The use of the cytolytic action of the immune responses (humoral or cell mediated) in cancer treatment has been considered for a long while but the knowledge of tumour-associated antigens has stimulated fresh interest in the immunological approach to this treatment (Foley, 1953; Prehn, 1963; Old and Boyse, 1964; Klein, 1966).

In order to confine discussion to the trials that have been or can be conducted on man, we shall deal only with the immunological approaches to curative treatment and omit those questions of immunological prevention which are not answered by current research.

We call passive immunotherapy the administration of antibodies, adoptive immunotherapy the administration of immunocompetent cells, and active immunotherapy the stimulation of reactions of the organism.

PASSIVE IMMUNOTHERAPY
In the context of passive immunotherapy, clinical attempts to use the serum or immunoglobulins of allogeneic or heterospecific donors in the treatment of various cancers and the endeavours to treat chronic lymphocytic leukaemia or acute lymphoblastic leukaemia by antilymphocytic serum (see Mathé et al., 1967) have been of dubious value.

Nevertheless, recent experimental data, including those of Gorer (Gorer and Amos, 1956), have been encouraging. In our group, Motta (1970) is trying to make passive immunotherapy more specific, the object being to obtain the most potent antisera possible, specifically directed against the transplantation leukaemia antigens. He has produced immunisation by a mixture of tumour cells of Charlotte Friend leukaemia with antisera directed against normal cells of the same strain. The activity against normal cells is very markedly diminished, and sometimes practically suppressed by the passive administration of antisera anti-normal cells at the moment of immunisation. On the other hand, the activity of the sera against the leukaemic cells is not diminished. In mice, one can calculate that the quantity of specific
antibodies directed against the tumour antigens of Friend leukaemia is four times greater than that obtained after conventional immunisation.

ADOPTIVE IMMUNOTHERAPY

Research into adoptive immunotherapy has advanced further. It deals with the introduction into the cancer patient of immunocompetent cells able to react against the tumour cells. Since they are derived from foreign organisms, either allogeneic or heterospecific, they will react against the strain or species histocompatibility antigens of the tumour cells (a reaction that can destroy the cells by non-specific immunotherapy) and against the tumour associated antigens (a reaction destructive to cells by specific immunotherapy).

The following experiment shows the importance of immunising the donor against the tumour associated antigens in lymphocyte transfusions (Mathé
AkR mice received $10^4$ or $10^6$ lymphocytes of C57B1/6 donors, one group of which were not immunised and the other group immunised specifically against the tumour antigens (with the irradiated cells of E\(\delta\) G2 leukaemia, which had been induced in C57B1/6 mice by Gross virus). It can be seen in Fig. 1 that the antileukaemic effect, non-existent when they receive $10^4$ lymphocytes of non-immunised C57B1/6 mice, becomes obvious when they receive $10^6$ cells of non-immunised donors or $10^4$ cells of immunised donors. It is even more remarkable when they receive $10^6$ cells from immunised donors. Hence the number of lymphocytes transfused, and the possible specific immunisation of donors, are important factors in adoptive immunotherapy.

In man, we have obtained some remissions in acute leukaemia patients resistant to available chemotherapy, either by transfusions of white blood cells from donors with chronic myelocytic leukaemia (Schwarzenberg et al., 1966) (Fig. 2), or by transfusions of lymphocytes of normal donors, whose
blood cells had been separated by the IBM continuous flow separator (Schwarzenberg et al., 1970). There is a good correlation between the frequency of remissions obtained, and the manifestations of the secondary disease tied to the graft versus host reaction, which, from our experience of grafting bone marrow, we have learned to recognise (Mathé et al., 1965a).

It is impossible today to use the transfusion of allogeneic lymphocytes from immunised donors to man. One can only utilise heterospecific lymphocytes. We have tried intravenous transfusions to humans of sheep lymphocytes immunised against their tumour cells but these have been poorly tolerated.
As yet we have had to limit our trials of specific adoptive immunotherapy to neoplastic ascites or pleuritis, the sheep lymphocytes being injected locally. In a total of 10 cases, we have observed two complete regressions (Figs. 3 and 4) (Mathé et al., 1967), induced by intra-pleural or intra-peritoneal injections of lymphocytes from sheep immunised with the patients’ cells.

The remissions obtained in leukaemia after lymphocyte transfusions are short, of the order of seven days to four months. We wondered if the grafting of allogeneic bone marrow with a persistent graft-versus-host reaction would achieve a longer effect. The experimental studies conducted in 1958,
1959, 1960, 1962 and 1964, on grafted (L 1210), viral (Friend), and spontaneous (AkR) leukaemias (Mathé and Bernard, 1959; Mathé et al., 1962a; Mathé and Schwarzenburg, 1968; Mathé et al., 1962b; Mathé and Amiel, 1964; Mathé and Bernard, 1958; Mathé et al., 1960) have supported this hypothesis. Our attempts in man were first carried out on bone marrow grafts in leukaemic patients, conditioned by total irradiation (Fig. 5). Remissions lasting from five to nine months had been obtained after partial and transitory (three months) haematochimerism. In one patient, who was a complete haematochimera twenty months after the graft, the leukaemia had not yet reappeared but he died of an infectious complication of the secondary disease (Mathé et al., 1963, 1965b). Since we lost other patients from acute or subacute secondary disease, we abandoned total irradiation. Even if this means of conditioning is not directly responsible for the secondary disease, it greatly enhances it. Cyclophosphamide, in doses proposed by Santos et al. (1970), seems to have the same effect.

Thus, we have tried to use antilymphocyte serum (ALS) as a means of conditioning the recipient. This method rendered the bone marrow grafts

![Diagram of remissions in four patients treated by allogeneic bone marrow grafts who had not died from bone marrow aplasia or the secondary disease.](image-url)
free from secondary disease, but, at the same time, we did not observe any antileukaemic effect (Mathé et al., 1970b). As a result, we are at present trying the association of ALS and cyclophosphamide, administered in lower doses (45 mg/kg for 4 days) than those used by Santos. The first trials are encouraging: complete remissions have been obtained, but the grafts have been followed by a secondary disease. Not enough time has elapsed for us to judge the real value of this method.

ACTIVE IMMUNOTHERAPY
Active immunotherapy is also making its first steps. There is a considerable literature on the preventive effect of adjuvants (Old et al., 1959; Biozzi et al., 1959; Amiel, 1967; Mathé and Pouillart, 1969), or of irradiated cells (Glynn et al., 1963; Mathé and Pouillart, 1969) against grafted tumours.

We are only concerned here in the research on an eventual curative role for active immunotherapy; that is to say, a form of therapy that can be applied after the appearance of the disease.

Taking L 1210 leukaemia as a simple model (Mathé, 1968; Mathé and Pouillart, 1969), we have shown that:

1. The treatment of animals with irradiated leukaemic cells or by BCG applied 24 hours after the graft of $10^4$ living leukaemic cells delays and reduces the mortality (Fig. 6).
2. BCG acts only if given repeatedly, while the repeated injection of irradiated cells has the same effect as a single injection.
3. BCG seems to be the most efficient of all the presently employed adjuvants, some of which can kill the animals more quickly by toxic phenomena and not by immunological enhancement for there was no alteration in tumour volume.
4. There is, however, an important limitation to this action of active immunotherapy, BCG or irradiated tumour cells or the combination of both (Fig. 6) being effective only if the number of living leukaemic cells grafted is not more than $10^5$.
5. Irradiated leukaemic cells were more efficient than BCG, and the combination of the two was more efficient than the use of irradiated cells alone: the irradiated cells, especially if given with BCG, were still effective if they were given four days after grafting the leukaemia.
6. After transplanting, leukaemic cells at first grow fast then slowly, and this immunotherapy acts only on the slow phase; sometimes it diminishes the slope, sometimes it transforms it into a plateau, and sometimes the curve descends and the animal will be cured. Very rarely, relapses (2 out of 150 mice)
can occur after some months; we wondered if the tumour cells could be maintained by immunotherapy in the ‘go’ state. Studies of the cell cycle by Lhérítier and Bullens (1970) have shown that it is not so: all the cells are in the cycle, which suggests that, in the case of a plateau, destruction equals production.

Fig. 6. Cumulative survival of mice grafted with $10^2$ to $10^7$ L1210 leukaemia cells: not treated or treated in the 24 hours following the graft by BCG, or irradiated leukaemic cells, or association of BCG and leukaemic cells.

In these experiments, tumour cells were rejected subcutaneously, and the leukaemia used had been transmitted over so many generations that it might have produced a certain withdrawal of histocompatibility with the mice to which it had been grafted. We must add that the latter were F1 (DBA/2 × C57B1/6) mice. So we performed two other experiments in which we treated mice injected intravenously with $10^4$ living cells of very recently induced
leukaemias (RC19, induced by Rauscher virus, and E♀K1, induced by Gross virus in C57B1/6 mice). Active immunotherapy, non-specific or mixed, applied 24 hours after the isogeneic intravenous graft of RC19 has a considerable action. The association of specific and non-specific active immunotherapies, applied in the same manner in mice grafted with E♀K1 leukaemic
cells, leads to a moderate, but significant antileukaemic action (Mathé et al., 1970c).

The experimental studies have formed the basis of a clinical therapeutical trial in patients suffering from acute leukaemia. This disease was suspected to be an unwise choice for a trial of active immunotherapy, because of its
possible immune tolerance, of which we are aware because of its supposed existence in spontaneous leukaemia of AkR mice, and because of the vertical transmission of Gross virus (Levy, 1969). Nevertheless, we have chosen this leukaemia for the following reasons:

(a) This tolerance has not been proven.
(b) Doré et al. (1967) have found in the serum of some patients antibodies against their own leukaemic cells.
(c) If this tolerance existed at the beginning of the illness, it might be possible to break it down by chemotherapy, as Orbach (1968) was able to suppress tolerance to sheep red cells in rats by methotrexate, cyclophosphamide, and 6-mercaptopurine.
(d) We even doubt this alleged tolerance of AkR mice towards the tumour antigens of Gross virus induced leukaemia for various reasons, the main one being the possible production of antibodies, after immunisation with K36 cells directed against AkR leukaemic cells (Doré et al., 1970).

The experiments cited above suggested that the optimum condition for immunotherapy applied to man was that the patients should be carrying the smallest number of leukaemic cells possible. To achieve this, we first reduce the cell number by chemotherapy, to induce a remission. Then, we try still further reduction by sequentially administering all the different forms of chemotherapeutic drugs available (Mathé et al., 1966).

In our first trial, 30 patients, whose ages varied from three to fifty years, were treated in this way; they received, after remission induction, a sequential complementary cell-reducing chemotherapy (one drug at a time) combined with intrathecal administration of methotrexate and meningeal radiotherapy. Four groups were formed randomly. In the first, 10 control patients did not receive any further treatment after complementary chemotherapy was stopped. In the second, 8 patients were treated by BCG (Figs 7 and 8) and received, on every fourth day and then on every eighth day, twenty cutaneous scratches, each 5 cm long, and arranged in a square. Two ml of a suspension containing 75 mg/ml of living bacteria were put into the scarified area. In the third group, 5 patients received each week, both intradermally and subcutaneously, \(4 \times 10^7\) leukaemic cells, which had been obtained from a pool of allogeneic donors suffering from acute lymphoblastic leukaemia. These cells had been preserved at \(-70^\circ C\) in DMSO. For the first six injections, the cells were treated with a 4 per cent formol solution to inactivate any possible virus and, for the remaining injections, they were irradiated with 4,000 rads \textit{in vitro}. In the final group, 7 patients were given both forms of immunotherapy.

Each of the 10 patients, left without treatment after chemotherapy was
stopped, relapsed. The average duration of the remission was 66 days, the median lying between the 70th and 77th day. The limits were 30 and 130 days.

At the 130th day, only 9 of the 20 patients given immunotherapy had relapsed, and the difference between those two groups is highly significant ($\chi^2 = 6.18, p = 0.02$). Later, three other patients relapsed.

An examination of the relapses gives rise to the following ideas:

(a) The majority of them appeared to be early: 9 before the 100th day, 5 before the 30th day, which is comparable with the experimental observations in mice, and suggests that the number of tumour cells left after the chemotherapy was greater than the maximum number that could be controlled by active immunotherapy.

(b) Four of them were late in onset. One occurred at the 210th day, another at the 324th day, and another at the 950th day (which is comparable with those exceptional relapses we had noted in our experiments with mice). The fourth, which occurred at the 315th day, was in an infant in whom the BCG treatment had been stopped 44 days previously (because of a severe and intractable phlyctenular keratoconjunctivitis which had required the treatment to be stopped several times). This last patient, when considering the possibility of late relapses, answers the question as to whether immunotherapy should be continued indefinitely. Seven patients are still in remission more than two years after chemotherapy has been terminated; in four it is more than three years, in one four years, and in one for more than four years.

Figure 9 shows the actuarial curves, demonstrating differences between the groups submitted to immunotherapy and the control group. They indicate that, although the median for the remission duration in our patients given immunotherapy is of the same order as those given intensive chemotherapy (Holland and Glidewell, 1970), the shape of the curves of these two groups is quite different. That of the patients given immunotherapy tends to straighten out and continue as a plateau.

There were no significant differences between the group given BCG (5 relapses out of 8 patients; treatment had been stopped in one), that given leukaemic cells alone (3 relapses in 5 patients), and the group given both these forms of immunotherapy (5 relapses in 7 patients).

The remarkable results and the failures of this trial require further discussion. With regard to the successful results, it is important to remember that the patients who underwent immunotherapy had been highly selected. That is, they all had received prolonged chemotherapy, with little effect. It is possible that these patients belonged to a special category and perhaps were destined for long spontaneous remissions. However, it should be noted that the control individuals relapsed very rapidly, confirming the inefficiency of
chemotherapy. In regard to the design of the trial, it is the chemotherapy that should be reviewed, in terms of the poor choice of certain drugs which rarely act against acute lymphoblastic leukaemia, and in terms of sequential administration of only one drug at a time.

The protocol, which we have subsequently followed and in which we erroneously abandoned meningeal radiotherapy, confirms the ideas of the preceding discussion. The results are, in fact, disappointing, mainly because of several meningeal relapses.

This is why our present protocol requires not only a more intensive systemic chemotherapy, comprising, after remission induction, a complementary cell-reducing chemotherapy (3 sequences of 2 drugs in each), but also an intensive intrathecal chemotherapy with methotrexate and cytosine arabinoside complemented by central nervous system irradiation.

These are the clinical results we have so far obtained with immunotherapy. Most of them concern leukaemias and not solid tumours: we have been limiting our initial trials to leukaemias because it is considered that immunological enhancement is less feared in these diseases than in solid tumours.
Our study on placental choriocarcinoma (Mathé et al., 1964; Amiel et al., 1967) as well as the first data collected by Hellström et al. (1968) strongly suggests that this phenomenon does exist in man.

The adjuvants of immunity that we have been using are not very powerful in man, mainly because we are not able to give a dose equivalent to the dose given in mice or to use the same route of administration. We hope that the introduction of the Poly IC (Mathé et al., 1970d), a polynucleotide that is an adjuvant in mice (Braun et al., 1968; Mathé and Hayat, 1970, in preparation) and is able to induce remissions in patients with acute leukaemia if they have only a small number of tumour cells (Mathé, 1968), will notably improve the efficiency and the management of active immunotherapy.

This article is based on a paper read by Dr Mathé at the Conference on the Treatment of Malignant Disease held at the Royal College of Physicians in July 1970.

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Rules for the Apothecary

Must fyrst serve God, forsee the end, be clenyly, pity the poore. Must not be suborned for money to hurt mankynde. His place of dwelling and shop to be clenyly to please the sences withal. His garden must be at hand with plenty of herbes, seedes, and rootes. To sow, set, plant, gather, preserve and kepe them in due tyme. To read Dioscorides, to know ye natures of plants and herbes. To invent medicines to chose by colours, tast, odour, figure etc. To have his morters, stilles, pottes, filters, glasses, boxes, cleane and sweete. To have charcoles at hand, to make decoctions, syrumpes etc. To kepe his cleane ware close, and cast away the baggage. To have two places in his shop—one most cleane for thephisik, and a baser place for the chirurgie stuff. That he neither increase or diminish thephisician’s bill and kepe it for his own discharge. That he neither buy nor sel rotten drugges. That he peruse often his wares that they corrupt not. That he put not in quid pro quo without advysement. That he may open wel a vein for to helpe pleuresy. That he meddle only in his vocation. That he delyte to reede Nicolaus Myrepsus, Valerius Cordus, Johannes Placaton, the Lubik etc. That he do remember his office is only to be ye phisician’s cooke. That he use true measure and weight. To remember his end, and the judgement of God: and thus do I commend him to God, if he be not covetous, or crafty, seeking his own lucre before other men’s help, succour, and comfort.

(By Sir William Bulleyn, Elizabethan physician.)