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# Adjuvant immunotherapy with BCG in squamous-cell bronchial carcinoma. Immune-reactivity in relation to immunostimulation (preliminary results in a controlled trial)

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**Jansen, H M, The, T H, de Gast, G C, Esselink, M T, van der Wal, A M, and Orie, N G M (1978).** *Thorax*, **33**, 429–438. **Adjuvant immunotherapy with BCG in squamous-cell bronchial carcinoma. Immune-reactivity in relation to immunostimulation (preliminary results in a controlled trial).** Twenty-nine patients with, at operation, evidence of locally advanced primary squamous-cell bronchial carcinoma (stage II, UICC, Geneva, 1974) had lung resection to remove all the visible tumour. Postoperatively a randomly chosen group of 16 patients received adjuvant BCG immunostimulation by scarifications, while the control group received no adjuvant treatment. Follow-up studies were done from three to 23 months. Immune-reactivity in vivo with PPD and DNCB skin tests, and in vitro with E-rosetting tests and lymphocyte transformation tests with PHA, Con A, diphtheria toxoid, and PPD was monitored in 10 treated and in seven untreated patients. Recurrence rates decreased appreciably in the BCG-stimulated group after a six to 23 months' follow-up ($P<0.005$). A pronounced increase in both in-vivo and in-vitro immune-reactivity went in parallel with a more favourable clinical outcome in the BCG-treated group. In these cases there was a significant increase in skin reactivity to PPD three months after surgery ($P<0.025$) and a statistically significant rise in lymphocyte reactivity to Con A ($P<0.05$), diphtheria toxoid ($P<0.01$), and PPD ($P<0.05$) but not to PHA 12 months after surgery. DNCB skin reactivity increased as well in the BCG-treated group, but the number of individuals was too small for statistical evaluation. Increase in immune responsiveness did not occur in the control group and appeared to be independent of the initial immune state of the patients. No differences were found in the numbers of E-rosetting lymphocytes in relation to immunotherapy. It is concluded that adjuvant BCG immunotherapy used in patients with minimal residual bronchial carcinoma improves the prognosis and a favourable clinical outcome is mirrored by an increase in cellular immune reactivity.

Several studies dealing with human as well as animal tumours have shown the existence of an immune response in the hosts directed against the malignant cells (Hellström and Hellström, 1974; Herberman, 1974; Price-Evans, 1976). Despite this, tumour growth takes place due to several escape mechanisms neutralising an effective cell-mediated tumour killing. Masking of receptors on the lymphocyte membrane (Han, 1975), activation of suppressor-T-cell activity (Herberman *et al*, 1976), or serum blocking factors (Sjögren *et al*, 1972) have been suggested as mechanisms in counteracting tumour cell killing.

In experimental tumour models several attempts have been made to re-establish the host immune response by using the non-specific immunostimulant Bacillus Calmette-Guérin (BCG) (Bast *et al*, 1974; 1976). Its precise mode of action on the immune system is not yet understood. Apart from non-specific activation of macrophages,

which may selectively eradicate neoplastic cells (Hibbs *et al*, 1972; Alexander, 1973; Cleveland *et al*, 1974), it probably potentiates the helper-T-cell system in a more specific way (Hawrylko, 1975; Scott *et al*, 1976). BCG can only be expected to be effective if the tumour load is low and if the contact between BCG and the tumour cells is as close as possible (Mathe, 1971; Bast *et al*, 1976). Systemic administration, however, can also inhibit tumour growth, especially when the tumour burden is diminished after surgery (Morton *et al*, 1976).

In several clinical trials of BCG in the treatment of bronchial carcinoma and other neoplasms relatively little attention has been paid to randomisation of the patients, selection of the different histological types, and the extent of the tumour burden (Bast *et al*, 1974; Hersh *et al*, 1974). We aimed to evaluate in a randomised controlled trial the benefit of systemic, adjuvant BCG immunostimulation in patients with locally advanced, primary squamous-cell bronchial carcinoma. The patients were at high risk of recurrence after surgery because of the tumour cells that are likely to have been left behind after operation, although all the visible tumour was removed. In addition, in-vivo and in-vitro immunological tests have been applied to decide whether they are useful for measuring a possible immunostimulating effect of BCG in the individual patient.

## Patients and methods

The study comprised 29 patients with, at operation, evidence of locally advanced, primary squamous-cell bronchial carcinoma. That means, according to the criteria of the UICC-TNM classifications (UICC, Geneva, 1974), tumour sizes ranging from T2.N1.M0 to T3.N0.M0, and T3.N1.M0, established by histology immediately after the operation. Lung resections were performed in all the patients by the same surgical unit (head: Professor Dr J Homan van der Heide). All the visible tumour was removed.

The patients were at high risk of recurrence because of residual tumour and had a very bad prognosis (Homan van der Heide *et al*, 1974). Postoperatively a group of 16 patients, chosen by drawing lots, received adjuvant BCG immunostimulation, while 13 control patients received no adjuvant treatment. None of the patients received radiation therapy or chemotherapy. Corticosteroids were not used for longer than 10 days. Every three months all patients had a complete clinical examination—chest radiograph, blood cell counts, blood chemistry studies, sputum analysis, and urine analysis.

In addition, if there was any suggestion of recurrence or metastasis, skeletal survey, bone scan, liver scan and brain scan, or repeat bronchoscopy and sputum cytology were performed. If positive, BCG treatment was discontinued, and most of the patients received palliative radiotherapy, chemotherapy, or corticosteroids. The observation time of the patients ranged from three to 23 months after surgery.

### BCG-IMMUNOSTIMULATION

BCG vaccine taken from a dispersed culture in the logarithmic phase and then freeze-dried at −70°C (Dr J L Sirks, Rijksinstituut voor de Volksgebondheid, RIV, Bilthoven, the Netherlands) containing $160\times10^6$ viable micro-organisms per dose was administered to scarification sites of 5×5 cm on the volar side of the arms and the legs. BCG administration was started two to three weeks after surgery, repeated at weekly intervals for six weeks, and then subsequently twice every three months at a week's interval for an indefinite period.

### IMMUNOLOGICAL STUDIES

Immunological studies were performed two to three weeks before surgical treatment, three months after surgery (which was about two weeks after the sixth BCG administration), and subsequently in patients without recurrence or metastasis at 12 months after the operation. The studies were also performed on a group consisting of 20 individuals with low-grade chronic obstructive lung disease (COLD) matched on age, sex, and smoking habit and without history or clinical evidence of malignant disease. None of these subjects received corticosteroids. Immunological data obtained in only 17 of the 29 patients studied can be reported for technical reasons.

### PPD SKIN TESTS

Studies performed included delayed-type hypersensitivity (DTH) tests with purified protein derivative (PPD) injected intradermally: skin test results were recorded at 48 hours as millimeters of induration. A response with a diameter >5 mm was taken as positive.

### DNCB (2,4-DINITROCHLOROBENZENE) SKIN TESTS

Sensitisation and challenges were performed as described by Bleumink *et al* (1974): 2000 μg of DNCB, recrystallised twice, was applied within a 2 cm polythene ring to the volar side of the arm. Two weeks later challenges were performed by

patch testing the patients on their backs with 30, 10, and 3 $\mu$g of DNCB in acetone. The reactions were graded 0+ to 4+. To avoid the influence of booster effects, rechallenging was performed only once at three months after surgery with the lowest dose of 3 $\mu$g.

E-ROSETTING TEST

Rosetting technique was performed as described by Jondal *et al* (1972), that is, monocytes were removed from heparinised venous blood by carbonyl iron treatment. Lymphocytes were purified on Ficoll-Isopaque gradients (Boyüm, 1968). The final concentration of the lymphocyte suspension in RPMI medium was adapted to $2\times10^6$ cells/ml. Fresh sheep red blood cells (SRBC) stored in Alsever Buffer 1 : 1 were washed three times in RPMI medium. 0·1 ml of the resuspended SRBC-pellet were added to 4·9 ml RPMI medium. Using glass tubes (50×7·5 mm) 0·05 ml of the SRBC suspension and 0·1 ml of the lymphocyte suspension were added to 0·05 ml fetal calf serum (FCS), which had been absorbed against an equal volume of the same SRBC for 30 min at 4°C and heat-inactivated at 56°C for 30 min. The cell suspensions were gently mixed and incubated at 37°C for 10 min, centrifuged at 200 g for 5 min, placed in an icebucket for 60 min, resuspended by gently turning in the fingers, and stained by adding one drop 0·1% Brilliant cresyl blue. Counting was performed on a haemocytometer chamber. Two-hundred cells from each sample were counted, and the percentage of SRBC-rosettes was determined. Cells were recorded as rosettes only when at least three SRBC were bound. Total E-rosettes forming lymphocytes per mm$^3$ peripheral blood were scored.

LYMPHOCYTE STIMULATION TEST

Lymphocyte cultures were performed according to a micro-culture technique in round bottom Cooke(R) microtitre plates as described by Du Bois *et al* (1974). Phytohaemagglutinin (PHA-Burroughs-Welcome, 5 $\mu$l/ml) and Concanavalin A (Con A-Calbiochem 1 $\mu$g/ml) induced lymphocyte transformation was recorded in $30\times10^3$ lymphocytes/well. Antigen stimulation with diphtheria toxoid (RIV, the Netherlands, 10 Lf/ml) and with PPD (RIV, the Netherlands, 3 $\mu$g/ml) was recorded on $100\times10^3$ lymphocytes/well.

Lymphocyte transformation was recorded in the presence of 25% normal human control serum. The sera were inactivated for 30 min at 56°C and stored at −20°C. Lymphocyte cultures were incubated at 37°C in humidified 5% $CO_2$ in air atmosphere for 5 days. On the fourth day, 25 $\mu$l [6−$^3$H] thymidine (Radiochemical Centre, Amersham, England, 0·5 $\mu$Ci: specific activity 400 mCi/mM) was added and incubated for 16 hours. Harvesting was done on the fifth day with a multiple cell culture harvester (Skatron, Norway) using glassfibre filters. The filters were dried at 60°C for 60 min and transferred to counting vials. After the addition of 5 ml of scintillation fluid, counting was performed in a liquid scintillation counter (Packard Tricash-2450). The following formula was used to determine the disintegrations per minute (dpm) per mm$^3$pf peripheral blood.

$$\frac{\text{dpm/well in stimulated cultures} - \text{dpm/well in unstimulated cultures} \times \text{number of ly/well}}{\text{number of ly/mm}^3 \text{ peripheral blood.}}$$

DIFFERENTIAL LEUCOCYTE COUNTS

Quantitative differential leucocyte counts were performed by using the spin-smear method to avoid unequal distribution of the different cell types in the spreads on the glass slides. So we considered the counted leucocytes as a random sample of those in the smear and made the extent of the inaccuracy that is inevitably inherent in the method used for differential counts as small as possible (Rümke *et al*, 1975). Two hundred cells were counted twice in different randomised chosen fields. The mean value of the counted percentages of lymphocytes was used.

STATISTICAL ANALYSIS

Differences between recurrence curves of BCG-treated patients and controls were evaluated by a generalised Wilcoxon test for comparing arbitrarily single-censored samples (Gehan, 1965).

The Fisher exact two-by-two contingency test and the McNemar test were used to evaluate the differences in skin test results. The Student *t* test on paired observations was used to compare the serial results on the same patients in the lymphocyte stimulation tests. To test the hypothesis that there was no correlation between the outcome and initial results in lymphocyte stimulation tests, the Spearman rank correlation test was performed. P values less than 0·05 were considered significant.

## Results

The clinical and histological data are summarised in table 1. No difference can be seen between the 16 BCG-treated patients and the 13 in the control group. The mean age in the BCG group was 56 years (range: 25–70; SD 12·33) and in the control group 63 (range: 43–78; SD 9·79). This difference

Table 1 *Lung cancer patients—age and histological type. TNM classification and operation in the BCG-treated patient group and in the non-immunotherapy patient group*

| *BCG* | | | | | *Control* | | | | |
|---|---|---|---|---|---|---|---|---|---|
| *Patient* | *Age* | *Histological type* | *Stage* | *Operation* | *Patient* | *Age* | *Histological type* | *Stage* | *Operation* |
| 1 | 25 | Squamous | T2N1M0 | bilob ect | 1 | 68 | Squamous | T3N1M0 | pn ect |
| 2 | 57 | Squamous | T2N1M0 | pn ect | 2 | 73 | Squamous | T2N1M0 | lob ect |
| 3 | 45 | Squamous | T2N1M0 | pn ect | 3 | 67 | Squamous | T3N0M0 | lob ect |
| 4 | 40 | Squamous | T2N1M0 | pn ect | 4 | 52 | Squamous | T3N0M0 | pn ect |
| 5 | 53 | Squamous | T3N1M0 | pn ect | 5 | 69 | Squamous | T2N1M0 | bilob ect |
| 6 | 53 | Squamous | T3N1M0 | pn ect | 6 | 43 | Squamous | T2N1M0 | pn ect |
| 7 | 64 | Squamous | T2N1M0 | pn ect | 7 | 71 | Squamous | T2N1M0 | pn ect |
| 8 | 55 | Squamous | T3N0M0 | pn ect | 8 | 64 | Squamous | T3N0M0 | pn ect |
| 9 | 64 | Squamous | T3N0M0 | pn ect | 9 | 57 | Squamous | T3N0M0 | pn ect |
| 10 | 70 | Squamous | T3N0M0 | lob ect | 10 | 53 | Squamous | T3N1M0 | pn ect |
| 11 | 69 | Squamous | T2N1M0 | pn ect | 11 | 78 | Squamous | T3N0M0 | bilob ect |
| 12 | 67 | Squamous | T3N1M0 | pn ect | 12 | 61 | Squamous | T3N0M0 | pn ect |
| 13 | 65 | Squamous | T3N1M0 | pn ect | 13 | 58 | Squamous | T2N1M0 | pn ect |
| 14 | 69 | Squamous | T3N0M0 | pn ect | | | | | |
| 15 | 60 | Squamous | T2N1M0 | pn ect | | | | | |
| 16 | 49 | Squamous | T2N1M0 | pn ect | | | | | |

was not significant. Only one patient was under 40. The results reported here cover the follow-up period of three to 23 months after surgery. No patient has been lost to follow-up due to other causes than the primary disease.

The effect of BCG treatment on the duration of the recurrence-free period can be seen in fig 1. Only recurrence-free periods were considered because after the appearance of recurrence or metastases the patients get palliative radiotherapy, chemotherapy, or corticosteroids, and the BCG treatment was discontinued.

From the patients treated with BCG, 31% remain clinically tumour-free after more than 12 months' follow-up. In contrast, none of the patients from the control group remained recurrence-free within that period. After nine and six months' follow-up these data were respectively 56% and 0% and 81% and 23%. Figure 2 shows the "cumulative" percentage of tumour recurrence free patients treated with BCG and of the controls during the follow-up study using the "life table method." After three to 23 months' follow-up the difference between the groups was highly significant ($P<0.005$).

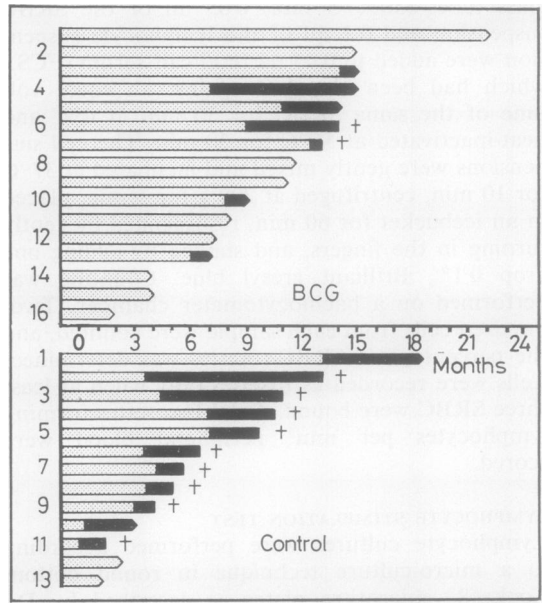


Fig 1 *Effect of BCG treatment on duration of period, free of recurrence or metastasis, after surgical resection of lung cancer patients with locally advanced disease.* ▒▒▒ = *Free of recurrence or metastasis;* ▬ = *Recurrence or metastasis;* + = *death.*

SKIN TESTS

The results of the skin tests are shown in table 2. In patients with bronchial carcinoma a decreased reactivity in PPD and DNCB skin tests was found compared with the COLD control group. In the BCG-treated patients, retested three months postoperatively, significant improved PPD skin reactions ($P<0.025$) were found and also an increase in DNCB reactivity, but the number of tested individuals were in that case too small for statistical evaluation. The control patients remained anergic after surgery. In addition, patients who were recurrence free for longer than 12 months showed the strongest rises in PPD-skin reactivity. In DNCB tests such a relation with the recurrence-free period was not found.

E-ROSETTING TEST

The numbers of E-rosette-forming cells per $mm^3$

Table 2 *Skin test responses to PPD and DNCB in BCG-treated patients and in non-immunotherapy control patients before and three months after operation and compared with COLD control group. Values are numbers of positive reacting subjects (percentage in brackets) versus numbers in each group tested*

| | *COLD* | *BCG* | | *Control* | |
|---|---|---|---|---|---|
| | | *Before operation* | *Three months after operation* | *Before operation* | *Three months after operation* |
| PPD≥5 mm | 11/20 (55) | 3/10 (30) | 8/10 (80) | 2/9 (22) | 2/9 (22) |
| DNCB 3 μg≥1+ | 11/15 (73) | 2/8 (25) | 6/8 (75) | 1/7 (14) | 2/7 (28) |

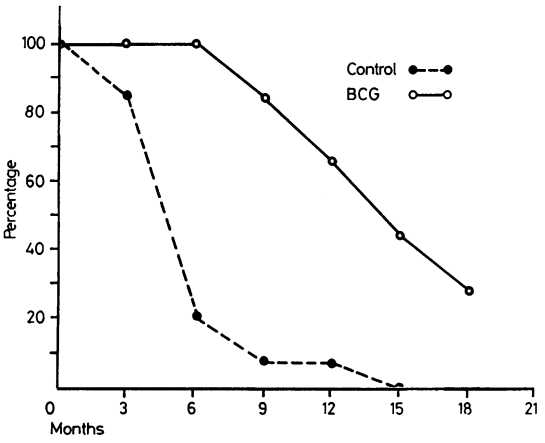


Fig 2 *Cumulative percentages of lung cancer patients free of recurrence or metastasis on different moments after surgery in BCG-treated group, compared with non-immunotherapy control group. Difference between these groups was highly significant at six to 18 months after surgery* (P<0·005).

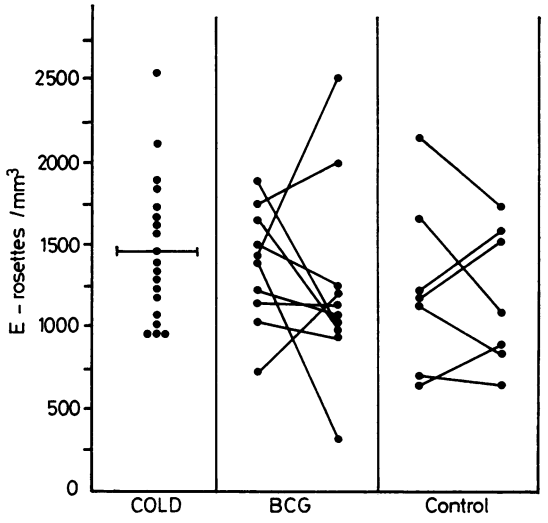


Fig 3 *E-rosette numbers/mm³ of peripheral blood in BCG-treated patients and in non-immunotherapy control patients before and three months after operation compared with COLD control group. Cross bar indicates mean score. There is no difference between numbers in lung cancer patients and in COLD group, nor between numbers before or after operation and BCG treatment.*

blood did not show any significant changes in 10 BCG-treated patients nor in the seven patients of the control group who were tested before and three months after the operation and compared with the COLD group (fig 3).

LYMPHOCYTE TRANSFORMATION TEST

The results of mitogen- and antigen-induced lymphocyte transformation tests are presented in figs 4–7. Results from 10 BCG-treated patients and seven controls were compared with those found in the COLD control group. The mean values and standard deviations of the test results are summarised in table 3. The Wilcoxon's rank sum test, which is most applicable in comparing values obtained in groups with high individual variations, showed no statistical difference between the lymphocyte reactivity found in BCG-treated patients and in controls, tested before treatment (P values >0·05). After three months of treatment, only a slight tendency to improvement in cellular immune reactivity in BCG-treated patients was seen.

BCG-treated patients however who were recurrence free for longer than 12 months after surgery showed an appreciable increase in lymphocyte reactivity in all the tests after one year, to values within the normal range of the COLD group. The differences in results in this group before and 12 months after the onset of immunotherapy were statistically significant with Con A (P<0·05), diphtheria toxoid (P<0·01), and PPD (P<0·05) but not PHA, where the initial values were relatively high. Immune responsiveness did not improve in the control group. The Spearman

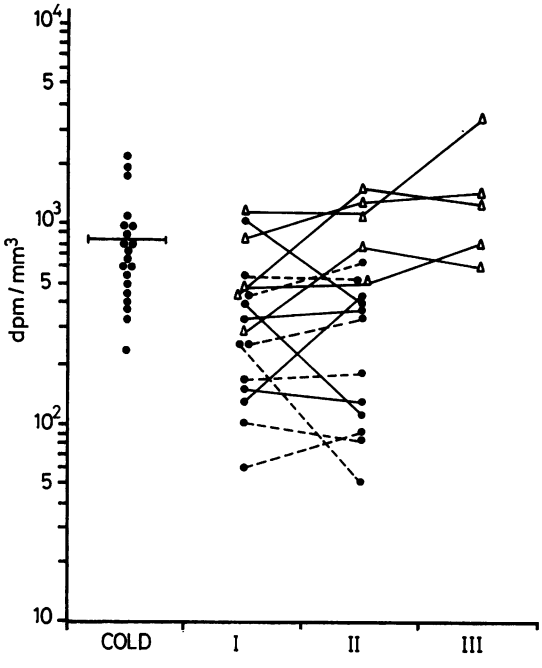


Fig 4 *In-vitro lymphocyte response to 1μL/ml PHA in lung cancer patients, before (I), three months after (II), and >12 months after surgery (III) compared with the COLD control group. Cross bar indicates mean score.* △——△ *=Sequential values in BCG-treated patients with favourable outcome. Difference in serial results I and III was not significant.* ●——● *=Sequential values in BCG-treated patients with unfavourable outcome.* ●- - -● *= Sequential values in non-immunotherapy patients.*

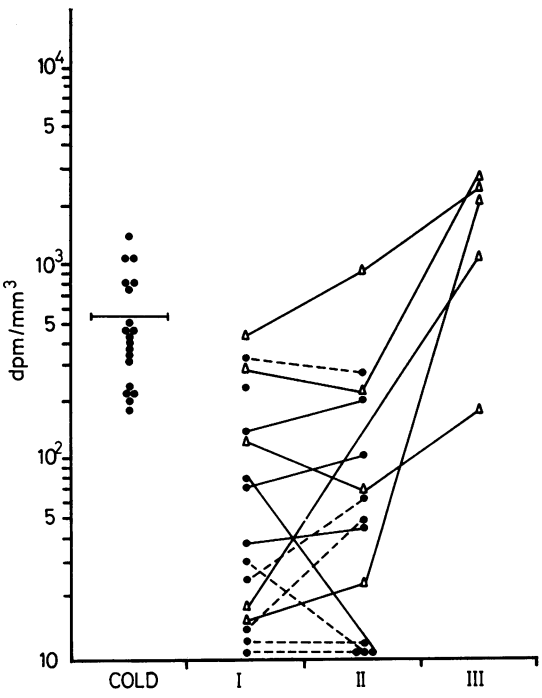


Fig 5 *In-vitro lymphocyte response to 1 μg/ml Con A in lung cancer patients, before (I), three months after (II), and >12 months after surgery (III), compared with COLD control group. Cross bar indicates mean score.* △——△ *=Sequential values in BCG-treated patients with favourable outcome. Difference in serial results between I and III was significant* (P>0·05). ●——● *=Sequential values in BCG-treated patients with unfavourable outcome.* ●- - -● *=Sequential values in non-immunotherapy patients.*

rank correlation test showed no correlation between the initial values obtained in lymphocyte stimulation tests before treatment and the subsequent results obtained after three and 12 months. So the individual alterations in test results were not related to the initial values obtained.

## Discussion

Several lines of investigation support the use of immunotherapy in lung cancer patients. One of the approaches entails adjuvant, non-specific immunostimulation to prevent tumour recurrence after surgical treatment (Hersh *et al*, 1974; Cohen, 1975).

This applies to squamous-cell carcinoma in particular since chemotherapy has had only slight effect (Stott *et al*, 1976). Our results in treating patients where resection proved to be incomplete with adjuvant radiotherapy have also been unsuccessful (Homan van der Heide *et al*, 1974).

Randomised controlled clinical trials in carefully chosen patient groups, taking account of the histological type and the extent of the tumour burden, can give us more information about the usefulness of adjuvant immunotherapy and about the immunological effects of this treatment. Our results using BCG as an immunostimulant in this study show a significant prolongation of the recurrence-free period after surgery in the treated group compared with the non-immunotherapy group.

Because we studied only patients with locally advanced disease, who therefore as a group had a very bad prognosis, most of them showed recurrence or metastasis quickly. For this reason

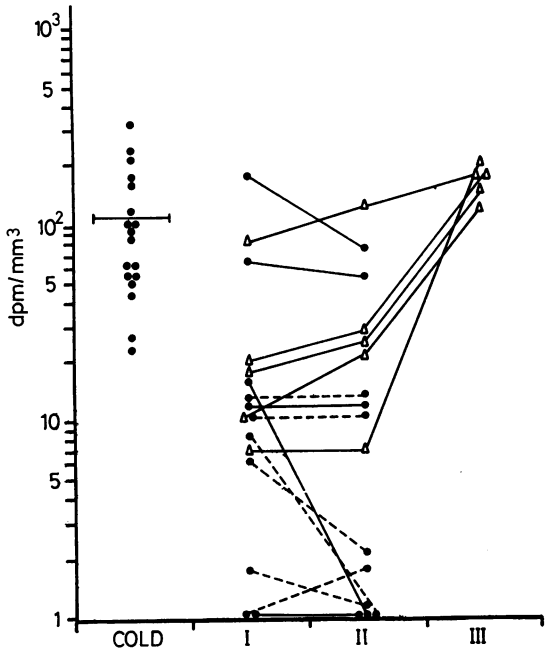


Fig 6 *In-vitro lymphocyte response to 10 Lf/ml diphtheria toxoid in lung cancer patients, before (I), three months after (II), and >12 months after surgery (III), compared with COLD control group. Cross bar indicates mean score.* △——△ *=Sequential values in BCG-treated patients with favourable outcome. Difference in serial results between I and III was significant* ($P<0.01$). ●——● *=Sequential values in BCG-treated patients with unfavourable outcome.* ●----● *=Sequential values in non-immunotherapy patients.*

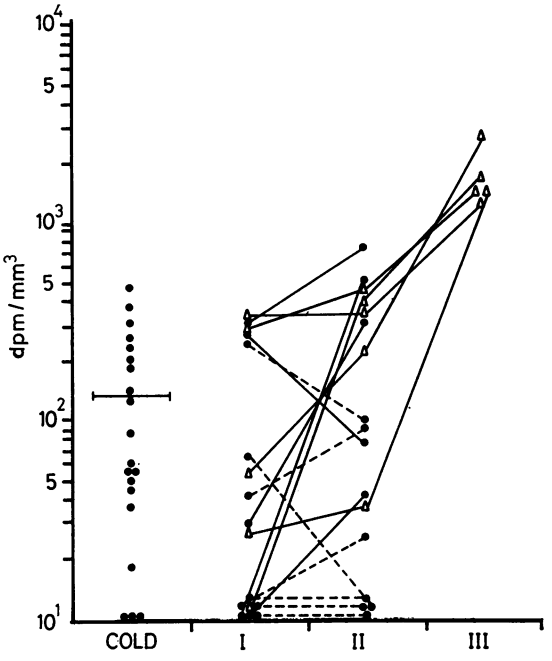


Fig 7 *In-vitro lymphocyte response to 3 μg/ml PPD in lung cancer patients, before (I), three months after (II), and >12 months after surgery (III), compared with COLD control group. Cross bar indicates mean score.* △——△ *=Sequential values in BCG-treated patients with favourable outcome. Difference in serial results between I and III was significant* ($P<0.05$). ●——● *=Sequential values in BCG-treated patients with unfavourable outcome.* ●----● *=Sequential values in non-immunotherapy patients.*

already after six months' follow-up the difference in the duration of the recurrence-free period appeared highly significantly in favour of the BCG-treated individuals. Five patients of this latter group remained recurrence-free for longer than 12 months and the longest treated patient (25 years old with a metastatic lymph node at the hilum) has now been recurrence-free for longer than 23 months.

It is difficult to compare our data with other reports. Adjuvant BCG immunotherapy has been used in bronchial carcinoma mostly combined with chemotherapy or radiotherapy (Mastrangelo *et al*, 1976; Price-Evans, 1976). Gross *et al* (1976) did find increased PHA responsiveness during adjuvant BCG-treatment, but some of the patients described received adjuvant radiotherapy as well, and the study was not controlled. McKneally *et al* (1976; 1977) did not find survival improved after treating patients once with intrapleural BCG administration in stage III of the disease and after removal of most of the tumor load. Immunological studies in these patients were not performed. Adjuvant immunotherapy with oil-attached BCG-CWS (cell wall skeleton) in lung cancer patients after curative surgery improved survival, as shown by Yamamura *et al* (1976), but detailed results of the immunological tests performed in this study are lacking. Skin tests with secondary antigens (PPD) or primary antigens after sensitisation (DNCB) reflect the patients' cell-mediated immune responsiveness. Patients with lung cancer appeared less responsive than controls (Krant *et al*, 1968; Israël, 1974). The results of this study confirm and extend these findings. The increase in skin reactivity and BCG treatment appear to be related.

Table 3 *Lymphocyte responses to mitogens and antigens in BCG-treated patients and in non-immunotherapy control patients, before and three and 12 months after operation, compared with a COLD control group. Values are means and standard deviation (SD) in dpm/mm³ of peripheral blood*

| | *COLD* | *BCG* | *Control* | *BCG* | *Control* | *BCG* |
|---|---|---|---|---|---|---|
| | n mean (SD) | Before operation n mean (SD) | Before operation n mean (SD) | Three months after operation n mean (SD) | Three months after operation n mean (SD) | > 12 months after operation n mean (SD) |
| PHA: 1 μl/ml | 20 872·8 (539·7) | 10 540·3 (358·8) | 7 262·3 (180·3) | 10 695·3 (530·1) | 7 284·1 (246·5) | 5 1576·9 (1182·2) |
| Con A: 1 μg/ml | 19 541·2 (347·8) | 10 148·0 (140·4) | 6 65·9 (121·0) | 8 209·1 (322·5) | 6 72·3 (112·8) | 5 1790·1 (1123·3) |
| Diphtheria toxoid: 10 Lf/ml | 18 113·4 ( 84·2) | 10 42·4 ( 57·0) | 6 7·2 ( 5·1) | 10 36·1 ( 40·4) | 6 5·2 ( 6·3) | 5 169·3 ( 30·1) |
| PPD: 3 μg/ml | 20 137·5 (134·3) | 10 136·2 (151·9) | 7 53·6 ( 94·1) | 10 326·3 (240·1) | 7 34·0 ( 43·3) | 5 1716·5 ( 707·4) |

n = number of subjects in each group.

It is interesting that this applies also for DNCB which is, unlike PPD, not related to BCG. These results differ from those obtained by Gutterman *et al* (1973). In their tests with recall antigens for measuring the secondary response some defects may have escaped detection. Skin tests using primary immunogens like DNCB appear to be more sensitive.

Different studies suggest that in patients with bronchial carcinoma the relative proportion of E-rosettes is decreased before treatment (Fudenberg *et al*, 1975; Gross *et al*, 1975). In our study no difference exists in the number of E-rosettes per mm³ found in carcinoma patients before surgery compared with the COLD group. No consistent patterns as an effect of BCG treatment on the number of rosette forming T-cells in the peripheral blood of the treated patients were found. This confirms the results of Anthony *et al* (1975).

The mitogen- (PHA and Con A) and the antigen- (PPD and diphtheria toxoid) induced lymphocyte transformation showed comparable results as found in the skin test. The lymphocyte reactivities, especially in patients with a favourable outcome of their disease, improved considerably after treatment with BCG. In-vitro studies, using animal or human lymphocytes, have shown different BCG strains to act as synergistic agents on lymphoproliferation induced by mitogens and LPS (lipopolysaccharide) (Bruynzeel *et al*, 1977). This synergistic activity of BCG was maximal when suboptimal concentrations of Con A or LPS were used, and the strongest activity was observed with the BCG-RIV-strain, used in our study. Until now the right dose and route of administration of immunostimulants has not been unequivocally shown. In animal tumour models manipulation of the immune system with high doses of immunostimulants may actually increase the rate of tumour growth (Bast *et al*, 1974). These possible tumour growth-enhancing effects might not be predicted by nonspecific measurements of immune-reactivity (Shibata, 1976). Data suggest that the number of viable organisms in the BCG vaccine, the ratio of viable to dead organisms, the presence of free antigen, and the frequency and route of administration are all important factors that should be taken into account for optimal immunotherapy in human cancer (Gutterman, 1976).

Intrapleural administration of BCG is an effective adjuvant treatment in patients with bronchial carcinoma after curative surgery (McKneally *et al*, 1976; 1977). In contrast to our results, however, this was not the case in their patients with locally advanced disease. The relatively long time necessary to restore the immune-reactivity of these patients, as shown in the present study, even after continuous BCG treatment might be the cause of this. Intralesional administration of BCG is very effective in melanoma (Rosenberg *et al*, 1976), but the inaccessibility of bronchial carcinoma makes this route of administration difficult, although preliminary experiences were reported by Holmes *et al* (1977). Furthermore, the side effects of BCG given into a tumour or intravenously are considerable (Sparks, 1976). In our study, using scarification techniques, few side effects were noticed.

Some days after BCG administration, areas of local skin reaction were seen with transient regional lymph node expansion. These were generally more intensive after the successive weekly scarification. Some of the treated patients developed slight and transient fever. One of the patients showed very severe local reactions and, after repeated treatment, erythema multiforme. Intradermal injection of BCG appears to give more severe complications, such as chills, fever, malaise, hepatic dysfunction, or even disseminated BCG infection (Aungst *et al*, 1975).

In comparing lymphocyte reactivity results of the sequentially studied patients with those found in a COLD group, matched on sex, age, and smoking habit, we found in the cancer patients,

before surgery and BCG treatment, considerably lower immune-reactivity. In patients with a favourable outcome after 12 months of BCG-treatment the lymphocyte reactivity in the tested systems was the same, or appreciably higher than in the COLD controls. So when we can tilt the balance in favour of the immune-suppressed patient, the immune-reactivity can be restored to values equal or higher than those found in a matched control group. The results obtained in this follow-up study show that adjuvant BCG immunotherapy improved the prognosis of bronchial carcinoma patients at least temporarily. Parallel to a favourable clinical outcome, there appeared to be an improvement in the immune-reactivity of the immune suppressed patients that was independent of the initial immune state of the patients. Because of the few patients studied, we cannot conclude that the immunological tests performed are useful as monitoring devices in immunotherpy but, given the variability of the tests used, our study shows the existence of a correlation between adjuvant BCG-treatment, favourable clinical outcome, and improved immune-reactivity in patients with squamous-cell bronchial carcinoma.

## Postscript

After patient no 30 entered the trial, adjuvant intrapleural administration of BCG as an initial treatment with subsequent scarifications performed monthly was started. Therefore no further comparable test results are available in the first group of patients, and only the length of observation in this group could be increased. After six to 29 months' follow-up one of the patients with favourable outcome of the disease after 12 months showed a recurrence 14 months after surgery. Two of the control patients who were recurrence-free after three and four months' follow-up showed recurrence of disease within the additional period.

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