Papers and Originals

Immunity to Malignant Disease in Man*

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Probably no subject in medicine in this century has undergone such radical changes as immunity to malignant disease. During this time there have really been three phases, which have been described by many scientists working in this field, and most recently by Klein (*Lancet*, 1968), as the optimistic, followed by the pessimistic, and finally the realistic, in which we are now and hope to remain. The optimistic phase started at the end of the last century and continued into the first 15 years of this. Distinguished scientists such as Ehrlich (1909) and in this country Bashford (Bashford and Russell, 1910), who first studied the phenomenon of the rejection of transplanted tumours, laid the foundation not in fact for further work on cancer research but instead for the study of the rejection of all forms of homograft. Wade (1908), describing the rejection of a transplanted sarcoma in dogs, wrote: "The final disappearance of the tumour is associated with a flooding of the tumour tissue with lymphocytes and plasma cells. The tumour is borne away on a lymphocytic tide." Da Fano (1912) stressed the importance of plasma cells as well as of lymphocytes in the rejection of tumour grafts.

Between 1913 and 1929 much work was done, and Woglom (1929) reviewed 600 papers published during this time, of which he wrote: "A small number are of permanent value, many record unconscious and unnecessary repetitions of previous experiments, and far too large a proportion are merely ridiculous, for few investigators seem to realize that cancer research is a discipline requiring some apprenticeship, and that not everyone with an inoculating needle and a dozen white mice can plunge in and emerge with a discovery." His final conclusion was that nothing at that time suggested that cancer could be modified by immunological procedures. The phase of pessimism had begun, and lasted in fact much too long.

The reason that no progress had been made was that all the experiments had been done by transplanting tumours into genetically different animals, where they were rejected like any other homograft. It was only when tumours began to be transplanted into genetically identical (syngeneic) animals that the presence of tumour-specific antigens could be proved and that immunological methods for modifying the growth of tumours could be investigated. It is usually stated that the first such experiment was conducted by Foley (1953), but, as Old and Boyse (1964) pointed out, it was in fact Gross (1941, 1943) who first showed that immunity could be produced in pure line animals with the use of a chemically induced tumour, and paved the way for the clear demonstration by Prehn and Main (1957) that tumour-specific antigens must exist. The age of realism had begun, though with the all-too-frequent delay in the advance of medical knowledge it had taken 16 years for the true significance of Gross's work to be appreciated.

No historical review would be complete without paying tribute to a remarkable scientist, Peter Gorer, who worked in

this country. At the age of 5 his parents consulted one of the greatest physicians in London at that time, who recommended that he should be sent to a school for backward children. Fortunately, as occasionally happens with medical advice, this was not taken; eventually he qualified in medicine, though he failed on the first attempt in gynaecology and obstetrics after an argument with one of the examiners about genetics, an ironic situation, as Medawar (1961) was to record following Gorer's premature death at the age of 54: "He was the world's foremost authority on the serological genetics of tissue transplantation." Gorer's two major contributions to tumour immunology were to show in collaboration with Kaliss (Gorer and Kaliss, 1959) and with Amos (Gorer and Amos, 1956) that the tumour cells in a transplantable leukaemia contained a tumour-specific antigen, and that antisera raised against these cells in a genetically dissimilar (allogeneic) strain would cause tumour regression. However, in a different system, and with the use of a transplantable sarcoma, tumour regression occurred with large doses of antisera, but enhancement could occur with low doses.

For there to be an immunological reaction by the host against its own tumour, the latter must contain antigens not present in the host—that is, tumour-specific antigens. I propose, firstly, to review the evidence that such antigens of a transplantation type exist on the surface of the malignant cells both in man and in animals, and that the host is capable of mounting an immunological reaction against malignant disease; and, secondly, to consider by what means such a reaction could be increased.

Evidence for Immunity in Animals

Now that pure-line (genetically identical) strains of animals have been established a tumour can be transplanted from one animal to many others in which it will grow. With such a system it is possible to demonstrate the presence of tumour-specific antigens and to immunize an animal against a tumour. The three principal methods of immunization are: (1) to transplant the tumour and after it has reached a certain size to remove it surgically or by ligation; (2) to inject irradiated tumour cells, which are no longer capable of cell division, but are still alive in that metabolism continues and they are impermeable to dyes; and (3) to immunize the animal with a dose of tumour cells which is too low to cause a tumour.

The production of immunity is shown by the fact that the animals pretreated by one of these procedures fail to develop tumours when challenged with an inoculum of cells which causes tumours in unimmunized animals.

All the tumours induced by any one oncogenic virus have the same tumour-specific antigens, so that one tumour can be used to immunize against transplants of all other tumours produced by that virus (Old and Boyse, 1965). With the chemically induced tumours the situation is the exact opposite: cross-resistance is extremely rare, if it occurs at all (Klein and

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Klein, 1962), so that one tumour will immunize only against transplants of that particular tumour and not against others produced by the same chemical carcinogen.

Immunity against malignant disease can also be shown by the presence of circulating antibody and by the cytotoxic action of immune lymphocytes against tumour cells. Again there is a difference between tumours of viral origin and those induced by chemical and physical agents.

Circulating antibodies which are cytotoxic to the tumour cells in vitro have usually been found in virally induced animal leukaemias, but have not been detected in animals immunized against chemically or physically induced sarcoma and carcinoma (Klein and Klein, 1964; Old and Boyse, 1964).

The injection of antisera prepared against experimental tumours may cause inhibition in the growth of some tumours, enhancement of others, while leaving many unaffected (Gorer, 1961). Immune sera have no effect on the growth of established solid tumours, but in some tumours experimental ascitic lymphomas may protect the animal against a subsequent inoculation of the tumour cells and even cause retardation of the growth of an established tumour (Alexander *et al.*, 1966a; Old *et al.*, 1967).

Regression of chemically induced tumours may occur after the injection of lymphocytes prepared by immunizing another animal with part of the tumour. These lymphocytes can be shown to be cytotoxic to the tumour cells in vitro as well as in vivo. It is very remarkable that the same results occur when animals of a different species are used. For example, Alexander and his colleagues have used the lymphocytes from sheep and goats to treat chemically induced tumours in rats in this way, and have now found that an extract from the immune lymphocytes, which contains ribonucleic acid, has the same effect (Alexander *et al.*, 1966b, 1967).

All the experiments mentioned so far have been concerned with transplanted tumours, but the vital question so far as man is concerned is whether there is a reaction in the host against his own primary tumour. Mikulska *et al.* (1966), using chemically induced tumours, showed that once the primary tumour had been removed it was difficult to re-establish the tumour in the original host by using the same number of cells which invariably caused tumours when injected into genetically identical recipients. Another interesting finding was that the spleen cells taken from a rat in which a primary sarcoma had been established were capable of preventing the transplantation of cells from this tumour in a syngeneic recipient only under certain conditions; they were cytotoxic only if they were taken from animals in which the tumour had been surgically removed three weeks earlier. When the spleen and tumour were removed at the same time, the spleen cells had no appreciable effect on the growth of the autochthonous tumour on transplantation. It would appear that the actively growing primary tumour exhausts the supply of anti-tumour lymphocytes that are produced by the host, but that after the tumour is removed the concentration of cytotoxic lymphocytes builds up and the animal can reject an autograft. If this situation applies to malignant disease in man, it would explain the great difficulty in demonstrating an immunological reaction by lymphocytes against tumours when the bulk of the tumour tissue remains. The time to search for such an effect is *after* the tumour has been removed.

From this it is clear that it is possible to immunize animals against tumours and cause the regression of both primary and transplanted tumours by various immunological procedures.

Evidence for Immunity in Man

There are two reasons why it has not yet been possible to prove that tumour-specific antigens are present in human malignant disease. Firstly, pure-line human beings do not exist, with the exception of identical twins, and, secondly, even

if they did exist the experiments would be impossible for ethical reasons. However, there is indirect evidence that in man, as in animals, there may be an immunological reaction by the host against his own tumour. The evidence for this comes from many sources.

(1) Tumours and Abnormal Immunity Mechanisms

It is now clearly established that in patients with hypogammaglobulinaemia there is an increased incidence of lymphoma (Page *et al.*, 1963; Peterson *et al.*, 1966; Miller, 1968). There is also a connexion between autoimmune diseases and malignant disease both in man and in animals. For example, in chronic lymphocytic leukaemia and lymphosarcoma autoimmune haemolytic anaemia not infrequently occurs (Dacie, 1962), and in some cases the haemolysis has preceded the apparent onset of the malignant disease (Rosenthal *et al.*, 1955). It is interesting that animals of the NZB strain of mice, which spontaneously develop autoimmune haemolytic anaemia, also develop both malignant thymomas and malignant proliferation of reticulum cells leading to death from reticulum cell sarcoma (East *et al.*, 1965; East and Prosser, 1967). More recently, Schwartz and his colleagues have produced a similar situation in animals by producing chronic allogeneic disease, and in one such system there was a striking hyperplasia of the reticuloendothelial system with enlargement of the lymph nodes and spleen. The animals also developed autoimmune haemolytic anaemia and subsequently died from reticulum cell sarcoma (Schwartz, 1968). With other so-called autoimmune diseases, in particular the rheumatic diseases, there is an increased incidence of lymphoma (Abbatt and Lea, 1958; Lea, 1964; Talal and Bunim, 1964).

Burnet (1967) has stressed many times that an intact immunological system is an essential mechanism for the elimination of unwanted (malignant) cells arising by a process of somatic mutation. It is therefore not surprising that Woodruff stated there are now five patients who have developed reticulosarcoma while on immunosuppressive drugs following a renal homotransplant (*Lancet*, 1968), two of whom have been reported in detail (Doak *et al.*, 1968).

Further evidence for the growth of malignant disease in patients with impaired immunity mechanisms comes from graft experiments in man.

(2) Tumour Grafts in Man. (a) Homografts

The subcutaneous transplantation of human cancer cells into normal people almost invariably results in transient growth followed by complete regression during the third and fourth weeks (Southam *et al.*, 1957). As with other tissues rejected by the homograft reaction, further transplants lead to an accelerated response. Rejection of homografts of malignant cells in patients with advanced cancer is delayed sometimes indefinitely, and in one patient with advanced cancer who received a homotransplant of another tumour the latter actually metastasized to the regional lymph nodes (Southam *et al.*, 1957). This was not simply due to general debility, as in patients with debilitating non-neoplastic disease the rejection of homografts of malignant cells is the same as in normal controls (Levin *et al.*, 1964).

However, as with animals, malignant cells transplanted into genetically dissimilar normal recipients occasionally take. Scanlon *et al.* (1965) took a small biopsy of a melanoma from a woman of 50 and transplanted it into her 80-year-old mother, who subsequently died of widespread metastases from this tumour. The fact that nine months after the implant the Mantoux test was negative, and that the recipient was old, raises the possibility that there may have been some impairment in her immune responses which encouraged this tumour to

metastasize. It is also interesting that five years before this she had had a carcinoma of the uterus which had been successfully removed. Another explanation could be that because the donor and recipient were so closely related there was a high degree of histocompatibility.

Grafts of malignant tissue into recipients receiving immunosuppressive drugs may be accepted. D. C. Martin *et al.* (1965) successfully transplanted a cadaveric kidney from a patient with carcinoma of the bronchus, and four months later malignant cells were found in the transplanted organ; the recipient died from widespread metastases of the transplanted carcinoma five months after the graft. Presumably in this case the graft of malignant cells took for the same reason that the kidney transplant was successful—that is, that the immunosuppressive treatment with azathioprine, prednisolone, and actinomycin C was so effective.

An even more remarkable case has been reported by Wilson *et al.* (1968). They transplanted a cadaveric kidney from a donor who died from a bronchial carcinoma, and 18 months later tumour was found surrounding the transplanted kidney. This proved to be a carcinoma morphologically identical with that of the donor. Immunosuppressive treatment was stopped, and the kidney underwent rejection and was removed. The tumour then regressed and an exploratory operation six months later failed to show any trace of tumour. A further renal transplant was carried out, immunosuppression was resumed, and 10 months later there had been no recurrence of the carcinoma.

In view of this it is clearly dangerous in some circumstances to transplant human malignant tissue into apparently healthy recipients, and is now ethically unjustifiable.

(b) Autografts

The subcutaneous implantation of malignant cells or biopsy material from the patient's own tumour may give rise to "takes," depending on the experimental conditions. For example, Grace (1964), using biopsy material, found that only 2 out of 16 implants grew, and Nadler and Moore (1965) obtained only 11 takes out of 82 transplants. Southam (1965), by injecting cell suspensions, found that the development of a subcutaneous tumour depended on the number of malignant cells injected; 10^8 cells always produced a nodule, whereas 10^4 cells invariably failed to do so. With 10^5 – 10^7 cells the number of tumour cells required to produce a successful autograft was related to the ability of the patient to reject a homograft of tumour cells, and this in turn was related to the immunological state of the patient, as judged by the tuberculin test, the ability to develop delayed hypersensitivity to dinitrofluorobenzene, and the macrophage response in inflammatory exudates. The greater the immunological defect, the fewer cells were required to establish a tumour autotransplant. Hughes and Mackay (1965) found that patients with malignant disease and anergy to tuberculin have a worse prognosis than those who respond to tuberculin.

Patients with carcinomas may respond to the injection of cell-free extracts of their own tumours in about 25% of cases by a delayed hypersensitivity response and in a small number of cases by an immediate response (Hughes and Lytton, 1964; Stewart, 1968).

(3) Histological Evidence

The natural history of malignant disease is related to the morphology of the tumour. Black *et al.* (1955, 1956) and Black and Speer (1958) showed that the prognosis in carcinoma of the breast and stomach was related to the degree of nuclear differentiation, the amount of lymphoid infiltration in the primary tumour, and the presence of sinus histiocytosis in the

regional lymph nodes. The greater the degree of differentiation, lymphoid infiltration, and sinus histiocytosis, the better the prognosis. Hamlin (1968) has produced convincing evidence that the prognosis in carcinoma of the breast is dependent on the host's reaction to the tumour as judged by the number of lymphocytes and plasma cells surrounding the tumour and the presence of a marked reactive hyperplasia in the drainage nodes. A similar situation arises in Hodgkin's disease, where lymphocytic depletion is associated with a poor prognosis and widespread disease; lymphocytic and/or histiocytic proliferation is associated with a better prognosis and localized disease (Lukes, 1964).

(4) Changes in Lymphoid Cells in Peripheral Blood

Because of the relationship between the degree of lymphocytic infiltration in biopsy material and the prognosis in Hodgkin's disease (Lukes, 1964), and the fact that delayed hypersensitivity is impaired to a greater extent in this disease than in any other malignant disease (Fairley and Matthias, 1960; Aisenberg, 1966), we decided to study the lymphoid cell population in the peripheral blood in this disease, and found three abnormalities (Crowther *et al.*, 1967). Firstly, there was an increase in the number of large lymphoid cells actively synthesizing deoxyribonucleic acid; secondly, there was an increase in the number of medium-sized lymphocytes with intensely basophilic cytoplasm; and, thirdly, an occasional plasma cell was seen. These changes are identical with those found under conditions of known antigenic challenge in man—for example, after immunization and during infections—and in the so-called autoimmune diseases, rheumatoid arthritis, and systemic lupus erythematosus.

We concluded that since the change in the lymphoid cell population in the peripheral blood was identical with those found under conditions of known antigenic stimulation, this could represent an immunological reaction by the patient against his own disease. We were therefore disappointed to find that the percentage of morphologically "active" lymphoid cells in the blood was not related to the stage of the disease, but the changes were in fact related to the activity of the disease. In those patients who responded to treatment the lymphoid cells returned to normal, and in those who failed to respond the changes became more pronounced (Crowther *et al.*, 1969). A study is now being made in patients with other malignant diseases. In view of these findings it might seem surprising that in Hodgkin's disease there should be such gross impairment in cellular immunity, but it may be that most of the lymphocytes are committed to react against Hodgkin's tissue and are therefore unable to react against other antigens.

Hellström *et al.* (1968), using their colony inhibition test, have shown that blood lymphocytes from both children with neuroblastomas and their mothers inhibited the growth of the malignant cells in vitro.

(5) Circulating Antibodies

In virally induced animal leukaemias antibodies directed against the neoantigens of the virus have been found, and the search for both a virus causing leukaemia and lymphoma in man and antibodies to such an agent have been sought (Henle, 1968). One of the most exciting sagas in this field has been the recent work on the Burkitt lymphoma. Epidemiological work suggested that an infective agent, possibly transmitted by a mosquito, might be responsible for the disease (Burkitt and Davies, 1961; Burkitt, 1962, 1969; Dalldorf *et al.*, 1964). Then followed a world-wide collaboration between several centres in Africa, from which biopsy material and sera from the patients were flown to Epstein in Britain, to Klein in

Sweden, and to Dr. and Mrs. Henle in the U.S.A.; and now a team from the National Institutes of Health have established a research unit in Kampala. As a result of this Epstein *et al.* (1964, 1967) described the presence of a herpes-like virus in several of the cultures of cells derived from Burkitt's lymphoma, now known as the EB virus. With fluorescent techniques antibodies have been found in the sera of patients from Burkitt's lymphoma which react with the EB virus and also with cells in tissue culture (Henle *et al.*, 1966; Klein *et al.*, 1966), and recent work has linked these together, since it has been shown that in tissue culture it is the cells which contain the virus which fluoresce (Zur Hausen *et al.*, 1967).

This appeared to be the first time that a virus could be incriminated as an aetiological agent in human malignant disease, but there was one disquieting fact that antibodies to both the EB virus and to the lymphoma cells in tissue culture could be found in the sera of some normal people in Africa, Sweden, and the U.S.A. Old *et al.* (1966) also found a precipitating antibody to an antigen present in cultured Burkitt's lymphoma cells in 95% of cases of nasopharyngeal carcinoma in the U.S.A. This was clarified when Henle *et al.* (1968) observed that the mononuclear cells from a technician in their laboratories when cultured developed immunofluorescence after an attack of glandular fever; she also developed antibodies against the EB virus, whereas before glandular fever she had none. Subsequently they proved (Niederman *et al.*, 1968), in a prospective study in students at Yale University, that 100% of those who developed glandular fever also developed antibodies to the EB virus. This antibody is different from that which causes the positive Paul-Bunnell reaction. Finally, it has now been shown that in tissue culture some glandular fever cells contain herpes-like virus particles indistinguishable from the EB virus of Burkitt's lymphoma (Hinuma *et al.*, 1967; Moses *et al.*, 1968).

The present position would appear to be that 100% of patients who have had glandular fever and 100% of those with the Burkitt lymphoma have antibodies directed against the EB virus. Klein *et al.* (1968a, 1968b) have now produced evidence that in Burkitt's lymphoma two separate antibodies are produced, one directed against the EB virus and one directed against an antigen present on the cell membrane. In glandular fever both these antibodies are produced, together with a third—the heterophile antibody which is responsible for the Paul-Bunnell reaction. The significance of these observations remains obscure. Possibly both diseases are caused by a similar virus, perhaps as suggested by Dalldorf *et al.* (1964) and by Burkitt (1969) a superadded chronic infection such as malaria could lead to the appearance of a malignant disease in infected recipients, or it might even be that the EB virus happens to grow well in both Burkitt's lymphoma and glandular fever cells.

There is evidence that antibodies exist in other human malignant diseases. Lewis (1967), in a fascinating study, has shown that in human malignant melanoma in Uganda the serum of some patients with a long history and no metastases is cytotoxic to the autologous melanoma cells but not to other melanoma cells in tissue culture. With disseminated disease the serum is not cytotoxic. This suggests that there may be antibodies against malignant cells in some cases of melanoma, and Morton *et al.* (1968) found antibodies against human melanoma by immunofluorescence. Doré *et al.* (1967) and Tanigaki *et al.* (1967) reported findings suggesting that new antigens are present in human malignant cells, and Dmochowski *et al.* (1967) reviewed the evidence that virus particles may be present in human leukaemic cells and plasma.

(6) Phenomenon of Spontaneous Regression

There are now a number of cases of malignant disease in man in which the disease has regressed unexpectedly, though

not necessarily without any form of treatment (Everson and Cole, 1956; Smithers, 1962; Everson, 1964).

Everson and Cole (1956) defined spontaneous regression of cancer as "the partial or complete disappearance of a malignant tumour in the absence of all treatment or in the presence of therapy which is considered inadequate to exert a significant influence on neoplastic disease." Regressions have been recorded in carcinomas of the bladder, breast, kidney, lung, ovary, stomach, and uterus and with melanoma and neuroblastoma.

Everson (1964) collected 130 cases in which the evidence of spontaneous regression of malignant disease was beyond all doubt, and 28 of these had occurred in neuroblastoma, 21 in hypernephroma, 13 in choriocarcinoma, and 12 in malignant melanoma. Many of these regressions occurred in metastases after the primary tumour mass was removed, and in these cases it may be that the host reaction against the tumour is being overwhelmed by the mass of malignant tissue, and removal of part of this tissue might enable the host to deal with the remainder.

Bodenham (1968), in a personal survey of 650 cases of malignant melanoma in the South-Western Region of England, recorded that even during the course of fatal dissemination of the disease some lesions may spontaneously heal while others appear.

(7) Evidence from Treatment

A total of 157 patients with undoubted acute leukaemia, who have survived from periods of 5 to 17 years since diagnosis, have been collected by Burchenal (1968): 87 out of 127 children and 16 out of 30 adults were alive and well with no evidence of leukaemia more than five years after diagnosis. They received a variety of treatments, and as yet no common factor has been detected in these patients.

A more dramatic situation has now arisen with the Burkitt African lymphoma. Regressions of tumour masses with freedom from recurrence from up to five years have followed the single injection of methotrexate, cyclophosphamide, or vincristine (Burkitt *et al.*, 1965; Burkitt, 1966). It is extremely unlikely that this treatment could have killed all the malignant cells, and this is further evidence that there is host resistance as well, which could be immunological. Klein *et al.* (1966) found a correlation between their antibody test and the response to chemotherapy, those with the highest titres against antigens on the cell membrane having the best response to chemotherapy.

It is against this background that there may be an immunological reaction in man directed against the patient's own malignant disease that one has to consider how such a reaction could be strengthened. The problem of immunotherapy is now considered.

Possibilities for Immunotherapy

As already mentioned in the previous section, it is now possible to protect animals against an inoculum of malignant cells by immunizing them with both virally and chemically induced tumours. With the former protection can be obtained by immunization with irradiated tumour cells, immune lymphocytes, killed virus particles, and specific antisera, but with the latter only irradiated tumour cells and immune lymphocytes can be used.

While these are now routine procedures in many laboratories, it is not so easy to influence the growth of established tumours. Indeed, until recently the evidence that immunotherapy might have some place in the treatment of malignant disease in man was non-existent. Just as in animals, immunotherapy could take the form of specific or nonspecific treatment.

Nonspecific Immunotherapy

Stimulation of Reticuloendothelial System

It has been known for many years that various agents, including oleic acid, B.C.G., and zymosan, which are capable of stimulating hyperplasia of the reticuloendothelial system, also have the effect of retarding the growth of some tumours, particularly mouse mammary tumours (Murphy, 1926), as well as accelerating homograft rejection. This approach was neglected until Old *et al.* (1961) showed that B.C.G. would retard the growth of chemically induced tumours, and Weiss *et al.* (1961) demonstrated a protective activity with B.C.G., using living and killed organisms and methanolic extracts of the bacillus against mammary carcinomas and two different sarcomas. Subsequently Halpern *et al.* (1963) showed that *Corynebacterium parvum* had a similar effect to B.C.G. on the reticuloendothelial system, and Woodruff and Boak (1966) and Smith and Woodruff (1968) showed that immunization with *C. parvum* delayed the appearance of a transplantable mouse mammary carcinoma in a syngeneic system; Halpern *et al.* (1966) obtained the same result in a mouse sarcoma system and with Ehrlich and Krebs ascites tumours. It is of great interest that Woodruff obtained a better result with a mouse mammary carcinoma, to which no immune reaction could be demonstrated, as the animals are tolerant to the vertically transmitted tumour-specific antigen, than with a chemically induced tumour against which the host is capable of reacting.

This type of immunotherapy has been used by Mathé *et al.* (1968), who treated patients with acute leukaemia with a variety of drugs, and when the patients were in complete remission they treated them with regular immunization with B.C.G. One patient has now been in complete remission for over three years. Vaccination with vaccinia virus has also been used in malignant melanoma, and in some cases has produced regressions not only of the local lesions but also of other cutaneous and visceral metastases (Belisario and Milton, 1961; Burdick and Hawk, 1964).

Use of Non-specific Lymphoid Cells

Woodruff and Nolan (1963) treated eight patients with advanced carcinomatosis by injecting spleen cells from another individual after preparatory treatment of the recipient with a cytotoxic drug designed to delay rejection of the foreign cells. All cases showed some slight improvement. These cells had, of course, not been immunologically activated, as the donor had not been immunized with tumour cells.

Schwarzenberg *et al.* (1966) treated 21 patients with acute leukaemia with leucocyte transfusions from donors with chronic myeloid leukaemia and obtained nine remissions, though these were only temporary. They concluded that the antileukaemic effect was dependent on the immunologically competent cells in the transfused population, as the remissions were related to the onset of a secondary syndrome identical with that complicating marrow grafts in man.

Specific Immunotherapy

Vaccines and Antisera

Kellock *et al.* (1922) attempted to influence the course of malignant disease by immunizing patients with material from their own tumours without any real benefit. Graham and Graham (1959) prepared a "vaccine" from the patient's own tumour, injected it mixed with Freund's adjuvant, and concluded that it might exert an effect in potentiating subsequent radiotherapy—a situation similar to that already described in animals by Haddow and Alexander (1964). More recently,

Czajkowski *et al.* (1967) immunized patients with tumour cells coupled to a foreign protein (rabbit gammaglobulin) with bisdiazobenzidine. Of the 14 patients treated, two were alive and tumour-free four years later, and biopsy of their tumours after treatment showed necrotic changes. Mathé *et al.* (1968) maintained remissions for a long time in acute leukaemia by immunizing with irradiated leukaemic cells, alone or in combination with B.C.G.

Antisera produced against human malignant tissue in animals have not been successful in modifying the course of cancer in man, with the possible exception of choriocarcinoma, and this form of treatment carries the potential danger of tumour enhancement.

Of all malignant tumours in man, choriocarcinoma should be the most antigenic, for it is derived from the placenta and presumably carries antigens derived equally from each parent. Doniach *et al.* (1958) were the first to attempt treatment by immunizing the patient against her husband's cells by skin grafting and by repeated injections of his leucocytes. Cinader *et al.* (1961) described the case of a patient in whom chemotherapy had been unsuccessful, and who was subsequently successfully treated with active immunization of her husband's leucocytes and antisera prepared in rabbits against the husband's seminal fluid. In both cases immunotherapy was used in addition to orthodox treatment, and it is difficult to assess the benefits.

Since the work of Klein *et al.* (1966) showing the presence of antibodies against Burkitt lymphoma cells, Ngu (1967) treated two patients with sera obtained from two other patients who had been free of disease for over two and a half years. In one case this "immune" sera had a marked effect and in the other only a moderate effect.

Use of Specifically Stimulated Lymphoid Cells

Following the experience with primary rat tumours by Alexander (1968), Nadler and Moore (1966) took pairs of patients with incurable malignant disease. A biopsy of the malignant tissue was taken from each patient and implanted subcutaneously into the other patient, and vice versa. After 10 to 14 days white cells were collected from the recipients of the tumour grafts and injected back into the donor. They used the white cells from 500 ml. of blood on each occasion, and repeated the transfusion once or twice daily for three weeks. Seven out of 26 patients derived some benefit from this, and two patients had complete objective remission, which in one case lasted for two years at the time of publication.

It is vitally important to remember that under certain circumstances vaccines and antisera may cause tumour enhancement rather than regression (Gorer, 1960; Milner *et al.*, 1964) and that any treatment in man must be carried out with great care. Further, the injection of foreign immune lymphocytes could cause a graft versus host reaction if the immune system of the recipient is impaired, which is why the experiments of Alexander *et al.* (1967), using the ribonucleic acid extracted from stimulated lymphocytes, may form a safer basis for immunotherapy in man.

From all this evidence it is reasonable to postulate that in some instances there is a host reaction in both man and animals against malignant disease, and it has been suggested that in certain circumstances it might be disadvantageous to irradiate or remove the drainage lymph nodes with a localized carcinoma—for example, Crile (1965) has shown that the survival with stage I carcinoma of the breast is slightly better when a simple mastectomy is performed than after a radical mastectomy. However, with other tumours—for example, seminoma—the practice of irradiating the pelvic and abdominal lymph nodes has revolutionized the prognosis (Whittle, 1957; Hope-Stone *et al.*, 1963; L. S. J. Martin *et al.*, 1965). Presumably the efficacy of irradiating the drainage lymph nodes depends on

the sensitivity of the tumour to radiotherapy. With radio-sensitive tumours the treatment may harm the tumour cells more than the lymphocytes, which may be reacting against them. On the other hand, with radioresistant tumours irradiation may harm the lymphocytes more than the malignant cells.

Before proceeding to summarize the present position one very important question must be answered: if tumours are antigenic, why should they ever arise?

Factors Permitting Growth of Tumours

As stated earlier, Burnet (1967) has postulated that an intact immune system is necessary to eliminate any malignant cells which could arise by somatic mutation, and this hypothesis is supported by the high incidence of malignant disease in patients with impaired immunity mechanisms and in the elderly. This, however, does not explain the occurrence of tumours in immunologically normal people. There are several possibilities:

Firstly, tumours may arise in immunologically protected anatomical sites. For example, in animals inert films of plastic when first implanted become surrounded by a thick wall of connective tissue, which may prevent the tumour-specific antigens of the sarcoma forming at this site from stimulating a host response until the tumour is too large to be affected.

Secondly, specific tolerance to the tumour-specific antigen may be present, as occurs with the "vertical" transmission of mammary carcinoma or leukaemia from mother to offspring. Further, tolerance may develop in the course of the growth of a chemically induced sarcoma—for example, Mikulska *et al.* (1966) have shown that it is only after the tumour has been removed that the spleen cells can be shown to be cytotoxic to the tumour cells.

Thirdly, the tumour may grow so fast that it reaches a size when it is unaffected by any immune response before an adequate response has had time to develop.

Finally, circulating antibodies may coat the malignant cells which prevent them being destroyed by cytotoxic lymphocytes. This could explain why antibodies sometimes cause enhancement.

Conclusions

From all this evidence it is reasonable to conclude that in man, as in animals, many tumours contain tumour-specific antigens against which the host is capable of reacting.

One of the reasons why attempts to demonstrate such a reaction have so far been unconvincing may be that it is only after the tumour has been removed that the presence of an immunological response to the tumour antigen can be demonstrated.

For the same reason attempts at immunotherapy in patients with advanced disease are doomed to disappointment, for animal experiments suggest that one needs a powerful immunological reaction to combat even a small number of malignant cells. This may be the explanation of the fact that the most impressive results of immunotherapy to date have been those of Mathé *et al.* (1968), who treated patients with acute leukaemia in remission after intensive chemotherapy, at a time when they had a minimal number of malignant cells left. At the present time immunotherapy should be used only as an adjuvant for the removal of residual malignant cells after other forms of treatment have been used.

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Significance of the Changes in the Circulating Lymphoid Cells in Hodgkin's Disease

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Summary: The lymphoid cell population in the peripheral blood in Hodgkin's disease differs from normal blood in three ways. Firstly, the number of large lymphoid cells actively synthesizing deoxyribonucleic acid is increased; secondly, the number of medium-sized lymphoid cells with intensely basophilic cytoplasm is increased; and, thirdly, occasional plasma cells are seen. These changes are related to the activity but not to the stage of the disease.

Similar changes are found under conditions of known antigenic challenge—that is, in infections and after immunization, and in rheumatoid arthritis and systemic lupus erythematosus.

Introduction

In 1967 we published our preliminary data showing that there were morphological changes in the lymphoid cell population in the peripheral blood in Hodgkin's disease similar to those seen in infections following immunization and in two of the

so-called autoimmune diseases, systemic lupus erythematosus and rheumatoid arthritis (Crowther *et al.*, 1967).

Since then we have made further morphological and metabolic studies on the lymphoid cells in these diseases, and the purpose of this paper is to describe these results and to discuss in particular their significance in Hodgkin's disease.

Patients

Hodgkin's Disease.—Twenty-nine patients with completely untreated Hodgkin's disease were studied. The histological diagnosis was beyond doubt in every case. Eleven had localized disease without symptoms (stages I and IIA) and the remaining 18 had localized disease with symptoms or generalized disease (stages IIB to IV, Rye classification (Peters *et al.*, 1966)). Bone-marrow biopsy and abdominal lymphangiography were used routinely in staging the patients.

Infections.—Eleven patients with viral and bacterial infections, including acute upper respiratory tract infections, acute pyelonephritis, pneumonia, tuberculosis, and staphylococcal empyema, were studied.

Autoimmune Diseases.—Six patients with active seropositive rheumatoid arthritis and three with systemic lupus erythematosus having L.E. cells and positive antinuclear factor tests were studied.

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