients with gastric carcinoma[73] and may have similar uses to CEA. A specific pancreatic oncofetal antigen has been described[74] and it is possible that several others will be discovered.

In addition to these well established antigens, several others have been described. Glycoprotein antigens, distinct from CEA, have been demon-

strated in gastric cancer and gastric secretions[63,75,76]. Using immunofluorescent techniques an antigen has been located in gastric cancer and in gastric metaplasia[77].

The conclusion from this section of the review is that gastrointestinal tumours do produce new antigens and these may be of use in the diagnosis and monitoring of tumour growth.

Specific Immune Responses against Gastrointestinal Tumours

It is an interesting paradox that though the general immune responses in patients with gastrointestinal tumours may be depressed, it may still be possible to detect specific, tumour-directed immunity.

LYMPH NODE REACTIONS IN GASTRIC CANCER

One indirect piece of evidence for a specific immune response against a tumour is the histology of the draining lymph nodes. Sinus histiocytosis and follicle formation may occur and in gastric neoplasms such reactive changes have been found[78,79]. This response may be correlated with a good prognosis.

SPECIFIC SKIN TESTING IN GASTROINTESTINAL NEOPLASMS

By preparing soluble antigens from gastrointestinal tumours and injecting such extracts subcutaneously, delayed hypersensitivity reactions may be observed[80,81]. Colonic tumours have been studied extensively and it is of interest that CEA alone does not appear to be reactive in this way. Stewart[82] showed that 33% of stomach cancer patients gave positive reactions to autologous tumour extracts.

CELL-MEDIATED IMMUNE RESPONSES

Several techniques have been used to demonstrate cellular immunity in colonic cancer patients. Using the method of inhibition of leucocyte migration[83,84] it was noted that patients did show a specific immune response. As with non-specific stimuli the presence of autologous serum modified the reaction. Mixed mononuclear migration has also been used in this way[85].

With the more direct technique of colony inhibition assay[86], it has been shown that lymphocytes from patients with colonic cancer will kill autologous cultured tumour cells[87–89]. In this system it has been possible to demonstrate cross reactivity indicating that colonic tumours may share a common antigen, and there is tentative cross-reactivity with other gastrointestinal tumours. With this same system, it has been found that serum factors may block this cytotoxicity. The implication is that although patients may have active cellular immunity against their tumours, the presence of serum factors may block this response, facilitating or enhancing tumour growth.

SPECIFIC ANTIBODY RESPONSES

Reference has already been made to serum factors demonstrated in the colony inhibition test in colonic tumours. In these patients, however, it has been found that serum factors may also be cytotoxic for tumour cells[87–90]. Anti-IgG antibodies have been described in colonic cancer pa-

tients[91]. It is suggested that this anti-antibody may block the cytotoxic effect of antitumour antibody and again enhance the growth of the tumours. As the tumour progresses so the titre of anti-IgG antibody seems to rise.

Immunotherapy for Gastrointestinal Neoplasms

Immunotherapy of gastrointestinal cancer has attracted less attention than it has for other human tumours notably leukaemia, malignant melanoma, and breast cancer[9–11]. However, several reports have appeared which indicate that there may be possibilities for this form of treatment in gut neoplasms.

Taylor and Odili[92] showed that, following injection of multiple doses of tumour with Freund adjuvant, evidence of tumour destruction, assessed histologically, was noted. Symptoms disappeared in over 50% of patients.

By coupling a tumour cell extract with a highly antigenic substance, bisdiazobenzidine[93], it was demonstrated that following injection of this complex there was a clinical response in four out of 14 patients with advanced disease. In the two patients with gastrointestinal neoplasms, there was no response.

A further method of immunotherapy involves the cross-immunization of patients with extracts of tumour cells. Leucocytes obtained from these tumour partners are then transfused back. Some responses have been obtained with this method in patients with colonic tumours[94–97]. In a series of 25 patients with colonic tumours treated in this way, five objective responses were obtained in patients with advanced disease. The ethical aspects of this procedure require careful consideration.

An alternative method has been to immunize pigs with human tumour cells. The mesenteric lymph nodes are then removed and a lymphocyte suspension is made[98] and is then infused back into the patient. Three patients with colonic tumours were treated and two were judged to have had a definite clinical response. One patient with cancer of the pancreas showed no response.

Autologous, irradiated tumour cells have been injected into a variety of patients with advanced cancer[99]. In one patient with colonic cancer an objective response was obtained.

The only controlled trial of immunotherapy for large bowel cancer has recently been reported in a preliminary way[100]. It compares the effect of BCG, used as a non-specific immunopotentiating agent, with BCG and 5-fluorouracil. The preliminary report indicates that toxicity was minimal but unfortunately no results are given. These are awaited with interest.

A recent study of immunotherapy in non-Hodgkin's lymphoma included some patients with gastrointestinal lymphomas[101]. Some benefit was obtained in these patients.

During BCG therapy for non-gastrointestinal neoplasms, a granulomatous hepatitis has occurred in a few patients[102]. The gastroenterologist may therefore be involved in the consequences of immunotherapy given for tumours at other sites.

While on the subject of complications it has been reported with several different kinds of neoplasms that the nephrotic syndrome may occur[103,104]. It has been suggested that this is related to the deposition of antigen-antibody complexes in the kidney. A case has been reported of a patient with colonic

cancer who developed this complication and in whom it was shown by immunofluorescent studies that such complexes were present in the kidney. Immunologically these were identical to CEA[105]. This is an instance in which the antigenic character of the tumour can be demonstrated in an elegant way.

Immunological Aspects of Small Bowel Tumours

The rarity of small bowel tumours contrasts sharply with the incidence of cancer in adjacent parts of the gastrointestinal tract[106]. While several mechanisms have been implicated, none takes account of the fact that the incidence of small bowel tumours is increased in patients with coeliac disease[107,108] and possibly also in Crohn's disease[109–110]; these conditions are associated with an abnormal local immune response. Small animal experiments have suggested that if the local immunity becomes depressed, then the incidence of tumours may rise[111]. There is a clear case for the further study of gastrointestinal immunity in patients with diseases predisposing to small bowel neoplasms.

Summary and Conclusions

This review set out to answer several questions related to tumour immunology and the gut. It is evident that in patients with gastrointestinal cancer there is a general depression of the immune response and this seems to be correlated with the stage of the disease. Paradoxically a specific immune response against definable tumour antigens can be demonstrated, both cellular and humoral mechanisms being involved although the complexities of this paradox require further analysis. Immunotherapy has been employed in gastrointestinal tumours in a sporadic way. The results suggest that gastrointestinal neoplasms may respond at least as well as other tumours. A firm conclusion awaits the results of controlled trials in which the bulk of the tumour has been effectively dealt with by other means or where combined immunochemotherapy is being used.

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