# The Place of Immunological Methods of Treatment in the Management of Acute Leukaemia

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The development of the syngeneic animal system permitted tumour transplantation experiments to be conducted without interference from genetically determinaled antigens, and provided systems in which acquired 'neo-antigens' could be studied. Under carefully manipulated conditions several different immunological manouevres were shown to be capable of slowing the growth of tumours in experimental animals (see review, Alexander, 1974), and the two most promising methods that emerged which were applicable to man were non-specific stimulation of the immune system using agents such as B.C.G. (Halpern, et al 1959) and specific stimulation with tumour cells (Haddow and Alexander, 1964).

In all the animal experiments successful anti-tumour effects were only seen if the tumour load was very small. Leukaemia in man is therefore a particularly good model in which to test immunotherapy because patients in remission have undetectable numbers of cells remaining but if left untreated they ineviatably relapse. A prerequisite for active specific immunotherapy for leukaemia in man was the demonstration (see Powles review 1974a) using mixed cell cultures of surface components on human leukaemia cells which behaved like tumour specific transplantation antigens (T.S.T.A.'s). This provided a rationale for using killed leukaemia cells in addition to B.C.G. for immunotherapy in man. Thus, in the last ten years specific (killed tumour cells) and non-specific (B.C.G.) immunotherapy have been used in a number of controlled clinical trials in man.

The first comparative study was initiated by Mathé (Mathé, 1969), who selected a group of patients with A.L.L. who had been in remission for at least two years. For some all treatment stopped and the rest were given weekly Pasteur B.C.G., killed allogeneic A.L.L. cells, or both B.C.G. and cells. All 10 of the untreated patients relapsed within 130 days, whereas half of the 20 immunotherapy patients remained in remission for greater than 295 days, some of them for many years. The numbers were too small to decide which of the immunological regimes was best.

Several attempts have been made to confirm the value of B.C.G. alone in A.L.L. during remission. In Britain, the Medical Research Council arranged a trial (M.R.C. 1971) which compared the use of twice weekly Methotrexate with B.C.G. or no treatment. They found no benefit from the use of B.C.G. but it must be remembered that a different form of B.C.G. (i. e. Glaxo) was used. A similar study

in the U.S.A. by Leukaemia Study Group A, (Heyn et al 1973) also failed to show benefit from B.C.G. More recently the Houston Group (Gutterman et al 1974) have used Pasteur B.C.G. for the maintenance of remission of all forms of adult leukaemia, and although they report benefit in A.M.L. (see below) there was no evidence that Pasteur B.C.G. prolonged remission in A.L.L. In the related disease Burkitt's Lymphoma, Ziegler (Ziegler and Magrath, 1974) used Pasteur B.C.G. given for a limited period by scarification and also found no therapeutic effect in maintaining remission in these children.

**Table I: Bart's 2, 3, + 4 trial of maintenance chemotherapy versus maintenance chemotherapy plus immunotherapy.**

| Days | Proportion in remission | | Proportion surviving* | | Proportion Surviving after relapse | |
|---|---|---|---|---|---|---|
| | C** | C+I*** | C | C+I | C | C+I |
| 0 | 100 | 100 | 100 | 100 | 100 | 100 |
| 100 | 78 | 82 | 96 | 100 | 40 | 74 |
| 200 | 50 | 68 | 70 | 90 | 0 | 44 |
| 300 | 32 | 54 | 50 | 72 | 0 | 24 |
| 400 | 18 | 40 | 32 | 60 | 0 | 10 |
| 600 | 14 | 26 | 14 | 42 | – | – |
| 800 | 10 | 8 | 14 | 36 | – | – |
| 1000 | 10 | 4 | 10 | 14 | – | – |
| No. pts. | 22 | 28 | 22 | 28 | 20 | 27 |
| No. Remaining | 2 | 1 | 2 | 5 | 0 | 4 |
| P. value**** difference C to C+I | | N. S. | | 0.03 | | 0.0005 |

* From onset remission.
** C Chemo maintenance only.
*** C+I chemo plus immunotherapy maintenance.
**** Log rank non-parametric method (Peto and Peto, 1972).

At present, the place of immunotherapy for A.L.L. remains speculative since only Mathé has reported a therapeutic effect and no other study has done exactly as he did in giving Pasteur B.C.G. and cells. Whether it is at present necessary to use immunotherapy as a primary method of treatment for A.L.L. in the face of the outstanding results produced by intensive combination chemotherapy and prophylatic treatment of the central nervous system as developed by Pinkel and his colleagues (Simone, 1974) remains to be tested. Initially A L.L. was selected as the best disease to test immunotherapy because so few patients with Acute Myelognous Leukaemia A.M.L. obtained remission with chemotherapy and so it was impossible to conduct a trial. This situation, however, has changed.

A joint Barts Hospital/Marsden Hospital study (B. 2, 3, 4.) was started in

1970 to establish the effect of immunotherapy in the maintenance of patients with A.M.L. (Powles, et al 1973). This was a controlled trial in which all patients in remission were given 5 days maintenance chemotherapy every month for one year but a randomized group of these patients were also given weekly immunotherapy consisting of Glaxo B.C.G., administered percutaneously using a Heaf Gun, and allogeneic irradiated A.M.L. cells intradermally and subcutaneously in three other sites. Results, summarised in Table 1, show that remission length and overall survival was prolonged in those patients who received immunotherapy and there was also a very significant prolongation of survival of the immunotherapy patients after they relapsed. The mechanism of action is obscure because for technical reasons it has not been possible to measure the immune reaction of the host directed against the T.S.T.A. of A.M.L. although tests employing the mixed leucocyte reactions (Powles, et al, 1971) indicate that immunization with leukaemia cells increases the ability of A.M.L. patients to recognise their own leukaemia cells (which have been kept stored). Two possible mechanisms deserve consideration; the first is an increased immune reaction to T.S.T.A.'s produced by administration of cells which would account for the prolongation of first remission, but it is difficult to see how such a mechanism could be involved in the prolongation of survival after relapse. A second mechanism which could produce this latter effect is a non-specific stimulation of the bone marrow which permitted immunotherapy patients who had relapsed to tolerate the high doses of cytotoxic chemotherapy which were then required. Such an effect has been seen in animal systems (Wolmark et al 1974, Dimitrov et al 1975) and would be effective in man because patients who have relapsed usually die due to bone marrow failure.

An obvious question concerns the relative importance of the B.C.G. and cells and to answer this would require three arms to a trial, i. e. chemotherapy alone, chemotherapy plus B.C.G., and chemotherapy plus B.C.G. plus cells. This was not possible in the initial study because of the limited number of patients available.

Three other groups have documented the effects of giving various forms of immunotherapy to remission patients with A.M.L. and all described clinical benefit. The Manchester group (Freeman, et al, 1973) repeated exactly the sort of immunotherapy given in the Barts/Marsden study. Although their patients did well by contemporary standards they were not able to make this a controlled clinical trial due to the small number of patients available. This group did, however, comment on the ease of obtaining second remission in these patients and also remarked that survival after relapse appeared to be long.

The first report of B.C.G. alone as immunotherapy for maintaining patients with A.M.L. appeared last year from the U.S.A. (Vogler and Chan, 1974). They described the work of the South-East Co-operative Group for the study of leukaemia in which 41 patients in remission were treated with Methotrexate and 18 of these patients also received B.C.G. (Tice strain) twice weekly for 4 weeks. They noted in the initial follow-up period a significant prolongation of remission length in the immunotherapy group, but the use of Methotrexate alone to maintain remission only produced a remission length of 26 weeks which is inferior to recent chemotherapeutic programmes. It may be that this study, when it is completed, will show whether B.C.G. adds significantly to chemotherapy in prolonged remission. Another study involving the use of B.C.G. which was essentially similar

to the Vogler report has been described by the Houston Group (Gutterman, et al 1974). In this they suggested that there was a distinct benefit to be obtained from the use of immunotherapy for maintaining A.M.L. patients in remission. However, criticism of the statistical analysis and data presented in this study (Peto and Galton, 1974) must lead to some reservations at present concerning the significance of its conclusions.

At the time the Barts/Marsden (B2, 3, and 4) study was commenced it was not ethical to have untreated remission patients with A.M.L. and so the only way to establish the controlled trial for immunotherapy was by giving them all maintenance chemotherapy. When the preliminary results of this trial suggested that immunotherapy might be of value, it was justified to test the effetcs of immunotherapy alone. Due to a need to obtain further information about methods of improving the drug regime to obtain remission in A.M.L., two new slightly different forms of induction chemotherapy were used (B.F.2, B.F.3) before starting the immunotherapy and so a direct comparison with the initial Barts/Marsden study (B234) was not possible. Table 2 shows the results obtained in such a group of

**Table II: Remission duration and survival of patients receiving immunotherapy only for maintenance of remission. All patients receive weekly B.C.G., BF2 patients also receive weekly unirradiated cells, BF3* patients weekly irradiated cells.**

| Days | Proportion in remission | | Proportion surviving | |
|---|---|---|---|---|
| | BF2 | BF3 | BF2 | BF3 |
| 0 | 100 | 100 | 100 | 100 |
| 100 | 72 | 91 | 95 | 100 |
| 200 | 32 | 58 | 77 | 83 |
| 300 | 18 | 17 | 72 | 76 |
| 400 | 5 | – | 32 | 65 |
| 500 | 5 | – | 23 | 65 |
| No. pts. | 22 | 24 | 22 | 24 |
| No. remaining | 1 | 8 | 4 | 18 |

* BF3 was sequentially after BF2 so these patients have not had sufficient time to die.

patients with A.M.L. and it is important to point out that this was not a controlled trial. The immunotherapy was similar to that described in the B2, 3, and 4 study except that the cells had been both unirradiated (BF2). (Powles, 1974b) and irradiated (BF3) (Powles, 1973).

Although remission length in both groups of immunotherapy alone patients was shorter than those who had received immunotherapy plus chemotherapy, all three of these groups had similar overall survival and prolonged periods of survival after relapse. In this study there was no significant difference in results between the use of unirradiated and irradiated cells but more time must elapse before we can be definite about this because the two groups have been sequentially studied and B.F.3 has only been including patients for $1^1/_2$ years.

Throughout the world there are now several other trials under way, the outcome of which should help identify the place of immunotherapy in A.M.L. As yet it is unclear whether intensive chemotherapy (Whitecar et al, 1972, Clarkson, 1972) is better for the patient than low dose intermittent chemotherapy and/or immunotherapy.

Some variations of the procedures just described are currently being studied clinically. There is experimental evidence to suggest that after sialic acid has been removed from tumour cell surfaces using neuraminidase they become more immunogenic (Currie and Bagshawe, 1968). A controlled trial is now under way for A.M.L. in which cells so treated are used for immunotherapy (Bekesi et al 1976).

Another approach is to use B.C.G. as a 'classical' adjuvant as has been tested with melanoma (Currie and Basham, 1974) and we are at present conducting a controlled trial using B.C.G. mixed with leukaemia cells for this purpose. In this study all patients receive weekly irradiated cells and B.C.G. as in the previous studies, but some of these patients also receive A.M.L. cells and B.C.G. mixed together intradermally during the first three months of remission. Preliminary results (Table 3) suggest there is no difference between these two groups for

**Table III: Proportion of patients of two groups remaining in remission at various durations after starting weekly immunotherapy. In one group cells and B.C.G. are mixed together, in the other they are given at different sites**

| Days | Proportion in remission | |
|---|---|---|
| | Cells + B.C.G. (separate sites) | Cells + B.C.G. (mixed) |
| 0 | 100 | 100 |
| 50 | 91 | 100 |
| 100 | 85 | 100 |
| 150 | 69 | 70 |
| 200 | 30 | 58 |
| 250 | 9 | 44 |
| 300 | – | 29 |
| Total number | 11 | 13 |
| No. in remission (Sept. 75) | 5 | 3 |

remission duration, but it is too early to examine the survival data. The possibility of treating patients whilst they still have detectable disease is also being explored and a preliminary study at Bart's (Hamilton-Fairley, 1975) indicates that this form of immunotherapy may help to obtain remission in patients who do not respond completely to induction chemotherapy.

There are three entirely new approaches which might soon deserve consideration in clinical studies. Specific xenogeneic antisera are now available for acute leukaemia cells (Mohanakumar et al 1974, Greaves et al, 1975) and although this has immediate application for monitoring the disease process, the possibility of using

such materials for passive serotherapy should be considered as their specificity would overcome many of the problems previously encountered with such methods. Another recent development has been the isolation in human leukaemia cells of R.N.A. sequences (Gallo et al, 1974, Spiegelman et al, 1974), which appear to have a common identity with Simian R.N.A. virus particles. Once the relevance of these observations to pathogenesis has been established the possibility of an immunological (and chemotherapeutic) approach to their presence could be considered.

Finally, in a rat leukaemia model, Thymosin – a hormone extracted from the Thymus – has been found effective in bringing about total remission (Khaw and Rule, 1973) and when this material becomes available in larger quantities the possibility of clinical evaluation may not then be too distant.

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