Active Immunotherapy in Treatment of Acute Leukaemia

R. J. GUYER,* M.B., M.R.C.PATH. ; D. CROWTHER,† M.B., PH.D., M.R.C.P.

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Summary: Active non-specific immunotherapy has been used to prolong remissions in acute lymphoblastic leukaemia. The series reported here used Bordetella pertussis vaccine in a controlled trial after intensive chemotherapy. Possibly immunotherapy delayed the onset of relapse in the treated patients, but no long-term remissions were obtained. Further work is needed to establish the role of immunotherapy in general, and the use of B. pertussis vaccine in particular, in the treatment of acute leukaemia.

Introduction

Non-specific immunotherapy may be effective in the treatment of acute lymphoblastic leukaemia in man (Mathé *et al.*, 1969). A number of substances, such as B.C.G., which have adjuvant properties can be used as non-specific immune stimulants, but some have certain undesirable side-effects. The least traumatic is *Bordetella pertussis* vaccine (Per/vac, Burroughs Wellcome), which has as its most obvious effect the production of a lymphocytosis in animals (Morse, 1965 ; Clausen *et al.*, 1968). Finger *et al.* (1967) found increased immunological activity after *B. pertussis* vaccination, and animal experiments suggested the efficacy of the vaccination in causing increased rejection of a transplanted tumour (Wissler *et al.*, 1968).

For these reasons it was decided to measure the effect of vaccination with *B. pertussis* in the prolongation of otherwise unmaintained remissions in acute lymphoblastic leukaemia.

Patients and Methods

Sixteen patients were divided into two groups matched for age and sex. The groups were also comparable in other respects ; the clinical findings were similar, with signs of systemic disease (adenopathy, splenomegaly, etc.) in three of the controls and two of the test patients, and high peripheral blast counts (over 10,000/cu. mm.) in two cases in each group.

All the cases were relatively newly diagnosed and the lengths of treatment before the remission were comparable (mean: control 6·5 months ; test 5·7 months).

All the patients had been brought into remission by chemotherapy consisting of prednisolone with either vincristine or mercaptopurine. Intensive "cytoreductive" chemotherapy was given to all patients immediately before the unmaintained period. The chemotherapy consisted of five courses lasting five days each of mercaptopurine and methotrexate in those patients who had never had mercaptopurine before. In those who had had previous non-intensive mercaptopurine (two in each group) the courses of mercaptopurine and methotrexate were alternated with courses of cyclophosphamide and methotrexate. In all cases the intensive treatment was completed in a period of three months, and all patients were in remission at the end of this therapy. The length of remission was measured from the last day of intensive chemotherapy.

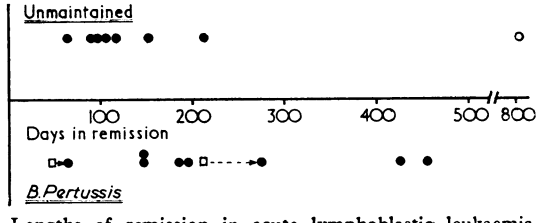
* Senior Registrar in Clinical Pathology, Royal Marsden Hospital, Sutton, Surrey.
† Member of Scientific Staff, Chester Beattie Research Institute, Sutton, Surrey. Present appointment: Registrar, Medical Unit, and Chief Assistant to Sir Ronald Bodley Scott, St. Bartholomew's Hospital, London E.C.1.

Half the patients were then treated with injections of *B. pertussis* vaccine, 0·5 ml. being given either weekly or twice weekly by intramuscular injection in a rotating system to the four quadrants of the body. The control group received no further therapy.

Follow-up on the two groups was the same. Total blood counts with white cell concentrates were examined every four weeks and bone marrow aspirations performed every eight weeks. Relapse was diagnosed either by haematological evidence from those tests or by unmistakable clinical evidence manifesting between tests.

Results

Of the 16 patients all except one control case have relapsed (see Chart). Seven control cases relapsed between 65 and 215 days, and the test cases relapsed between 65 and 455 days. Half of the controls relapsed within 112 days, whereas in the treated group half relapsed within 186 days.



Lengths of remission in acute lymphoblastic leukaemia after intensive chemotherapy, either treated by immunotherapy (*B. pertussis*) or unmaintained. ●=Cases relapsed. ○=Cases still in remission. □- - -→●=Onset of extramedullary lesion with delay of haematological relapse.

All those cases that relapsed were promptly brought back into remission by conventional means. Of the cases on immunotherapy one had meningeal leukaemia for six weeks before haematological relapse, and one had a testicular tumour irradiated nine months before haematological relapse. In both these cases vaccination was continued during the period in which the extramedullary lesion was treated, and in both of them this lesion was apparently eradicated before there was evidence of haematological relapse.

During the extensive exposure to *B. pertussis* vaccine no child suffered any complications attributable to the vaccine.

Discussion

In the series of cases of acute leukaemia reported by Mathé *et al.* (1969) active non-specific immunotherapy was given after chemotherapy to prolong remission. Mathé also showed that immunotherapy was effective only against a small tumour mass, and chemotherapy must therefore reduce the mass of leukaemic cells substantially before immunotherapy can act. In the animal this form of immunotherapy is most effective if the antigen is injected some time before the challenge of malignant cells, a situation not reproducible in human malignancy Mathé (1968) in Paris and I. Parr (personal communication) in London have, however, shown that a chemically induced murine leukaemia grafted on to an autologous animal can be

cured even when the antigen is given after the graft. Various bacterial antigens have been tried in the immunotherapeutic treatment of animal malignancies, both solid tumours and leukaemia, among them B.C.G. (Biozzi *et al.*, 1959 ; Old *et al.*, 1959 ; Amiel, 1967), *Corynebacterium parvum* (Woodruff and Boak, 1966), *B. pertussis* (Wissler *et al.*, 1968), and *Mycobacterium chelonei* (Mathé *et al.*, 1969). Only B.C.G. has been used in a trial in human leukaemia previous to this series, though *C. parvum* has been used in isolated cases. Since the series reported here has been completed it has been claimed that under certain circumstances *B. pertussis* may accelerate tumour growth (Floersheim, 1967).

It is apparent that the timing of both antigenic stimulation and challenge with tumour is of the utmost importance. Recent work by Parr (personal communication) suggests that immunization with *B. pertussis* vaccine combined with autologous irradiated tumour cells in the mouse L5178Y lymphoma system given 24 hours after the challenge dose may have a beneficial effect.

In whatever way immune assistance is evoked in treatment the initial chemotherapy is critical. Unless adequate cell destruction has been achieved no form of immunotherapy is likely to be effective. In our series the number of patients with remissions in excess of 250 days suggests that cytoreductive chemotherapy was adequate and that the residuum of cells at the end of chemotherapy was small and possibly zero in the one case with an unmaintained remission in excess of 800 days. The role of immunotherapy is more difficult to place, but it may have delayed the onset of relapse in this series. The observation of this partial response without the occurrence of a number of long-term remissions—for example, two to three years—suggests that this form of immunotherapy is not as effective as that described by Mathé *et al.* (1969) using B.C.G. Further experimental and clinical evidence is needed to establish the role of immunotherapy in general in the management of leukaemia, and in particular to examine whether *B. pertussis* vaccine has a part to play.

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REFERENCES

Amiel, J. L. (1967). *Revue Française d'Études Cliniques et Biologiques*, **12**, 912.
Biozzi, G., Stiffel, C., Halpern, B. N., and Mouton, D. (1959). *Comptes Rendus des Séances de la Société de Biologie et de ses Filiales*, **153**, 987.
Clausen, C., Munoz, J., and Bergman, R. K. (1968). *Journal of Bacteriology*, **96**, 1484.
Finger, H., Emmerling, P., and Schmidt, H. (1967). *Experientia*, **23**, 591.
Floersheim, G. L. (1967). *Nature*, **216**, 1235.
Mathé, G. (1968). *Revue Française d'Études Cliniques et Biologiques*, **13**, 881.
Mathé, G., *et al.* (1969). *Lancet*, **1**, 697.
Morse, S. I. (1965). *Journal of Experimental Medicine*, **121**, 49.
Old, L. J., Clarke, D. A., and Benacerraf, B. (1959). *Nature*, **184**, 291.
Wissler, R. W., Craft, K., Kesden, D., Polisky, B., and Dzoza, K. (1968). In *Advance in Transplantation, Proceedings of the First International Congress of the Transplantation Society*, edited by J. Dausset *et al.*, p. 308. Copenhagen, Munksgaard.
Woodruff, M. F. A., and Boak, J. L. (1966). *British Journal of Cancer*, **20**, 345.

Preliminary Communications

Nasal Mucosal Absorption of Tetracosactrin as Indicated by Rise in Plasma Fluorogenic Corticosteroids

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Summary : Tetracosactrin has been administered to six healthy dexamethasone-suppressed subjects by nasal insufflation. The pronounced increase of the plasma fluorogenic corticosteroids suggests that the peptide is well absorbed by this route.

INTRODUCTION

Tetracosactrin (Synacthen) is a synthetic peptide with the primary structure of the corticotrophic fraction of human adrenocorticotrophic hormone. It contains the first 24 amino-acids of all known corticotrophins. Previous reports show that some peptides are absorbed across mucosal surfaces—such as the octapeptides, vasopressin (Mimica *et al.*, 1968) and oxytocin (Ritchie and Brundell, 1967), and also pentagastrin (Wormsley, 1968). It was therefore decided to study the mucosal absorption of tetracosactrin.

METHODS

Six healthy subjects, all of whom were medical or paramedical staff and fully understood the nature of the experiments, were given 4 mg. of dexamethasone by mouth at 22.00 hours to suppress the hypothalamo-pituitary-adrenal axis, thus eliminating diurnal and individual variation in the resting levels of the plasma fluorogenic corticosteroids. Tests were started between 09.30 and 10.30 hours on each occasion on the day after the administration of the dexamethasone. Baseline specimens of heparinized venous blood were collected from indwelling needles. These were kept patent by injecting small amounts of heparin (1,000 units/ml.).

At a time designated zero, tetracosactrin powder was administered by nasal insufflation. Specimens of heparinized venous blood were collected at timed intervals thereafter. The specimens were centrifuged and the supernatant plasma was stored at about −20° C. until analysis.

The tetracosactrin powder was administered by simple insufflation into the nose by means of a Marvic inhaler. Each subject was given, on separate occasions, doses of 0·25 and 1·5 mg. The period of each insufflation was about five minutes. The low dose was administered in one insufflation and the high dose by three insufflations of 0·5 mg. each.

The plasma levels of fluorogenic corticosteroids were measured by the fluorimetric method of Spencer-Peet *et al.* (1965). Replicate analyses were performed.

RESULTS

The plasma fluorogenic corticosteroids showed an unequivocal rise within 60 minutes after the insufflation of tetra-