Middle Articles

Immunological Aspects of Cancer — Part II

On 16 and 17 October the Royal Medical Society of Edinburgh held a conference on this subject. Shortened versions of the first six papers were printed in last week's B.M.J. (14 November, p. 418). Similar versions of the remaining papers appear below.

Evidence for Antigenicity in Human Tumours with Reference to Both Melanoma and Acute Leukaemia

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There is now no doubt that tumour-associated antigens exist in experimental animal tumours, and that immunological reactions against these antigens can influence the course of the malignant disease.¹ Because of the difficulties of research in man, the situation is not as clear as in animals. Nevertheless, recent work has shown that tumour-associated antigens are present in human malignant tissue. The two diseases selected for special discussion are malignant melanoma and acute leukaemia.

Malignant Melanoma

For many years evidence has been accumulating that there may be a host reaction to malignant melanoma and that this reaction could be immunological. Firstly, melanoma is one of the malignant diseases which occasionally undergoes spontaneous regression.¹⁻⁸ Secondly, the disease may remain localized for a long time before it becomes disseminated.^{9 10} Thirdly, even in patients with disseminated disease some metastatic deposits can regress at the same time as new ones appear and others continue to grow.¹¹ Fourthly, Lewis¹² has shown in Ugandan patients that those with localized tumours often had cytotoxic serum antibody active only against the patient's own melanoma cells. This suggested that there may be tumour-specific antigens in melanoma similar to those found in chemically induced tumours in animals.

Correlation of the Stage of Malignant Melanoma with Presence of Tumour-Specific Cytotoxic Autoantibodies

	No. of Patients	% Having Auto-antibodies
Localized disease*	47	86
Disseminated disease	132	11

* The term "localized" disease includes all patients in whom the disease is either confined to the primary anatomical site, or has spread no further than the regional lymph nodes, with or without satellite nodules. "Generalized" disease is when tumour has spread beyond the regional lymph nodes.

Several groups of workers,¹³⁻¹⁵ using immunofluorescence, showed that there was cross-reactivity between melanoma cells and the sera from a number of different melanoma patients. For this reason we embarked on a large survey of patients with melanoma in collaboration with many centres in London, Manchester, Bristol, Cardiff, and Uganda. We tested the reaction between serum and tumour cells from the same and different patients in two different ways: (1) by the ability of sera to inhibit, in the presence of complement, the growth or metabolism of melanoma cells *in vitro*; and (2) by immunofluorescence to assess serum binding to the cytoplasmic components of dead cells and to the surface

membrane of living cells. We found that there were two kinds of autoantibody in over a third of melanoma patients. One reacted with the cell surface, and was also cytotoxic; the other reacted with components of the cytoplasm.¹⁶ The occurrence of such autoantibodies was closely correlated with non-dissemination of the tumour (Table).

Disappearance of Antibodies

In three patients in whom the serum was originally positive, later sera failed to inhibit growth of the culture of autologous melanoma cells when the disease became widespread. The disappearance of circulating antibodies with advancing disease could be due to loss of antigen(s) by the tumour, or inability of the patient to respond to the antigen(s), or for some other reason. We have not been able to show that there is any loss of antigenicity as the disease advances. Indeed, in three patients in whom the antibodies disappeared as the disease became widespread the positive sera which had been stored at -4° C. still reacted with tumour tissue taken at a time when antibodies were absent. To determine whether patients with generalized disease can still respond to the tumour we immunized 13 patients on one or more occasions with their own tumour cells, irradiated so that they could no longer divide. Provided sufficient cells were injected into multiple sites (more than 300 × 10⁶) the patients produced cytotoxic antibodies which also gave membrane immunofluorescence, though the response was only transitory.

These results¹⁷ raise the vital question of the role of such antibodies in this and other malignant disease. It seems likely that circulating antibodies, even if they are cytotoxic *in vitro*, have little effect on the tumour when it is growing as a solid mass, probably because the concentration of antibody within the tumour is lower than in the blood. Their presence, however, may prevent blood-borne metastases from forming. Possibly so long as cytotoxic antibodies are present in the serum malignant melanoma cells released into the blood are killed. Once these antibodies have disappeared, however, malignant cells are no longer killed in the blood and distant metastases form.

Such a theory is highly speculative, but it fits with the fact that this tumour may metastasize to the local lymph nodes, where it remains for a variable time before spreading via the blood stream. It also could explain why the disease disseminates by local lymphatic routes when antibodies are present, but by the blood only when antibodies are absent. A similar situation has been encountered¹⁸ in rats with primary fibrosarcomas induced chemically; these do not give rise to blood-borne metastases until antibody has been removed.

Clearly, more work is required to clarify the role of tumour-specific antibodies in human malignant melanoma.

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The reason why circulating antibodies disappear remains to be established; one possibility is that the nodes draining the tumour become incapable of responding to the antigen(s), allowing spread to occur.

Lymphocytes and Melanoma Cells

More recently, Dr. Graham Currie, working in our unit at Sutton, has been studying the effect of lymphocytes on melanoma cells using the microtechnique of Takasugi and Klein.¹⁹ The results have been difficult to interpret because some normal lymphocytes are cytotoxic to melanoma cells in vitro. For this reason he has been concentrating on comparing the cytotoxic effect of lymphocytes before and after the patients have been given an irradiated autograft of melanoma cells. In several patients lymphocytes taken before immunization were not cytotoxic to their own melanoma cells; but six days after the patients had been given an autograft of irradiated tumour cells the lymphocytes showed considerable cytotoxicity. Interestingly, the cytotoxic action of these autologous lymphocytes is not abolished by treating the melanoma cells with autologous serum at the same time—in other words, we have failed to show a blocking effect of antibody in this system.

There has been no effect on the course of the disease in any of the patients we have immunized with irradiated cells.

Acute Leukaemia

As with malignant melanoma some evidence suggests that there may be host resistance to acute leukaemia, even if it is only rarely effective. The finding by Burchenal²⁰ that 103 people in the world have had proved acute leukaemia and are alive and free from disease between 5 and 17 years later is of great interest. Some of these patients were given intensive chemotherapy, and some form of host resistance must be postulated.

There have been reports that antibodies to leukaemic cells are present in the serum of patients with acute leukaemia.²¹ We have been more concerned with attempts to show that there are tumour-associated antigens in human acute leukaemia and to study changes in the patient's lymphocytes following the injection of irradiated autografts of leukaemic cells. Dr. Raymond Powles, working in our department at the Institute of Cancer Research, has collected large numbers of leukaemic blast cells from the peripheral blood using the I.B.M. Continuous Blood Cell Separator and then tested their

ability to stimulate the lymphocytes from the same patient once a remission has been obtained. His preliminary results have shown, firstly, that leukaemic blast cells will stimulate autologous lymphocytes, thus confirming the work of others²²⁻²⁴ that tumour-associated antigens exist on the cell surface; and, secondly, that the response is greater after immunization with autologous irradiated blast cells, thus providing some rationale for the specific immunotherapy used by Mathe in human leukaemia.²⁵ His results will shortly be published.

In summary, firstly, undoubtedly tumour-associated antigens exist in at least some tumours in man, just as they do in those of animals. Secondly, it is possible to show a host response in the form of antibodies or reactive lymphoid cells following immunization. However, as Professor Alexander has just stated, with the exception of Mathé's results²⁵ in maintaining remissions in acute lymphoblastic leukaemia, it is premature to speak of immunotherapy in man at present. The most which can be expected is that by mounting a powerful immune response against the tumour antigens a few malignant cells will be killed. The place for immunotherapy is after the classical methods of reducing the number of tumour cells to the absolute minimum—in other words, surgery, radiotherapy, or intensive chemotherapy.

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Prospects for Immunotherapy of Cancer : Experience in Experimental Systems

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The experimental detection of tumour-specific antigens in the plasma membrane of malignant cells has raised the possibility that immunological procedures could be used in treatment. Antigenic substances specific to tumour cells have also been identified either inside the cell or associated with the extracellular matrix. Since some of these are released into the circulation they may become of great practical interest for diagnosis but they are less likely to be useful in relation to immunotherapy.

The identification of tumour-specific antigens associated

with the cell membrane presents many technical problems in man.¹ However, much has been learned about them in tumours and leukaemias of experimental animals. When the malignancy has been induced by chemical or physical agents then the tumour antigens are unique for each tumour and there is no cross-reactivity between the antigens of tumours induced by the same carcinogenic substance. On the other hand, all the tumours induced in animals by a particular oncogenic virus have the same new antigens, though they will be different for different viruses. Even so, it has not been possible to demonstrate tumour antigens in several tumours in mice and rats, and possibly not all malignant cells have such new antigens.

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