

Autologous x-irradiated tumour cells and percutaneous BCG in operable lung cancer

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ABSTRACT To determine the value of specific immunotherapy with adjuvant BCG in operable lung cancer, the immunological and clinical results of serial postoperative injections of autologous irradiated tumour cells and BCG were compared with those of a single preoperative injection of BCG in two randomly selected groups of patients undergoing resection of their tumours. There was a significant rise in tuberculin skin reactivity from seven weeks to 11 months after operation in the treated group. Actuarial curves for survival and freedom from tumour recurrence and median survival times showed an advantage for the treated patients who had stage I tumours, but these differences were significant only at the levels $p = 0.07-0.09$. Survival and duration of freedom from tumour recurrence was greater in autograft-treated patients whose skin responded to a weak test dose of dinitrochlorobenzene (DNCB) after sensitisation with 2% DNCB than in control DNCB-positive patients ($p = 0.02$). There were no significant differences in the actual proportion of patients from each group surviving at two years. The results show that this form of specific immunotherapy with adjuvant may have a beneficial effect in patients with stage I tumours and those who become sensitised to 2% DNCB after the first exposure.

Many investigations of immunotherapy in lung cancer have shown that it has at the most a weak therapeutic action. It is thus likely to be of value only where the tumour cell population has been reduced to a minimum, as after surgical resection.¹ With the exception of the unconfirmed results of intrapleural BCG,² non-specific immunological stimulants have produced little effect in surgical cases.^{3,4} A possible reason for this ineffectiveness may be failure to focus the available cytotoxic potential of activated lymphocytes and macrophages against residual tumour cells. Possibly this lack of specific activity could be overcome by injecting lung cancer cells into patients whose immunological systems have previously been stimulated by mycobacteria. We here describe the immunological and clinical results of injecting irradiated autologous tumour cells into skin areas previously treated with BCG in patients who have undergone resection of lung cancer.

Methods

The preparation of the suspension of lung tumour cells and the laboratory tests of immunological function were described in a previous paper.⁵ The scheme of the project is set out in figure 1.

IMMUNOTHERAPY

Informed consent was obtained from patients undergoing thoracotomy for suspected lung cancer at two Glasgow hospitals over three years from August 1976. One week before operation they were given a single preoperative treatment with percutaneous BCG Glaxo ($50-250 \times 10^6$ organisms per ml) to the deltoid region of the arm.⁴ The BCG was injected at five sites with a 20-needle Heaf gun adjusted to penetrate to a depth of 1 mm. Once the tumour was found at operation to be resectable, the patient was randomly allocated to the autograft or the control group. A cell suspension was prepared from the tumour of each patient in the autograft group. This was irradiated and divided into aliquots of approximately 3 ml. Provided that the diagnosis was confirmed by examination of the frozen section 3 ml of the cell suspension were injected intrader-

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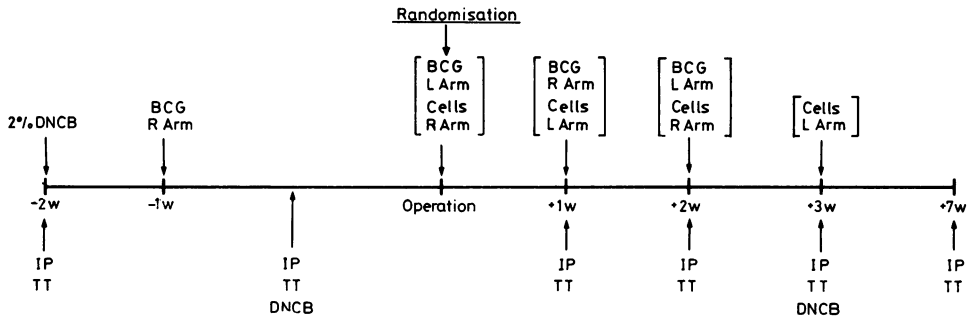


Fig 1 Scheme of trial. All patients received BCG preoperatively: those allotted to the control group at the time of operation received no further immunotherapy. Treatment enclosed by brackets refers to that given to the autograft group only. Regular measurements of the immunological profile (IP) and tuberculin reactivity (TT) were made before and after operation.

mally and subcutaneously into four sites in the deltoid region that had been treated with BCG one week before operation. The remainder was stored at -70°C . Serial injections of the same amounts of cell suspension and BCG were given during the ensuing three weeks, the cells always being injected into the area of skin that had been primed with BCG the previous week.

IMMUNOLOGICAL TESTS

Delayed hypersensitivity skin tests Thirty-five patients in each group were sensitised by application of 2% dinitrochlorobenzene (DNCB) to the skin at least two weeks before operation. Ten to 14 days later and before operation test doses of concentrations ranging from 62.5 to 1000 $\mu\text{g}/\text{ml}$ were applied. This was repeated three and seven weeks after operation. The reaction was graded from 1 to 4 according to the degree and extent of erythema and oedema produced at each site. Erythema or a higher grade of reaction to 500 $\mu\text{g}/\text{ml}$ was taken as a positive reaction. Dose-response curves were constructed for each patient according to his reaction to increasing strengths of DNCB. The DNCB index was read off from the curve as the strength of reaction at a concentration of 250 $\mu\text{g}/\text{ml}$. A standard tuberculin test using 10 tuberculin units of purified protein derivative (PPD, Weybridge) was carried out before and at regular intervals after operation.

Laboratory tests Counts of circulating total white blood cells and total T and B lymphocytes and estimation of lymphocyte transformation by phytohaemagglutinin (PHA), pokeweed mitogen (PWM), and purified protein derivative (PPD) were carried out before operation, at weekly intervals for three weeks after operation, at seven weeks after operation, and thereafter at three-monthly intervals.

STATISTICAL METHODS

Student's *t* test was used to determine significant differences and changes in all the immunological tests except those of lymphocyte transformation by mitogens and PPD. In the latter a Wilcoxon two-sample test was used because of the lack of normal distribution of the readings.

Actuarial life tables for survival and freedom from tumour recurrence were constructed by recording the number of patients in whom death or tumour recurrence occurred during each three-month interval after operation. The significance of differences between the curves obtained was determined by the log-rank test.⁶

STAGING AND HISTOLOGICAL CLASSIFICATION

The histology of the tumours was assessed on the basis of the WHO classification and the stage of the tumour was determined by an independent pathologist who had no knowledge of the clinical details.⁷

Results

IMMUNOLOGICAL

There was no difference in the mean values of peripheral blood cell counts and tuberculin skin reactions before operation between the autograft and control groups. In the same way median values of lymphocyte reactivity to PHA, PWM, and PPD and the prevalence of a positive reaction to the DNCB test were similar.

In both groups there was a fall in mean tuberculin reaction during the first three weeks after operation but this was significant only in the control group (fig 2). This was followed by a significant increase in the tuberculin reaction in the autograft group from seven weeks to 11 months. Thereafter the mean

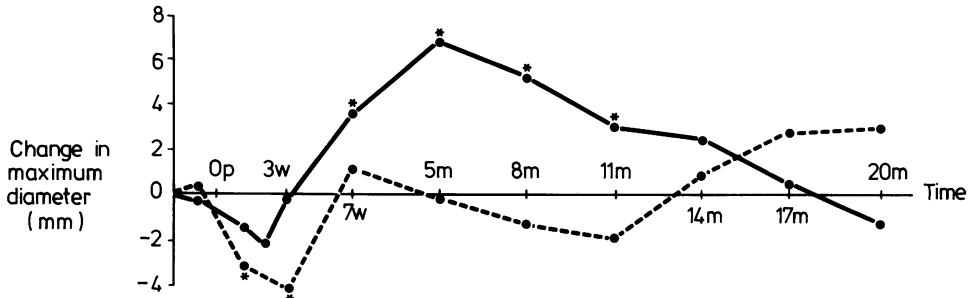


Fig 2 Mean change in maximum diameter of tuberculin reaction at 48 hours in main trial autograft (solid line) and control (interrupted line) patients during 20 months after operation (Op). Significant change ($p < 0.05$) indicated by asterisk.

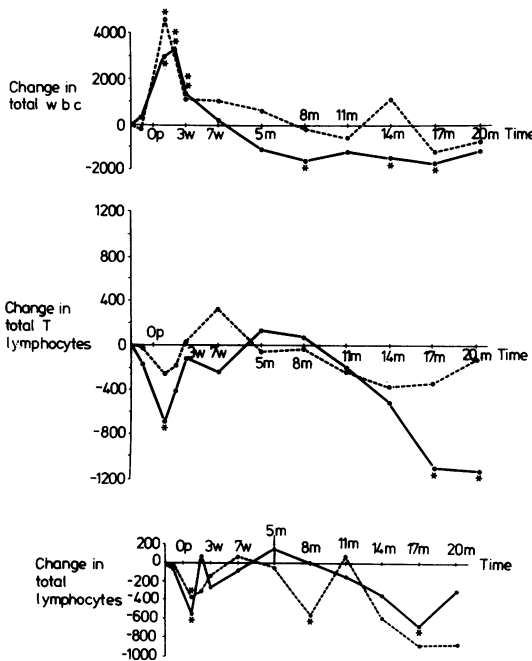


Fig 3 Mean change in total white blood cells (wbc), T lymphocytes, and total lymphocytes per mm^3 in autograft (solid line) and control (interrupted line) patients during 20 months after operation (Op). Significant change ($p < 0.05$) indicated by asterisk.

tuberculin reactivities of both were similar to each other and to the preoperative measurements.

At three weeks the mean increase in DNCB index of both groups was 0.5; at 7 weeks, it was 1.0 in the autograft group and 1.7 in the control group. The difference between the two groups was not significant.

There was a significant rise in total white blood cells and a fall in T lymphocytes during the first three weeks after operation in the autograft group

Table 1 Characteristics of patients with operable bronchial carcinoma in main trial

| | | Patients with autograft (n = 40) | Control group (n = 43) |
|---|--|----------------------------------|------------------------|
| Age (y): | Mean | 56.5 | 57.8 |
| | Range | 45-68 | 47-69 |
| Sex: | Male | 30 | 34 |
| | Female | 10 | 9 |
| Operation: | Lobectomy | 21 | 31 |
| | Pneumonectomy | 19 | 12 |
| Histology: | Epidermoid | 27 | 33 |
| | Large cell | 7 | 1 |
| | Adenocarcinoma | 4 | 7 |
| | Combined epidermoid and adenocarcinoma and other | 2 | 2 |
| Stage at operation: | 1 | 24 | 30 |
| | 2 | 1 | 1 |
| | 3 | 15 | 12 |
| Mean preoperative tuberculin reaction (mm) | | 15.8 | 17.9 |
| Mean preoperative dinitrochlorobenzene reactivity (units) | | 1.4 | 1.2 |

(fig 3). Similar changes were seen in the control group except that the fall in circulating T cell levels after operation was not significant.

Although the overall pattern of change in median lymphocyte reactivity to PHA, PWM, and PPD was that of depression during the first five months after operation followed by recovery, the variation in results at any one time was so large that these changes did not reach acceptable levels of statistical significance. There was no difference in median values between the two groups at any one time.

CLINICAL

From table 1 it can be seen that there was no significant difference between the clinical, surgical, or pathological features of the groups except for a higher proportion of pneumonectomies and large-cell carcinomas in the autograft group. There was no significant difference in the actuarial curves for survival and freedom from tumour recurrence when all

patients in both groups were compared. Patients with stage I tumours in the autograft group, however, fared better than controls in both respects, the significance of the difference between the curves being $p = 0.09$ (fig 4). This difference appeared at six months, was maximal at 18 months, and decreased thereafter. Similar differences were seen when median times for survival and freedom from tumour recurrence were calculated (table 2). Although a higher proportion of autograft patients were alive and free from tumour recurrence two years after operation, the difference was not significant (table 3).

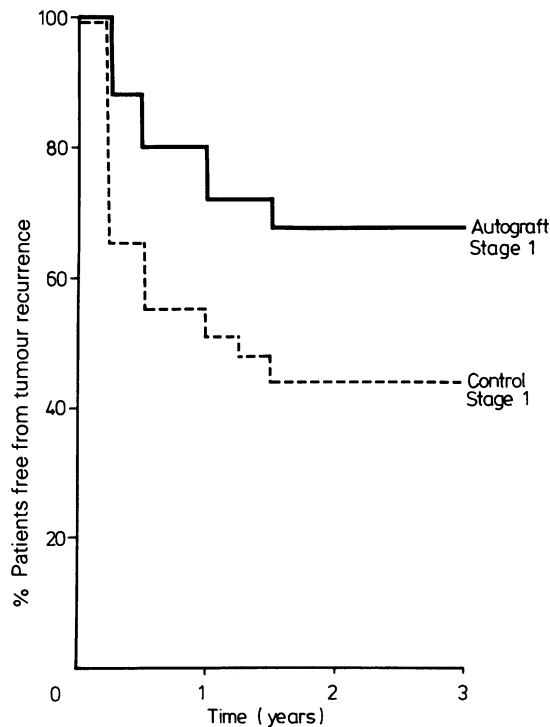


Fig 4 Actuarially calculated graph showing that the proportion of the 24 autograft patients with stage I tumours who could be expected to be free of tumour recurrence at a given time during the postoperative period was higher than that of the 30 control patients with stage I tumours ($p = 0.09$).

Table 3 Percentage of the patients in the autograft and control groups who were alive and free from clinical and radiographic evidence of tumour recurrence two years after operation (differences not significant)

| Group | | No (%) |
|-----------|------------------|------------|
| Autograft | All patients | 18/40 (45) |
| | Stage I patients | 17/24 (71) |
| Control | All patients | 16/43 (37) |
| | Stage I patients | 13/30 (43) |

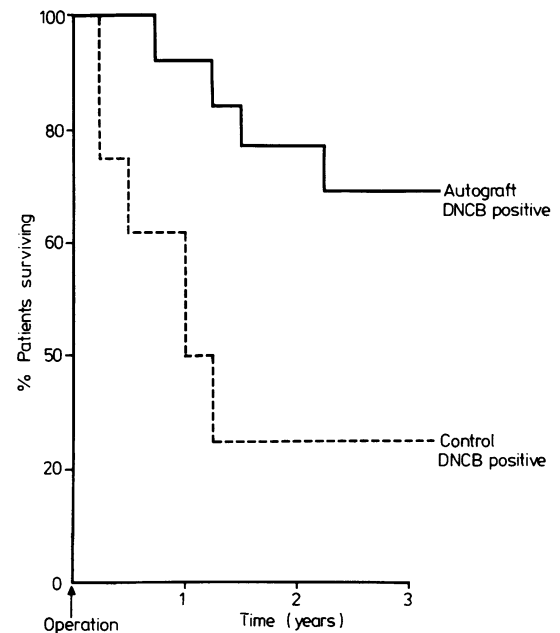


Fig 5 Actuarially calculated survival graph showing that a higher proportion of the 13 autograft patients who were DNCB positive before operation could be expected to survive at a given time during the postoperative period than of the 8 control DNCB-positive patients ($p = 0.02$).

Table 4 Pattern of tumour recurrence showing similar distribution in the two groups

| Group | Local | Distant |
|-----------|-------|---------|
| Autograft | 7 | 8 |
| Control | 10 | 10 |

Table 2 Median survival times and median times free from tumour recurrence in autograft and control groups

| | Patients | Autograft group | Controls | Significance |
|--|-----------------|-----------------|----------|--------------|
| Median survival time (months) | All | 25 | 15 | NS |
| | Stage I tumours | >36 | 17 | $p = 0.07$ |
| Median time free from tumour recurrence (months) | All | 14 | 6 | NS |
| | Stage I tumours | >36 | 14 | $p = 0.08$ |



Fig 6 Deltoid region of an autograft patient 3 weeks after treatment.

The clinical results were assessed in relation to the preoperative immunological tests. A significant finding was that autograft patients who had a positive skin reaction to DNCB before the operation survived and remained free from tumour recurrence longer than control DNCB-positive patients ($p = 0.02$) (fig 5) and longer also than control DNCB-negative patients ($p = 0.04$). There was no evidence that specific immunotherapy with BCG adjuvant decreased the ratio of distant metastases to local recurrences (table 4).

Percutaneous BCG produced five raised erythematous discs in the deltoid region. These lesions became maximal at three weeks (fig 6). They became itchy but were never painful or ulcerated. They faded, leaving a barely visible scar, after six months. There was no case of a tumour developing at the site of injection and no macroscopic evidence of local infection.

Discussion

Despite better methods of general anaesthesia and postoperative care, the results of surgical resection of lung cancer have improved little over the past 20 years.⁸ This is because small numbers of tumour cells are left behind or disseminated at operation or have already dispersed to other organs at the time of operation. These small populations can be eliminated by the host defence mechanism, as shown by occasional prolonged survival of patients in whom tumour resection has been incomplete⁹ or in whom tumour cells have been found on the margin of resected bronchi.¹⁰

That this host defence depends on specific adaptive mechanisms in which host lymphocytes play a part is suggested by the better prognosis of patients

with positive reactions to delayed hypersensitivity skin tests and laboratory evidence of active cell-mediated immunity. Tumour-associated antigens and antibodies and immune complexes have been found in association with lung cancer.¹¹ Stimulation of this defence mechanism has the theoretical advantage over adjuvant radiotherapy and chemotherapy that the activated lymphocytes and macrophages are able to locate and destroy tumour cell masses long before these become clinically obvious or detectable by scanning techniques.

The aim of adjuvant immunotherapy is to enhance the efforts of the host defence mechanism to eliminate residual tumour cells. BCG or *Corynebacterium parvum* are used to produce a general stimulation of lymphocytes and macrophages in the hope that their increased immunological activity will damage any "foreign" tumour cells that happen to be present. The long-term results of non-specific immunotherapy in lung cancer have, however, been disappointing. We have therefore sought to focus this cellular immunological reaction on to the residual tumour by injecting irradiated autologous tumour cells.

Use of autologous cells has the advantage that they are not destroyed by ordinary transplantation rejection mechanisms and so may have more time and opportunity to evoke an immunological attack on tumour cells left at operation. Other authors have reported beneficial results from injecting allogeneic lung cancer cells¹² and extracts^{13,14} with adjuvant. These authors, however, failed to show that their treatment had produced any immunological effect. In our study a significant increase in tuberculin skin reaction was produced from seven weeks to 11 months after operation. Moreover, the normal postoperative depression of T cell count was partly abolished in the autograft group. The lack of more specific tests of cytotoxicity against tumour cells has, however, been a weakness of this and most other trials of immunotherapy.

In our study actuarial curves and median times for survival and freedom from tumour recurrence did show that autograft patients with stage I tumours fared better. The significance of the differences in the clinical results between the two groups was, however, relatively low ($p = 0.07-0.09$). This finding was similar to that of two other groups,^{12,14} but the significance was less than in a third series.¹³ The results suggest that, while the overall therapeutic effect of specific immunotherapy is relatively weak, there may be a subgroup within the stage I group of patients who are capable of benefiting from this treatment.

In view of the promising initial results and lack of side effects, further trials of specific immunotherapy

should be considered. In these the treatment should be given over a long period. As the volume of tumour cells is necessarily limited, allogeneic cells or extracts would have to be used. More specific in vitro tests of the ability of macrophages and lymphocytes to kill tumour cells would also be highly desirable to show that benefit derived from the treatment was due to its effects on cell-mediated immunity.

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