PAPERS AND ORIGINALS

Active Immunotherapy Used Alone for Maintenance of Patients with Acute Myeloid Leukaemia

CHARLES B. FREEMAN, RODNEY HARRIS, COLIN G. GEARY, MICHAEL J. LEYLAND, JOHN E. MACIVER, IRVINE W. DELAMORE

British Medical Journal, 1973, 4, 571-573

Summary

A total of 32 patients suffering from acute myeloid leukaemia were initially treated with daunorubicin and cytosine arabinoside, and eight who achieved full remission were given a brief cytoreduction course of cyclophosphamide and thioguanine. Of these eight patients seven were then actively immunized with 10^6 irradiated allogeneic acute myeloid leukaemia cells and B.C.G. at weekly intervals. Six of these patients have survived in apparent good health for more than one year. Bone marrow changes suggestive of relapse were used as an indication for further short courses of chemotherapy, and except on single occasions in two different patients clinical relapse has been prevented. The average duration of first (bone marrow) remission appears to be comparable with the best achieved in trials using regular chemotherapy for maintenance, though criteria for determining relapse may be different. The rate of reinduction of remissions (bone marrow) in this series was high, with a subsequent increase in overall survival time. Possible explanations for the high rate of reinduction include, firstly, the effects of active immunization with specific leukaemia antigen; secondly, non-specific adjuvant effect; thirdly, avoidance of drug resistance; and, fourthly, early diagnosis of relapse by frequent bone marrow examinations.

Introduction

Human leukaemia cells may possess surface antigens capable of provoking an immune response in the host (Harris, 1973). Attempts have been made to increase this response both in experimental animals and more recently in man by various forms of active specific and non-specific immunization. In human acute leukaemia encouraging results are being obtained using a combination of active immunization with irradiated allogeneic cells and B.C.G. with or without *Corynebacterium parvum*. Such immunotherapy is administered only after remission has been induced and the leukaemic cell mass greatly reduced by means of chemotherapy. Using such treatment Mathé *et al.* (1972) claim that about 50% of patients with acute lymphoblastic leukaemia achieve a first remission of 12 months duration. Similarly Crowther *et al.* (1973) and Powles *et al.* (1973), treating patients with acute myeloid leukaemia, have reported that intermittent combination chemotherapy plus immunotherapy produces longer remissions than the same chemotherapy given alone.

In this preliminary report we describe a protocol in which immunotherapy is used alone during remission. Chemotherapy is reserved for initial remission induction and cytoreduction and for treating patients in whom routine bone marrow examination suggests that clinical relapse is imminent. In the small group of patients with acute myeloid leukaemia who were treated in this way normal bone marrow appearances were reinduced with remarkable ease and second, third, and fourth "remissions" were obtained with excellent overall survival and sense of well-being.

Patients and Methods

Altogether 32 unselected patients were treated between October 1971 and October 1972. These included 30 successive patients cared for at the Manchester Royal Infirmary plus 2 additional cases from Crumpsall Hospital. There were 19 with acute myeloblastic, 12 with acute myelomonocytic, and 1 with acute promyelocytic leukaemia. Their ages ranged from 13 to 72 years (mean 50 years) and there was an excess of male to female patients (19:13).

University Department of Medical Genetics, St. Mary's Hospital, Manchester M13 0JH

CHARLES B. FREEMAN, M.A., M.R.C.P., M.R.C. Research Fellow
RODNEY HARRIS, M.D., M.R.C.P., Reader in Medical Genetics

University Department of Clinical Haematology, Manchester Royal Infirmary, Manchester M13 9WL

COLIN G. GEARY, F.R.C.P., M.R.C.PATH., Consultant Haematologist
MICHAEL J. LEYLAND, M.R.C.P., Lecturer in Clinical Haematology
JOHN E. MACIVER, M.D., F.R.C.PATH., Consultant Haematologist
IRVINE W. DELAMORE, PH.D., F.R.C.P., Consultant Physician with Special Interest in Haematology

Remission induction was attempted with daunorubicin and cytosine arabinoside administered as described by Crowther *et al.* (1973) in trials 1 and 3 at St. Bartholomew's Hospital. Those patients who entered remission then received cytoreduction therapy with intravenous cyclophosphamide 200 mg/m² weekly and thioguanine 2.5 mg/kg daily by mouth. This was continued for up to six weeks but patients did not receive more than a total of three months of chemotherapy from presentation. Those patients remaining in remission received weekly immunotherapy with about 10⁹ irradiated allogeneic leukaemia cells plus B.C.G. The leukaemia cells were obtained from untreated cases of acute myeloid leukaemia using an N.C.I.-I.B.M. continuous-flow blood-cell separator (Buckner *et al.*, 1969). The cells were stored at approximately -179°C in a Union Carbide gas-phase liquid nitrogen container after programmed freezing in a G.V. Planer low-temperature refrigeration unit. Before injection leukaemia cells were thawed rapidly, washed twice in Ringer's solution, and placed on ice. They were then subjected to irradiation at 10,000 rads (3,000 rads/min) using an A.E.I. 4-MV linear accelerator. The irradiation was performed at 22°C, after which the cells were again placed on ice until injected. Leukaemia cells were injected intradermally and subcutaneously into three limbs within half an hour of thawing. B.C.G. (Glaxo) was administered by the multiple-puncture technique using a 20-needle Heaf gun fired twice into the fourth limb.

Complete remission was judged to have occurred when the marrow aspirate, usually obtained from the sternum, showed fewer than 5% blasts and promyelocytes with recovery of other normal marrow elements, a rising peripheral blood platelet and neutrophil count, a steady or rising haemoglobin level not maintained by transfusions, and absence of lymphadenopathy or splenomegaly. The stricter criteria of the leukaemia group B (Ellison *et al.*, 1968), including absence of clinical symptoms, a peripheral neutrophil count of above 1,500/mm³, and a haemoglobin of at least 12 g/100 ml (11 g/100 ml in women), could be used only retrospectively, after the start of immunotherapy, as cytoreduction chemotherapy was initiated before full recovery of haematological values. All patients receiving immunotherapy eventually fulfilled these criteria.

A marrow aspirate was obtained routinely at monthly intervals during immunotherapy, and more often if the patient developed clinical symptoms or changes in the peripheral blood counts suggestive of relapse. In most cases relapse was diagnosed on the basis of an increased level of abnormal blasts above 5% on bone marrow examination. When the blast count had risen only slightly, say 5-10%, without any changes in the peripheral blood counts or clinical deterioration, the bone marrow examination was repeated two weeks later. If there was any further increase in the number of abnormal blast cells chemotherapy was reinstituted. Reinduction chemotherapy was identical with that used for initial remission induction, but no cytoreduction therapy was given. Patients achieving a further remission again received immunotherapy with leukaemia cells from a different donor plus B.C.G.

Results

Of the 32 patients 8 (25%) achieved complete remission and 7 subsequently received immunotherapy (see fig.). The remission rates are shown in the table, which illustrates the high initial

mortality in patients with acute myeloid leukaemia. In half of the patients death occurred before an adequate number of courses of induction chemotherapy could be given. No patients with peripheral blood white counts over 50,000/mm³ entered remission, and it was noted that patients with rapidly rising peripheral blood blast counts at the time of diagnosis had a poor prognosis. Similarly a platelet count under 20,000/mm³, particularly when associated with bleeding, was a grave prognostic sign. A more detailed account of the results of remission induction will be reported elsewhere, and the present communication is concerned chiefly with the apparent ease of repeated reinduction after relapse.

Survival of 32 patients with acute myeloid leukaemia. Black areas indicate acute phase and relapse. White areas indicate remission. Hatched area indicates doubtful remission. + = Died.

The duration of first remission of the patients on immunotherapy was 14 to 56 weeks. Two of the patients were still in their first remission at 38 and 56 weeks respectively. The remaining five patients all relapsed at periods ranging from 14 to 28 weeks (mean 20 weeks). If the patients still in their first remission are included the mean duration of remission was 28 weeks.

Four of the five patients who relapsed achieved a second remission using daunorubicin and cytosine arabinoside as for the initial remission induction. At the time of writing one patient was in her fourth remission, and surprisingly each remission became easier rather than more difficult to achieve, requiring less chemotherapy on each occasion.

To date only one of the patients on immunotherapy has died, 43½ weeks after admission. The remaining six were alive from between 43 and 91 weeks, with a mean of 59 weeks.

While on immunotherapy and during remission all the patients remained completely well. The injections were tolerated well with the exception of one patient who had a local delayed type of hypersensitivity reaction to the injection of one particular batch of cells given on two occasions only. B.C.G. administration was rarely troublesome, though occasionally the scabs detached with some bleeding.

Complete Remission Rates

	All Ages	Under 50 Years	Over 50 Years
Patients not excluded	8/32 (25%)	3/14 (21%)	5/18 (28%)
Excluding patients dying during first month	8/21 (38%)	3/10 (30%)	5/11 (45%)
Excluding patients dying before receiving three full induction courses*	8/16 (50%)	3/4 (75%)	5/12 (42%)

*An average of three induction courses were required to achieve complete remission in the present series.

Discussion

The present series is too small to allow more than cautious optimism about the results of immunotherapy, and in contrast to the St. Bartholomew's group (Crowther *et al.*, 1973) the duration of first bone marrow remission did not seem to be obviously greater as compared with patients receiving maintenance chemotherapy alone. Nevertheless, immunotherapy has the great advantage that it has few side effects.

The high rate of reinduction achieved is very encouraging. The best reported figures for reinduction rates are around 30-40% (Wiernik, *et al.*, 1970; Crowther *et al.*, 1973), though in our hands the reinduction rate was previously not more than 12% (Bailey *et al.*, 1971). Moreover, third and fourth remissions are extremely rare. It is not clear whether the high reinduction rate in our patients treated with immunotherapy was a positive result of immunotherapy or attributable to the absence of maintenance chemotherapy and the avoidance of drug resistance. The latter explanation seems perhaps less likely in view of the fact that only one out of five patients who went into remission in the fourth and fifth M.R.C. trials on acute myeloid leukaemia and who for various reasons received no further treatment achieved a remission after subsequent relapse (Galton, 1973). Haddow and Alexander (1964) reported that the effectiveness of a single dose of 2,000 r of x-rays in retarding the growth of primary rat fibrosarcoma can be enhanced by an autograft of the tumour. One patient with acute lymphoblastic leukaemia treated with immunotherapy at the Royal Manchester Children's Hospital, Pendlebury (Morris Jones, 1973), was put back on to the same maintenance chemotherapy as he had received before immunotherapy. The same dose of drugs produced marked marrow hypoplasia, suggesting an increased sensitivity of the normal marrow elements to chemotherapy.

The ease of reinduction might also be attributed to the relatively early diagnosis of relapse in our series. In most patients relapse was based on the results of bone marrow examination which we performed monthly, and only occasionally was there any change in the peripheral blood counts. Only the one patient who eventually died and a further patient after his second relapse showed clinical signs of relapse.

Centres where bone marrow examination is performed less often and which depend more on clinical and peripheral blood

evidence of relapse may find greater difficulty in reinducing a remission. Though the apparent duration of first remission may be greater overall survival would then be shorter.

We are deeply indebted to our many colleagues in the department's of haematology and medical genetics for their clinical and technical help. Our thanks are due particularly to Miss G. Marriott and Miss M. Enright for their nursing and technical help, to Dr. D. Dawson at Crumpsall Hospital for allowing us to include in the trial two patients under his care, to Dr. J. Garrett, Dr. P. Massey, and Miss Souter and her staff for their help in the preparation and irradiation of the leukaemia cells, and to Miss Scholes at Manchester Royal Infirmary for her technical help. We are grateful to the Leukaemia Research Fund, the Medical Research Council, the United Manchester Hospitals Board of Governors, the research grants committee, and G. D. Searle and Co., for financial help and to the department of medical illustration, Manchester Royal Infirmary, for their help with the illustration.

ADDENDUM.—The latest data on patients receiving immunotherapy are as follows. Case 1 relapsed at 95 weeks and has since achieved a fifth remission (current overall survival 107 weeks). Case 14 died without achieving a further remission after a total of 72 weeks. Cases 19 and 29 are still in their first remission (duration 73 and 55 weeks respectively; current overall survival 78 and 60 weeks respectively). Case 21 remains in his second remission (duration 42 weeks; current overall survival 76 weeks). Case 26 relapsed for a second time at 64 weeks but has achieved a third remission (current overall survival 71 weeks).

References

- Bailey, C. C., *et al.* (1971). *Lancet*, 1, 1268.
- Buckner, D., Graw, R. G., Eisel, R. J., Henderson, E. S., and Perry, S. (1969). *Blood*, 33, 353.
- Crowther, D., *et al.* (1973). *British Medical Journal*, 1, 131.
- Ellison, R. R., *et al.* (1968). *Blood*, 32, 507.
- Galton, D. (1973). To be published.
- Haddow, A., and Alexander, P. (1964). *Lancet*, 1, 452.
- Harris, R. (1973). *Nature*, 241, 95.
- Mathé, G. *et al.* (1972). *National Cancer Institute Monographs*, 35, 361.
- Morris Jones, P. (1973). Personal communication.
- Powles, R., Kay, H. E. M., *et al.* (1973). *Procédes de Semaine Cancerologique*, 1972 de Paris. In press.
- Wiernik, P. H., and Serpick, A. A. (1970). *Medicine*, 49, 505.

Smoking in Pregnancy and Subsequent Child Development

N. R. BUTLER, H. GOLDSTEIN

British Medical Journal, 1973, 4, 573-575

Summary

A national sample of several thousand children has been followed longitudinally from birth. At the ages of 7 and 11 years physical and mental retardation due to smoking in pregnancy has been found, and this deficit increases with the number of cigarettes smoked after the fourth month of pregnancy. Children of mothers who smoked 10 or more cigarettes a day are on average 1.0 cm shorter and between three and five months retarded on reading, mathematics, and general ability compared with the

offspring of non-smokers, after allowing for associated social and biological factors.

Introduction

It is now widely accepted that maternal smoking in pregnancy is associated with both a reduction in birth weight and an increase in perinatal mortality (Butler *et al.*, 1972; *British Medical Journal*, 1973). Though there is still some dispute about the mechanism of the association the evidence from human and animal studies suggests that it is the smoking rather than the type of woman who smokes which is responsible (*British Medical Journal*, 1973).

The long-term effects of smoking in pregnancy on the surviving child are less well documented. Hardy and Mellits (1972) found very few significant differences in a number of body measurements and intellectual functions up to the age of 7 years between children of smokers and non-smokers. On the other hand, by using data from the National Child Development

Bristol Royal Hospital for Sick Children, Bristol BS2 8BJ
N. R. BUTLER, M.D., F.R.C.P., Professor of Child Health

National Children's Bureau, London EC1V 7QE
H. GOLDSTEIN, B.Sc., Statistician