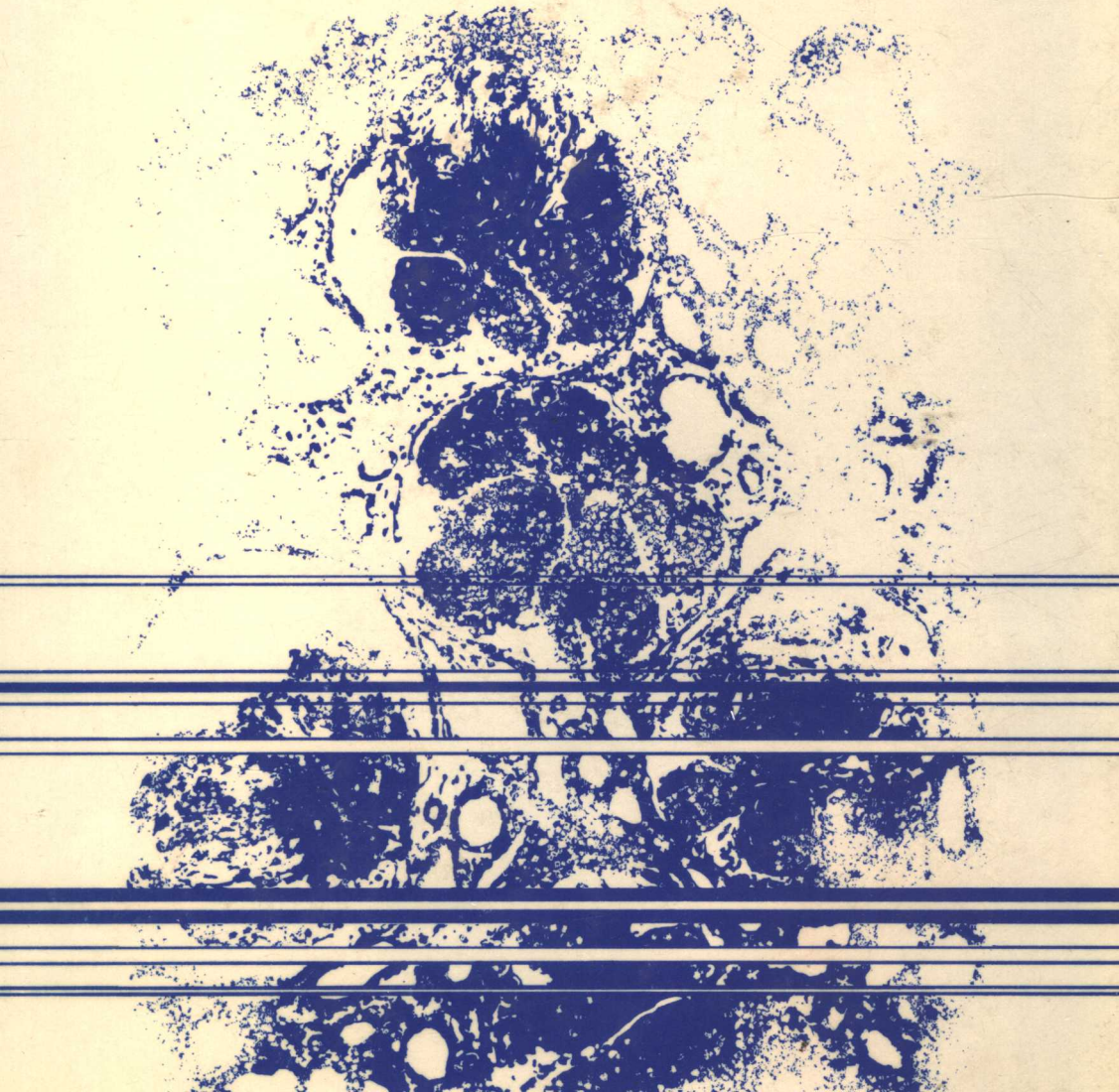
Immunological Engineering

Edited by D.W. Jirsch



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Foreword

Immunology has become one of the most important of the life sciences. As research unravels the mystery of the lymphocyte, a central role of the immune system in health preservation has become evident. The paediatric immunodeficiency disorders or 'experiments of nature' have demonstrated the division of cellular and humoral immunity; specific functional defects are now readily identified. The tendency of persons with immune dysfunction to develop neoplasms has suggested that surveillance mechanisms within the immune apparatus prevent tumour development. Malignancies, in fact, do seem to provoke certain immune responses, begging numerous therapeutic questions. Transplantation surgery, or the demand for 'new parts' has led to description of those antigens important in tissue-typing. Genetic loci have been found responsible for transplantation antigen display; as well, they influence clinical resistance or susceptibility to a wide variety of infections, auto-immune or neoplastic diseases.

Clinicians have been quick to recognize the therapeutic implications of laboratory work and to use this knowledge in disease treatment. Precise patient–tissue matching and immunosuppressive treatment make renal allotransplantation safer and more successful than ever before. Both paediatric and adult immune deficiency states are now often recognized; treatment may involve general immune support or specific manipulations with, perhaps, bone marrow or thymus grafts or treatment with lymphocyte transfer factor.

Transplantation of bone marrow has been used not only to correct certain immune defects but to correct marrow failure of diverse origin. Although the early hopes that cancers would melt away with administration of simple immune sera or cells were puerile and unfounded, augmentation of tumour immunity can benefit the cancer patient and is a welcome addition to oncotherapy.

These achievements may be considered examples of 'immunological engineering' in that diverse manipulations or alterations of immune responses are used therapeutically. In this text members of ten outstanding research communities survey these burgeoning fields of activity in depth.

No attempt has been made to cover the vast interface between immunology and illness, and certain repetition has been considered both valuable and provocative. The submissions document, I think, an extraordinary approach to the therapy of human disease.

Toronto, Canada
December, 1977 D.W.J.

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A Biological Approach to the Management of Acute Leukaemia in Man

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A decade ago it seemed reasonable to suppose that all diseases embraced within the general term 'leukaemia' were variants of the same fundamental pathological process.

Recently, it has become necessary to challenge the universal acceptance of this hypothesis in order to explain differences in the response to treatment of the various types of leukaemia. For example, chemotherapy with agents known to interfere with RNA and DNA (the 'usual' anti-cancer agents) has been able to render approximately 40% of patients with acute lymphoblastic leukaemia (ALL) disease free for a sufficiently prolonged period of time to assume they are now cured. With similar treatment using the same type of agents 50% or more of the patients with acute myelogenous leukaemia (AML) also become apparently disease free according to the methods of detection at present available, but it is a sad fact that all but a very small minority of these patients develop a rapid recurrence of their disease which then proves fatal. This failure to control the disease in seemingly a highly drug-sensitive condition is not only disappointing but also totally unexpected—in the carcinomas, for example, such a good response to initial treatment would be expected to be associated with a reasonable proportion of cured patients (as occurs with ALL).

There are many possible reasons (such as the emergence of a new clone of AML cells) for this failure to control AML with 'anti-nucleic acid' agents and these will be dealt with more fully later, but it is sufficient to say here

that a new approach to the management of this disease is clearly required. At present there are no agents (other than perhaps steroids and asparaginase) that are available with an anti-cancer action that do not interfere with nucleic acid function and this is largely due to screening programmes so far devised, which have always involved inhibition of proliferation of cancer cells. The possibility must be explored that regulation rather than proliferation may be the key problem for certain types of cancer (such as AML) and that it is the soil itself that is defective (i.e. the marrow environment) not the malignant clone of cells, which merely represents the end result of the process. This concept has led us to explore the possibility of a biological control of certain types of leukaemia and it is the purpose of this chapter to discuss one such line of endeavour, namely the manipulation of the host immune system to render the patient permanently disease free.

THE EARLY ATTEMPTS AT IMMUNOTHERAPY

Over 60 years ago Tyzzer[1] attempted to treat acute leukaemia in man with immunotherapy and similar anecdotal studies have been conducted for an even longer period for other forms of malignant disease. Much of this work was independent of animal experiments dating from the time of Paul Erhlich and others at the end of the last century, which appeared to show that immunological manipulation could cause the rejection of tumours. Unfortunately, the uncontrolled and largely unrepeatable clinical studies, followed by the realization that transplanted tumour rejection in 'unrelated' animals was the result of transplantation antigens and nothing to do with cancer, led to a feeling of great pessimism and a virtual abandonment of the subject until after the Second World War.

Interest in the field was revived in the mid-1940s when Ludwich Gross[2] took advantage of the recently developed inbred mice colonies produced by Leonell Strong[3] at Yale University. In this first study published by Gross[2], C3H mice were used. These had been bred by continuous brother to sister mating for more than 20 years, and had thus acquired a remarkable genetic uniformity that may for practical purposes be considered autologous. He studied a chemically induced sarcoma originally produced in an animal of the same line which eliminated the possibility that the immunity to tumour inoculation could be caused by genetic differences between the tumour cells and those of the host. He found that after inoculation of tumour-bearing animals with a suspension of this sarcoma, 20% showed tumour regression.

By the late 1950s carefully conducted animal experiments showed that tumour cells which had been induced by chemical carcinogens, by viruses or by physical carcinogens had in their plasma membranes macromolecules which were not present in the plasma membranes of normal cells. The host recognizes these substances as foreign and reacts against them by producing

antibodies and developing cell-mediated immunity. These tumour-specific macromolecules present in membranes are generally referred to as 'tumour-specific transplantation-type antigens' (TSTAs). Subsequent attempts in carefully controlled animal systems to treat tumours known to have TSTAs by immunological methods were disappointing until Haddow and Alexander[4] were able to show that primary sarcomas in rats which had been induced by implanting a pellet of the carcinogen 3,4-benzpyrene could be controlled by two types of immunological treatment after the bulk of the tumour had been removed either by surgery or by radiotherapy. The residual tumour cells could be held in check either by active immunization with irradiated tumour cells derived from the tumour to be treated[4], or by injection of lymphocytes obtained from other animals that had been immunized previously with a piece of the tumour to be treated[5]. It was quickly shown that these immunotherapy procedures were also effective against leukaemias in mice[6,7]

It must be stressed that only some experimental tumours respond well to these immunological treatments and that with many tumours, particularly those of spontaneous origin, no effective immunotherapy can be observed even when the tumour load is very small (e.g. spontaneous acute leukaemias in the rat[8]). Primary experimental tumours which give rise to distant metastases also respond badly, if at all, to immunotherapy and immunotherapy has also been very disappointing in the control of metastatic disease in animals. The possible inappropriateness of animal models in which effective immunotherapy has been demonstrated for the clinical situation may explain in part why, in properly conducted clinical trials using contemporaneous controls, immunotherapy has been shown to be virtually ineffective for the treatment of disseminated human malignant disease and reproducible clinical benefit from immunotherapy has so far only been seen in two situations: (1) The treatment of Stage I lung cancer (but not in later stages), where intrapleural injection of BCG appears to be of substantial benefit[9], and (2) acute myelogenous leukaemia, where some prolongation of life but probably no cures have been obtained using immunotherapy in conjunction with chemotherapy (see below).

METHODS OF IMMUNOTHERAPY

Passive immunotherapy

This method refers to the passive transfer of specific immune material into a tumour-bearing host. It has been highly effective in some animal systems but has not been systematically studied in controlled clinical trials for very good reasons. Until we can be much more sure that human tumours have TSTAs and that one can measure the activity of antibodies, cytotoxic cells, or products derived from them, no rational protocol can be designed.

Local immunotherapy

This relies on the destruction of tumour cells by inflammatory cells drawn locally into a tumour by either injection of BCG, *Corynebacterium parvum* or by using a delayed hypersensitivity reaction with tuberculin or dinitrofluorobenzene. This does not bring about systemic effects and there is no convincing evidence that such treatment affects any lesions other than those directly treated. However, unquestionably, BCG may drain into adjacent lesions or affect tumour cells in adjoining lymph nodes.

Active immunotherapy

Almost all carefully conducted trials of clinical immunotherapy and especially those involving leukaemias make use of this type of procedure, which consists of stimulating the existing immunological machinery of the host either specifically with tumour cells (or modified tumour cells) or non-specifically with substances such as BCG or *Corynebacterium parvum*, which cause hyperplasia of the reticuloendothelial system, or by a combination of the two.

CLINICAL STUDIES OF IMMUNOTHERAPY FOR ACUTE LEUKAEMIA IN MAN

Acute lymphoblastic leukaemia (ALL)

Animal experiments had shown that although immunotherapy was effective for 'prophylaxis' it only worked in tumour-bearing animals if the tumour load was very small. This led Mathé[7] to suggest that acute leukaemia might be an equivalent model to test for the effectiveness of immunotherapy in man. In this situation patients could be given conventional chemotherapy until there was no further detectable disease (the so-called remission state) but at a time when it is known that some leukaemia cells still remain because without further treatment relapse inevitably occurs. This remission state seemed to be an ideal moment to give immunotherapy. It is to Mathé's great credit that he selected the remission state of leukaemia to test the efficacy of immunotherapy and, in addition, he was one of the first oncologists to stress the importance of controlled clinical trials in cancer. In Mathé's later study[10] he selected a group of 30 children with ALL, all of whom had been in remission for at least 2 years. For some, all treatment was stopped whilst the rest were given weekly Pasteur BCG, killed allogeneic ALL cells, or both BCG and cells. All 10 of the untreated patients relapsed within 130 days, whereas half of the 20 immunotherapy patients remained in remission for more than 295 days, some of them for many years. The numbers were too small to decide which of the immunological regimes was best.

This study aroused great interest and several attempts have been made to confirm the value of BCG alone in ALL during remission. In Britain the

Medical Research Council arranged a trial[11] which compared the use of twice weekly methotrexate with BCG and no treatment. Figure 1.1 summarizes the results of this study[12] in which it was found that the duration of remission for 18 patients who received no further treatment after an initial 5½ months of chemotherapy was not significantly different from a similar group of 50 patients given weekly Glaxo BCG. Patients who received further chemotherapy (methotrexate) during the period following the initial 5½ months had longer remissions. Further follow-up of these patients 5 years later confirms these initial findings and only one patient in each of the BCG and no treatment arms remain in first remission. Of interest

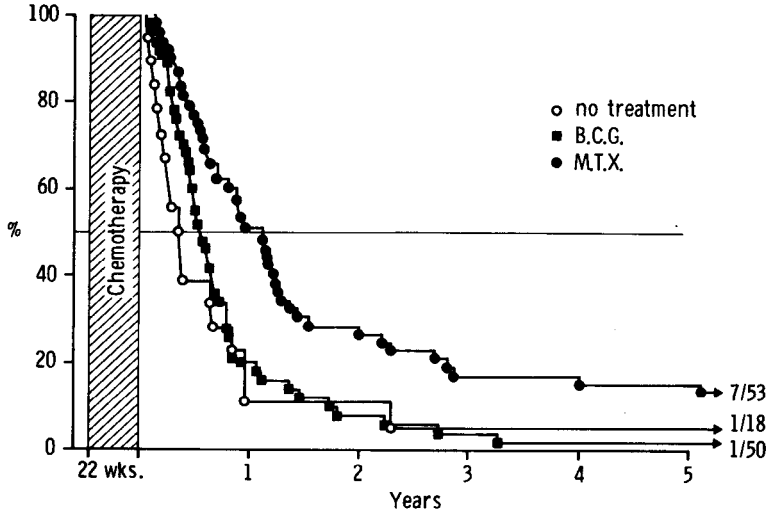

Figure 1.1 Effect of BCG on length of first remission for childhood ALL (MRC Trial 1969–70)

there is no difference in the overall survival curves of these three groups of patients. A similar study in the USA by Leukaemia Study Group A[13] was based upon the same lines as the British study, comparing BCG with no treatment (and with maintenance methotrexate). No difference in remission duration could be detected between those patients receiving Chicago BCG and those untreated although, again, those patients given methotrexate during remission tended to stay longer in remission. In this study the initial randomization was after 3½ months of chemotherapy but the patients receiving maintenance chemotherapy were further randomized after another 8 months into BCG, no treatment, or methotrexate maintenance. This further randomization showed no therapeutic advantage of BCG over no treatment for maintaining remission.

These three studies raise many questions. Table 1.1 summarizes the details and differences of Mathé's study from the two subsequent attempts designed

Table 1.1 Immunotherapy for acute lymphoblastic leukaemia of childhood

Study	*Duration induction chemo-therapy (months)*	*CNS prophylaxis*	*BCG used*	*Number of patients in each treatment group (bracket = median remission lengths in months)*			
				BCG	*BCG+ cells*	*No treatment*	*Metho-trexate*
Mathé	24	No	Pasteur	—	20 (7)	10 (2)	—
MRC	5½	No	Glaxo	50 (6)	—	18 (4)	53 (14)
Heyns	3½	No	Chicago	34 (4)	—	31 (4)	285* (8)
,,	11½	No	Chicago	44 (5)	—	52 (6)	57 (14)
Poplack	—	Yes	Pasteur	—	21	—	35

* Patients in this arm still in remission after 8 months further randomized into study below

to test his claim. It can be seen that Mathé's study only included patients who had already been on chemotherapy and in remission for 2 years and this may be a factor in selecting patients with less residual leukaemia than in the other two studies. Animal studies show that immunotherapy is only effective if the mass is very small and recent chemotherapy programmes[14] confirm that chemotherapy of less than 2 years is associated with very early relapse, presumably due to a large number of remaining leukaemia cells. However, this is not the whole answer, because although the control arms in the British and American studies relapsed very quickly (Table 1.1) so did those in Mathé's control arm (median remission duration of the 10 patients was 2 months after 2 years of chemotherapy). Other factors that may be important in explaining the variance between Mathé and the other groups is that the BCG was different for the three studies, and Mathé used cells in addition to BCG for some (and ultimately all) of his patients. Clearly failure to irradiate prophylactically the central nervous system (CNS) is something that should not be allowed to influence possible future studies although careful inspection of the Heyn's Group A and MRC results do not suggest this factor unfavourably biased the results against a possible therapeutic effect of BCG. Many of these objections could have been clarified by a study from the Bethesda group[15] in which Pasteur BCG and (live) allogeneic leukaemia cells were used for immunotherapy for 21 children with chemotherapy-induced remission (including CNS prophylaxis—see Table 1.1). The immunotherapy was given for 2-month periods interspersed with 4 months of chemotherapy and the whole cycle was repeated. Alas, the 35 patients in the control arm were given methotrexate instead of no treatment during the corresponding 2-month period when the treatment group was being immunized. Both arms were indistinguishable for length of first remission and thus a definitive conclusion about the usefulness of immunotherapy in

this study is impossible; either immunotherapy is as effective as methotrexate for maintaining remission, or both treatments give no benefit at all.

In a non-randomized study the Houston Group[16] have used Pasteur BCG for the maintenance of remission of all forms of adult leukaemia, and although they report benefit with acute myelogenous leukaemia (see below) there was no evidence that Pasteur BCG prolonged remission in ALL. However, the number of patients studied was small and this was a sequential series of patients using historical controls and is thus open to some criticism. In the related disease Burkitt's lymphoma, Ziegler[17] used Pasteur BCG given to the patients by scarification for 10 weeks after cyclophosphamide induced remission. He reports that 11 of the 21 BCG-treated patients have relapsed which is no different from 11 of the 19 control patients. However, it is always difficult to evaluate negative studies particularly involving small numbers of patients having a disease with many staging parameters. For example, six of the 11 patients that relapsed in the BCG group did so in the CNS compared with only one of the 11 patients in the control group and although this was attributed to an imbalance of the distribution of stage B patients in the original randomization it nevertheless detracts from the significance of the data. It is probably futile to attempt to draw any conclusions from this study concerning BCG, one way or the other.

At present the place of immunotherapy alone for the maintenance of remission in ALL must remain speculative since only Mathé has reported a therapeutic effect and no other study has done exactly as he did in giving Pasteur BCG and cells after two years of chemotherapy. It seems unlikely that it is at present necessary to use immunotherapy alone as the primary method of treatment for ALL in the face of the outstanding results produced by intensive combination chemotherapy with prophylactic treatment of the CNS as developed by Pinkel and his colleagues[14], but the possibility of chemoimmunotherapy as used in adult myelogenous leukaemia deserves careful consideration in properly controlled studies and this has yet to be done.

ACUTE MYELOGENOUS LEUKAEMIA

The Barts/Marsden Study

Towards the end of 1969 recent improvements in chemotherapy had allowed approximately half of all patients with acute myelogenous leukaemia to achieve complete remission[18], but chemotherapy alone was proving disappointing for maintaining these remissions. In consequence a combined study was initiated between St Bartholomew's Hospital in London and The Royal Marsden Hospital, Sutton, Surrey, to see if remission lengths and survival could be increased when immunotherapy was included as part of the remission treatment. In this study remission patients with AML were

randomized into two groups, one group receiving chemotherapy alone for the maintenance of remission and the other group receiving the same chemotherapy plus immunotherapy. Any difference between these two groups was attributable only to the immunotherapy. The maintenance chemotherapy was chosen to avoid immunosuppression as far as possible. Laboratory studies had shown that this could be achieved by giving cytotoxic drugs in widely spaced courses of short duration and by avoiding the use of powerfully immunosuppressive agents such as cyclophosphamide. The immunotherapy given was irradiated allogeneic stored myeloblastic leukaemia cells and BCG. Animal data had suggested that BCG and cells given in combination produce a stronger immunotherapeutic response than that seen with either alone[19].

Because of the extremely troublesome logistic problems raised by attempting to include cells as active specific immunotherapy in a therapeutic programme we decided to conduct some preliminary experimental studies to determine if we might expect such treatment to be beneficial. From such studies[20] we learned that the host was able to recognize autologous leukaemia cells as foreign *in vitro* if cultures were set up in a manner similar to the mixed lymphocyte reaction, i.e. in these experiments we found that remission lymphocytes were transformed in the presence of killed autologous leukaemia cells taken when the patient first presented and cryopreserved in a viable state. If, however, remission marrow (not containing leukaemia cells) was handled in a similar manner, then no such stimulation occurred and we concluded that there was something on the surface of the leukaemia cells that behaved as if it was a leukaemia antigen. In addition we found that this host response to leukaemia cells *in vitro* could be enhanced if the patient was immunized with at least 1×10^8 irradiated autologous leukaemia cells, but this effect was transient and so repeated injections were required to obtain a sustained effect. It thus became apparent that large numbers of cells would need to be available for an effective programme to test the efficacy of active specific immunotherapy. In this aspect we were fortunate that the IBM blood cell separator had become available which was capable of removing very large numbers of leukaemia cells from the circulating blood of untreated acute leukaemia patients in a safe and efficient manner[21]. It was this consideration in fact that led us to concentrate our efforts in clinical immunotherapy on AML because this disease primarily affects adults and the use of separators is very much simpler under these circumstances and between 10^{11} and 10^{12} leukaemia cells (a packed volume of several hundred ml) could be removed from a single patient presenting with a high blood count. Cells removed from the patients at presentation were stored in a viable form by freezing in liquid nitrogen[22,23].

It is worth considering in some detail the exact nature of this study because the interpretation of other studies attempting to confirm or refute the results then becomes clearer.

Patient selection

All patients with AML who were first seen at St Bartholomew's Hospital between 10th August 1970 and 31st December 1973 were included in the study. Analysis was made of the data completed to 7th August 1975. Before any treatment was given to induce remission, all patients were allocated into one of two groups on an alternate basis to determine whether they would receive immunotherapy if they achieved remission. The total entry of new patients was 139, 107 of whom were included in the series described by Powles *et al.*[24], and the rest were seen subsequently. The final allocation of patients who attained full remission was 22 to chemotherapy and 31 patients to chemoimmunotherapy. The two groups do not have equal numbers because they were allocated when they first entered hospital, and the number in each group that attained remission happened not to be the same. Of the 31 patients allocated immunotherapy, three were not included in the analysis. One of these patients died of infection after attaining full remission but before immunotherapy was given; one patient was 74 years old and could not tolerate the repeated journey to and from the hospital, and the third patient passed into remission whilst receiving the immunotherapy, so it was felt she was not representative of the group.

Induction treatment

The induction protocol of drugs (for details see ref. 24) consists of daunorubicin and cytosine arabinoside given in slightly modified ways (Studies 2, 3, 4A and 4B in refs. 18 and 25). Fifty-three patients passed into full remission so that the overall remission rate during the trial period now stands at 38%. All patients in remission in Studies 2, 3 and 4A received identical maintenance chemotherapy, as described by Powles *et al.*[24], which consisted of 5-day courses of cytosine arabinoside and daunorubicin alternating with 5 days of cytosine arabinoside and 6-thioguanine. Between every 5 days of treatment there was a 23-day gap, and it was during this period that patients received immunotherapy. The patients in Study 4B were all aged over 60 years, and their maintenance chemotherapy consisted of 3-day courses every 2 weeks. All patients stopped maintenance chemotherapy after 1 year (12 courses) and thereafter the chemoimmunotherapy patients received only immunotherapy and the chemotherapy patients received no further treatment.

Immunotherapy

Immunotherapy was started whenever possible just before complete remission, at a time when the marrow was hypoplastic. In all instances, subsequent marrow biopsies confirmed that these patients had achieved a full remission. The immunotherapy, described in detail previously[24], consisted of weekly BCG (Glaxo) and 10^9 irradiated allogeneic myeloblastic leukaemia

cells given i.d. and s.c., and timed to avoid the 5-day courses of chemotherapy. All four limbs received the BCG in turn, once weekly, and the cells were injected into the other three limbs. Individual patients received cells from the same donor for as long as possible.

Treatment after relapse

When patients relapsed, the initial induction treatment with daunorubicin and cytosine arabinoside was repeated whenever possible. If no regression of leukaemia was seen, the treatment was usually changed to a combination of cyclophosphamide and 6-thioguanine. If remission occurred, the maintenance treatment was modified to a single injection of daunorubicin and 3 days of cytosine arabinoside followed 11 days later by 3 days of oral cyclophosphamide and 6-thioguanine. After another 11-day gap the whole cycle was repeated, with maintenance chemotherapy for 3 days every fortnight. Those patients who previously received immunotherapy were given further treatment with BCG and a different population of irradiated AML cells.

Results of the Barts/Marsden Study (As reported by Powles *et al.*[26])

At the time of analysis (August 1975) five of 28 patients in the chemo-immunotherapy arm remained alive, although four of these had relapsed; two of 22 patients on chemotherapy were alive, both still in their first

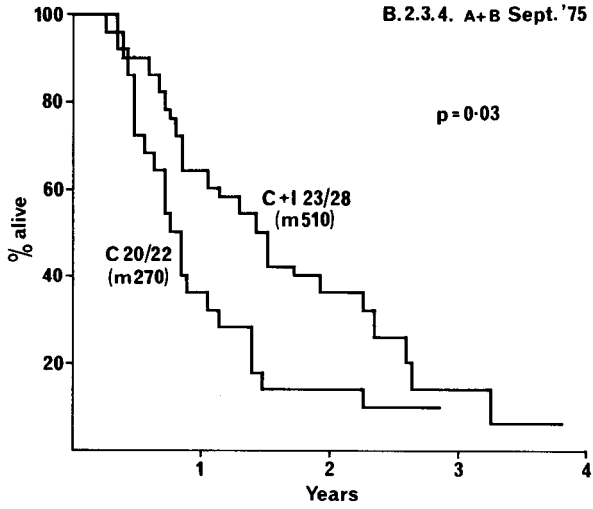

Figure 1.2 Survival following remission of two groups of patients with AML (Barts/Marsden Trial) allocated at presentation: one group receiving maintenance chemotherapy alone (C), the other group chemotherapy plus immunotherapy (C + I). The percentage surviving at different times has been calculated by standard actuarial methods, m = median survival in days. Difference between curves has $p = 0.03$