Papers and Originals

Approaches to the Immunological Treatment of Cancer in Man*

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Since only about 40% of malignant tumours can be eradicated by surgery or radiotherapy and only a few particular neoplasms destroyed by chemotherapy, there are pressing reasons why a complementary weapon for the treatment of cancer should be found. The fact that chemotherapy is effective only in tumours which seem to induce intense immune reactions, such as placental choriocarcinoma (Mathé, Dausset, *et al.*, 1964; Amiel *et al.*, 1967) and Burkitt tumour (Klein *et al.*, 1966), has focused attention on the development of immunological treatment.

A further reason which indicates the importance of an immunological approach to the treatment of cancer, in respect of both prevention and cure (Hamilton Fairley, 1969), is that tumour cells carry new antigens which are specific to each individual tumour—in the case of chemically induced tumours—and to each individual virus—in the case of tumours that are virally induced. Some experimental findings have indicated that these antigens are not peculiar to neoplastic cells and that they may be present in normal cells in which a chemical carcinogen or a virus has penetrated but not caused malignancy (Mathé, 1968a). Furthermore, certain animals have an immunological tolerance to tumours, particularly when a carcinogenic agent is introduced into the animal during or before the neonatal period (Klein and Klein, 1965). An immune insufficiency may frequently occur in human patients with cancer (Schneider, 1969) and in animals with a variety of different tumours. This insufficiency has also been observed during the preleukaemic period in strains of mice which have a high incidence of spontaneous leukaemia (Doré *et al.*, 1969; Schneider *et al.*, 1969); and in human patients it can be increased or induced by some types of treatment—in particular by certain forms of chemotherapy (Schneider, 1968). Moreover, antibodies which do not fix complement and which could facilitate the growth of a tumour can be produced in patients with cancer.

This phenomenon of immunological enhancement, which has mainly been demonstrated and studied in animals, should be given serious consideration in man, as it may be the most probable explanation for the correlation that we have observed in cases of placental choriocarcinoma between the frequency of serum agglutinins, the tolerance of a skin graft from the father during pregnancy, and the prognosis of the individual patient with this tumour (Mathé, Dausset *et al.*, 1964; Amiel *et al.*, 1967).

Possible Approaches

Many possible approaches to the curative or preventive treatment of cancer by immunological means exist. Most of them

are still at the stage of basic research, a few of them are at the stage of experimental therapeutic research, and, finally, some have passed on to the stage of their first clinical trials. The harmful immunosuppressive effects of chemotherapy can be prevented by not using those drugs which are particularly powerful immunosuppressives—such as the corticosteroids—or by giving these drugs by methods such as discontinuous administration (Schneider, 1968). Many studies have aimed at restoring immunity in patients suffering from immune insufficiency. Thus grafts of the thymus have a beneficial effect in radiochimeral animals with an allogeneic bone-marrow graft which have developed an immune insufficiency (Hrask *et al.*, 1968; Mathé, Nouza *et al.*, 1969). We are now testing the therapeutic effects of extracts of thymus in man. Orbach-Arbouys (1968a, 1968b, 1968c), working in our laboratory, has confirmed that it is possible to break tolerance by total body irradiation in rats who were tolerant to sheep red cells, and by giving various cytostatic drugs (cyclophosphamide, methotrexate, mercaptopurine) and antilymphocyte serum. Immunotherapy may therefore be spoken of as passive, adoptive, or active.

Passive Immunotherapy

Passive immunotherapy consists in the administration of antibody. The results of an attempt to use the IgG fraction of horse anti-human lymphocyte serum in patients with chronic lymphatic leukaemia was deceptive (Mathé, Schwarzenberg, and Amiel, 1967). The use of such a serum for the treatment of lymphatic leukaemia in mice gave variable and unpredictable results; a rabbit anti-mouse lymphocyte serum was ineffective against the L 1210 leukaemia but could completely eradicate the E δ G2 leukaemia, if fewer than 10,000 leukaemic cells were transplanted (Pouillart and Mathé, 1969); this latter leukaemia is more antigenic than the preceding one. The most serious difficulty in passive immunotherapy is the fear of enhancement of tumour growth, for up till now we have been unable to separate the cytotoxic antibodies from the enhancement antibodies. One line of progress would seem to be the preparation of specific antisera against the antigens of the tumour cells. Working in our laboratory, Motta (1969) was able to immunize animals with tumour cells that were isogenic with these tumour cells. In this way he was able to prepare a specific antitumour antibody.

Adoptive Immunotherapy

Adoptive immunotherapy, on the other hand, consists in transfusing or grafting the cellular machinery responsible for the immune reactions. When a short-term effect is required this can be done by transfusing lymphocytes, or, for a longer-term effect, by grafting the stem cells—that is, the bone marrow.

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This form of adoptive immunotherapy arose as a result of some experiments made on mice carrying the L 1210 leukaemia (Mathé and Bernard, 1959; Mathé, Amiel, and Niemetz, 1962; Mathé, Schwarzenberg, and Lapeyroue, 1968); on those with AkR spontaneous leukaemia (Mathé and Bernard, 1958; Mathé, Amiel, and Bernard, 1960); and on those with the Charlotte Friend virus leukaemia (Mathé, Amiel, and Friend, 1962; Mathé and Amiel, 1964). These experiments have shown the antileukaemic effects of a bone-marrow graft on the leukaemic cells of a recipient if the donor of the bone marrow is immunized with a leukaemogenic virus. In this case the marrow from the donor animal can exert both an antileukaemic effect on a leukaemia induced in the recipient by this virus and an antiviral effect on the leukaemogenic virus.

Finally, we hope that if this virus is not completely eradicated and if it is not genetically compatible with the tissues of the donor (as is the case with certain leukaemogenic viruses in mice which can induce leukaemia only in particular strains of mice) the allograft bone marrow will not induce a new leukaemia arising from these donor cells. This experiment has also indicated the difficulty of deriving benefit from this type of immunotherapy on account of the reaction of the graft against the antigens of the host. Such a reaction gives rise to the severe signs and symptoms which have been collectively called "the graft-versus-host disease," which is particularly severe in man, especially if the recipient has been pretreated by total body irradiation, the only procedure in use until about a year ago. Under these circumstances the sequelae of irradiation in addition to this graft-versus-host disease can cause a secondary disease that is often lethal.

The incidence of this secondary disease (or syndrome) and our experience with the allogeneic bone marrow grafting in man (Mathé, Bernard, *et al.*, 1959, 1960; Mathé, Amiel, *et al.*, 1963, 1965, 1968) are summarized in Table I. The positive

TABLE I.—Results of 24 Graft Attempts After Total Body Irradiation

Failure of the graft	7
Died with aplasia	5
Failure followed by isogenic restoration	1
Survival in aplasia until relapse of leukaemia	1
Grafts	17
Died with acute secondary syndrome	10
Died with subacute secondary syndrome	2
<i>Candida albicans</i> septicaemia	1
Varicella encephalitis	1
Died with chronic secondary syndrome at 20 months (herpes zoster encephalitis)	1
Complicated by a controlled secondary syndrome; died with leukaemia	4

take of the bone marrow graft was proved by the various tests listed in Table II. The antileukaemic effect has been analysed

TABLE II.—Proofs of Allogeneic Bone Marrow Grafts in Man

Erythrocytic antigens (Mathé, Jammet, Pendic, and Schwarzenberg, 1959)
Sex-linked granulocyte appendices (Mathé, Bernard, de Vries, <i>et al.</i> , 1960)
Mononuclear sex chromatin (Seman and Seman, 1961)
Sex chromosomes (Mathé, Amiel, <i>et al.</i> , 1963)
Groups of immunoglobulins (Inv) (Mathé, Amiel, <i>et al.</i> , 1963)
Specific immune tolerance based on the chimerism (Mathé, Amiel, <i>et al.</i> , 1963)

in four patients (Fig. 1) who survived the secondary syndrome. In three of them the graft was only partial and transitory; it produced only half of the red cells, and survived for about three months. A "complete" remission lasting for nine months was obtained in the first patient, and for five months and six months, respectively, in the second and third. In the fourth patient the graft was total and appeared definitive, to the extent that when the patient died 20 months after the grafting (from herpes encephalitis, an infectious complication of immune insufficiency related to latent secondary disease) all of the red cells had been produced by the graft. At the time of death the patient was still in complete remission, and at necropsy no leukaemic cells were found in any of his tissues.

The control of secondary disease in allogeneic haemopoietic radiochimeras is very difficult because of the frequent occur-

rence of infections. As irradiation plays an important part in producing secondary disease, we have replaced this procedure by other methods of conditioning the patient before the graft, particularly by using antilymphocyte serum. We have successfully carried out a graft in a patient with acute myeloblastic leukaemia in an aplastic phase. Both the recipient and the donor, his brother—chosen for his close histocompatibility—were treated with antilymphocyte serum. A graft was established and the degree of the secondary syndrome was far less than those we had been accustomed to see following total body irradiation (Mathé, Amiel, and Schwarzenberg, 1968). It is still too early to judge the future of this method, but the replacement of total body irradiation by the use of antilymphocyte serum may represent a considerable advance in the field of bone marrow grafting and, hence, in the realization of adoptive immunotherapy for treating leukaemia.

FIG. 1.—Visible phases and remissions in four patients treated by allogeneic bone marrow grafts and who had not died from bone marrow aplasia or the secondary syndrome.

The clinical and histological signs of secondary disease closely resembling that which follows allogeneic bone marrow grafting occur after white-cell transfusion as a form of symptomatic treatment, particularly for infections complicating agranulocytosis (Schwarzenberg *et al.*, 1965). This observation led us to think that a graft-versus-host reaction could occur in a patient who had not been irradiated, and we therefore attempted to obtain an antileukaemic effect from this reaction. We have treated patients suffering from acute leukaemia who had become resistant to all the available forms of chemotherapy with transfusions of white cells. As the signs of a graft-versus-host reaction appeared only when many leucocytes were transfused, we first used patients with chronic myeloid leukaemia as donors in order to obtain this large number of cells. We have now treated 21 patients, all of whom were resistant to every available form of chemotherapy, with this form of leucocyte transfusion (Schwarzenberg *et al.*, 1966). Of these, 10 had remissions, of which seven were complete (Fig. 2). In all the patients (except one who was also being treated with vincristine and had no previous resistance to this drug) the antileukaemic effect may undoubtedly be attributed to the transfusions of leucocytes. The antileukaemic effect correlated well with the clinical signs and symptoms of a graft-versus-host reaction (Table III) and strongly suggested that they were causally related.

We have now acquired the use of an I.B.M. continuous flow machine for the separation of white cells, which enables us to obtain easily up to 1.5×10^{10} lymphocytes from a normal donor at one session (Schwarzenberg *et al.*, 1968). Using these

TABLE III.—Immunotherapeutic Effect of Leucocyte Transfusions. Relation between the Antileukaemic Action and the Existence of a Secondary Syndrome

	Complete Remissions	Incomplete Remissions	No Effect
Secondary syndrome present	4	2	1
Absence of signs of secondary syndrome	1	2	11

normal cells, we have treated five patients suffering from acute leukaemia and have obtained only two very short remissions. This finding suggests that adoptive immunotherapeutic effects are rarer with normal lymphocytes than with chronic myeloid leukaemia cells.

FIG. 2.—Induction of a remission in a patient with acute lymphoblastic leukaemia by leucocyte transfusions from donors with chronic myeloid leukaemia.

In mice with ascites tumours remarkable results have been obtained when a local form of adoptive immunotherapy was used (Mathé, Schwarzenberg, and Lapeyraqe, 1968) by means of local injections of heterospecific lymphoid cells from animals immunized against these ascites cells. We have treated a few patients with malignant pleural effusions or ascites by using lymphocytes obtained from rabbits which had been immunized against the cancer cells isolated from the pleural or ascitic fluids from these patients. The administration of the rabbit cells, given either intrapleurally or intraperitoneally, was sometimes followed by a febrile reaction. One malignant pleural effusion in a patient with Hodgkin's disease and one in another with ascites appeared to be reduced by this treatment.

Having not yet started trials in man of systemic adoptive immunotherapy, using lymphoid cells from donors immunized with tumour antigens, I cannot comment on the remarkable results obtained in the rat by Alexander (1968). Nevertheless, we have recently confirmed in mice carrying K36 leukaemia the value of such a specific adoptive immunotherapy by using lymphocytes from donors specifically immunized against the Gross antigens (Mathé, Nouza, *et al.*, 1969).

Active Immunotherapy

In active immunotherapy the patient's own immune defences are stimulated, either non-specifically (in which case adjuvants are used to stimulate immunity) or specifically, by giving a specific antigen, which may consist of inactivated tumour cells, a cellular antigen extracted chemically, or a carcinogenic virus in the case of a virus-induced cancer.

The preventive effect of adjuvants (Old *et al.*, 1959; Biozzi *et al.*, 1959; Amiel, 1967; Mathé and Pouillart, 1969a), or of irradiated tumour cells (Glynn *et al.*, 1963; Mathé and Pouillart, 1969b) against grafted tumours has been widely reported. Charlotte Friend (1959) was able to protect mice against a leukaemogenic virus by vaccinating the animals with formalized virus. Martyre and Halle-Pannenko (1968), working in our institute, have been able to protect the mice against this same leukaemogenic virus by vaccinating them with the specific antigen in the form of a soluble lipoprotein.

Virus particles have been discovered—sometimes in large numbers—in human leukaemic cells, particularly by Seman and Seman (1968), and there is evidence that in the blood of 20% of patients with leukaemia antibodies may be found that are active against the patient's own leukaemic cells (Doré *et al.*, 1967). Nevertheless, it is still too early to contemplate the use of active preventive immunotherapy in man.

Using the L 1210 leukaemia as a simple model (Mathé, 1968b; Mathé and Pouillart, 1969b), we have shown that treatment of animals with donor-irradiated leukaemic cells or by B.C.G. applied 24 hours after the graft of the leukaemia delays and reduces the mortality, but only if the number of living leukaemic cells that were grafted was not more than 10^5 ; irradiated leukaemic cells were more efficient than the B.C.G., and the combination of the two of them was more efficient than using irradiated cells alone. The irradiated cells, and especially with B.C.G., were still effective if they were given four days after grafting the leukaemia. After leukaemic cells have been transplanted they first grow fast and then slowly.

Immunotherapy acts only on this slow phase, sometimes slowing it down or stopping growth, or sometimes killing the graft entirely, so that the animal is cured. Relapses (2 out of 150 mice) may rarely occur after some months in animals that have been thought to be cured. B.C.G. acts only if it is given repeatedly, which contrasts with treatment by injecting irradiated cells, where there is little difference between the effect of repeated and a single injection. Finally, B.C.G. seems to be the most efficient of all the various adjuvants being employed.

Therapeutic Trial

These experimental results form the basis of a clinical therapeutic trial of this type of therapy in patients with acute lymphoblastic leukaemia. Though several experts have considered that this particular leukaemia was a bad model for trials of active immunotherapy because of possible immune tolerance, we have chosen it as, firstly, this tolerance has not been proved; secondly, the serum of some patients has been shown to contain antibodies against their own leukaemic cells (Doré *et al.*, 1967); and, thirdly, if this tolerance was present at the beginning of the illness it might be broken by chemotherapy, since Orbach-Arbouys (1968a) found in rats that it was possible to break tolerance to sheep red cells by methotrexate, cyclophosphamide, and mercaptopurine.

The best conditions for using immunotherapy are when the patient's body contains the smallest number of leukaemic cells. To achieve this we have first reduced the number of cells by chemotherapy to induce a remission and subsequently tried to reduce them still further by sequentially administering all the different forms of available chemotherapeutic drugs (Mathé, Schwarzenberg, *et al.*, 1966). Thirty patients aged between 3 and 50 years have been treated in this way. They were divided at random into four groups.

The first comprised 10 patients who acted as controls and received no further treatment after stopping chemotherapy. The second group contained eight patients who were treated by B.C.G. by cutaneous scarification, initially every fourth day and then every eighth day. Each of the scratches was 5 cm. long, and they were arranged in a square; into this scarified area was placed 2 ml. of a suspension containing 75 mg. of living bacteria per ml. The third group, containing five patients, were given both intradermal and subcutaneous injections of 4×10^7 leukaemic cells, which had been obtained from a pool of allogeneic donors suffering from acute lymphoblastic leukaemia (these cells had been preserved at -70°C . in dimethylsulphoxide). For the first six injections the cells were treated with a 4% solution of formal to inactivate any possible virus, and for the following injections they were irradiated with 4,000 rads in vitro. The fourth group, of seven patients, were given both B.C.G. and injections of leukaemic cells.

Results

All the 10 control patients have relapsed. The average duration of the remission after the arrest of chemotherapy was 66 days; the median was somewhere between the 70th and 77th days, the limits being 30 and 130 days. On the other hand, 130 days after stopping chemotherapy (the date of the relapse of the last control patient) only 9 out of the 20 patients given immunotherapy had relapsed. The difference between these two groups is highly significant ($\chi^2=6.18$, $P=0.02$). On 1 June 1969 four other patients had relapsed. Analysis of the relapses in these treated patients suggests that most of them occur at an early stage. Thus nine occurred before the 100th day and five before the 30th day. This is comparable with the experimental observations, and suggests that the number of tumour cells left after chemotherapy was greater than the maximum number that could be controlled by active immunotherapy. Four relapses were of late onset; one occurred at the 210th day, one at the 315th day, and another at the 950th day. This feature is comparable with those exceptional late relapses which we had noted in our experiments with mice. In the fourth patient the relapse occurred at the 324th day; he was an infant in whom the B.C.G. treatment had been stopped 44 days previously because of the recrudescence of a severe phlyctenular keratoconjunctivitis. Seven patients are still in remission more than one year since stopping chemotherapy, in four it is more than two years, and in one more than three years. Hence there seems to be no reason for not continuing immunotherapy indefinitely.

Fig. 3 shows the actuarial curves, demonstrating differences between the groups submitted to immunotherapy and our groups of controls; and indicates that though the median length of remission after stopping chemotherapy in our patients given immunotherapy is about the same as that in those given intensive chemotherapy, the shape of the curve of these two

FIG. 3.—Actuarial curves of the duration of complete remission after stopping chemotherapy.

groups is quite different. That of the patients given immunotherapy tends to straighten out and continue as a plateau. There was no significant difference between the group given B.C.G. (five relapses out of eight patients, in one of whom treatment was stopped), those given leukaemic cells alone (three relapses in five patients), and those given both forms of immunotherapy.

Hence this trial proves that active immunotherapy may be successful in controlling acute lymphoblastic leukaemia. We now plan to apply this method to patients treated by chemotherapy to diminish the number of leukaemic cells even more than was possible using the sequential chemotherapy that we employed in this first trial.

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