Specific and Nonspecific Immunotherapy as an Adjunct to Curative Surgery for Cancer of the Lung

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Attempts to improve survival following curative surgery for non-small-cell lung cancer are reviewed. Most of these approaches have been designed to stimulate the resistance of lung cancer patients in a non-specific fashion. Living bacteria or products of dead bacteria have been given as adjunctive treatment. Various routes have been used; oral, intradermal, subdermal, or intrapleural, with either BCG or Corynebacterium parvum. No reproducible benefit has been observed. Levamisole has not been proven to be useful. Trials have yet to be completed to confirm the use of thymosin fraction V for small cell carcinoma in improving the effectiveness of chemotherapy.

A pilot trial using specific active immunotherapy is described. Prolongation of survival four years after closure of the trial in those patients immunized, compared with non-immunized patients, has prompted two further clinical trials. A small trial has confirmed the effectiveness of specific immunotherapy as adjunctive therapy for squamous cell carcinoma. A large multicenter trial in Canada and the United States should be completed and open to analysis in 1984 and may shed light on the role of tumor-associated antigens in stimulating specific resistance to lung cancer.

This paper reviews the results of nonspecific immunotherapy for lung cancer and then presents data from the cooperative study between Washington and Ottawa, using a specific approach to boost the immune resistance of patients with stage I lung cancer.

The pioneer in the use of living and later dead bacteria, whole or as extracts for the treatment of cancer, was the surgeon William Coley [1,2]. Such material was given into the tumor, subcutaneously or intravenously. This therapy, initiated before the advent of radiotherapy or chemotherapy, was occasionally followed by regression of even metastasizing tumors. Because of associated fever, nausea, diarrhea, and even collapse of the patient, Coley called his treatment "toxins," not immunotherapy. Loeb [3] conducted a survey of the efficacy of Coley's toxins, writing to surgeons other than Coley. He concluded that, in not quite 5 percent of cases treated, a positive result was obtained. In 1915 Harmer [4] made a critical analysis of 134 cases, confirmed microscopically to be malignant. He concluded that the round

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cell type of sarcoma had the greatest chance of benefit and that the small round cell sarcoma had a much greater chance of regression. In 1969 the conditions considered necessary to allow success with Coley's toxins were listed [5]. Essential would be histologic evidence of a dense infiltrate of the tumor by lymphocytes and plasma cells, representing an intense but arrested immune response by the host. We now know that in lung cancer such a response is the exception, seen in only 16 percent of cases [6]. Thus in 84 percent of cases the use of bacterial extracts would cause morbidity but little benefit to the patient. This reasoning led to a search for more specific means of boosting host immunity to lung cancer, and to cooperation between Ottawa and Washington.

THE USE OF BACTERIA OR BACTERIAL PRODUCTS
AS ADJUNCTIVE THERAPY OF CARCINOMA OF THE LUNG

*Bacille Calmette-Guerin (B.C.G.) Given by the Intradermal Route*

A five-year controlled study of B.C.G. and radiotherapy in inoperable lung cancer was reported by Pines [7]. Forty-eight patients received radiotherapy for advanced lung cancer. Thereafter 25 received B.C.G. regularly by Heaf gun; 23 patients received no B.C.G. Three years later all patients who received B.C.G. were dead; 22 of 23 controls were dead.

Another five-year study was reported by Edwards et al. [8]. Sixty patients received B.C.G. by subdermal injection, following surgery by one surgeon. Sixty consecutive surviving patients served as controls. No statistical evidence was found that B.C.G. influenced the survival rate nor was there any effect on involved lymph nodes or small metastases.

A study of 92 patients, strictly randomized following surgical resection of the tumor, was reported by Roscoe et al. [9]. Twenty-nine patients received B.C.G. by Heaf gun, 26 patients by intradermal injection, and 37 control patients received nothing. No significant difference in survival between the control group and the two immunotherapy groups was observed.

*B.C.G. Given by the Oral Route*

Miller et al. [10] evaluated a controlled clinical trial of 308 patients. Of these 155 received oral B.C.G.; 153 control patients received nothing. Over a three- to five-year follow-up no difference in survival was seen between the two groups.

*B.C.G. Given Intra Pleurally*

McKneally et al. [11] continue to report on the favourable effect of postoperative B.C.G. in stage I lung cancer patients. Their latest data shows 11 of 36 patients who received B.C.G. having recurrences, compared to 20 of 36 control patients \( (p = .003) \).

Lowe et al. [12] fail to confirm this favorable effect of intra-pleural B.C.G. in a report of a controlled randomized clinical trial of 92 patients. No evidence of benefit was seen with a period of follow-up of 14 to 36 months. This is in contrast to the striking difference between controls and the B.C.G. patients over a similar time period reported earlier by McKneally and his colleagues [13]. No significant benefit of intra-pleural B.C.G. has been reported [14] in a large multicenter trial sponsored by the National Lung Cancer Study Group. A total of 383 stage I patients were randomized to receive intra-pleural B.C.G. or saline. The 56 recurrences were nearly equally distributed between the two groups.
No significant benefit ($p = .11$) was reported by Wright et al. [15] in a study of 134 patients who received B.C.G. intra-pleurally compared to a group who received placebo.

**Intra-Pleural Corynebacterium Parvum**

Stjernsward [16] has reported on a randomized clinical trial comparing intra-pleural corynebacterium parvum (CP) versus placebo. Four hundred patients were evaluable. CP was found to be detrimental to the disease-free interval for well and moderately differentiated histologic diagnoses ($p = 0.034$). An increase in fever following CP was associated with a decrease in the disease-free interval.

It is of interest that the reports of naturally occurring empyema following surgical resection of lung cancer differ in their conclusions. The entire literature was reviewed by Brohee et al. [17]; six reports of individual cases and five reports of original controlled studies. They concluded that the literature was contradictory due to uncontrolled variables. In their own study 148 male patients on register were examined. In curative surgical resection empyema had no benefit on survival (14 cases). In reductive surgery (20 cases with empyema) survival time was shortened ($p = 0.025$). A careful study was reported by Minasian et al. [18] of 50 patients who developed empyema after resection for lung cancer. Such patients were individually paired with patients who had not suffered this complication. Pairs were matched for age, sex, extent of operation, histology of the tumor, extent of primary spread, extent of lymphatic spread, and the use of postoperative radiotherapy. The results suggested that any immunological suppression of carcinoma cells due to sepsis in the pleural space was ineffective in prolonging survival.

More optimistic has been the report of Yamamura et al. [19] on the use of B.C.G. cell wall skeleton. Some 455 patients with lung cancer treated by surgery, irradiation, and/or chemotherapy were given B.C.G. cell wall, oil attached, intratumorally, or intracutaneously. Historical controls were used and it was concluded that survival was significantly prolonged ($p = 0.0001$).

**Levamisole as Adjunctive Therapy of Carcinoma of the Lung**

Amery [20] has recently updated survival following the randomized trial of levamisole given to 96 patients, with 115 patients receiving a placebo. Benefit was seen in patients receiving a higher dose of levamisole, and was evident two years after surgery. Four years following surgery no effect was discernible.

Anthony et al. [21], using the optimal dosage of levamisole, studied 318 patients in a randomized blind trial. A significantly poorer survival for resected lung cancer patients was seen in the patients who received levamisole, with a 15 percent excess of deaths in those 99 patients.

Wright et al. [15] also report on shortened survival of patients who received intra-pleural B.C.G. plus levamisole, compared to controls. They conclude that the combination of levamisole and intra-pleural B.C.G. will be of no benefit.

**THYMOSIN FRACTION V**

In a study [22] of 46 patients with small cell lung cancer (SCLC) all were treated vigorously with remission-induction chemotherapy. The patients were randomized to receive twice weekly thymosin fraction V $60$ mg/m$^2$, $20$ mg/m$^2$, or no thymosin, during the initial six weeks of chemotherapy. Individuals receiving $60$ mg/m$^2$ had a significantly superior survival experience ($p = .017$). All but one of the 18 patients
who received 60 mg/m² are now dead, as are all those who received no thymosin or the lower dose of thymosin.

**SUMMARY**

The earlier enthusiasm for the use of bacterial products as adjuvants in treating lung cancer has waned, and one can see why. The reported effectiveness of levamisole has not been confirmed and confirmation of the effectiveness of thymosin in small cell lung cancer has yet to come, as emphasized by Cohen [23].

*The Rationale for Specific Immunotherapy of Lung Cancer*

When the clinical trial that will be described was designed in 1972 it was based upon the following observations. Healthy animals can be induced to mount a selective and destructive immunologic attack on various target organs if these animals were immunized with an homogenate of Freund's complete adjuvant plus appropriate target organ antigen. Thus encephalomyelitis, neuritis, uveitis, thyroiditis, and orchitis could be induced [24]. The correlation between the appearance of a delayed hypersensitivity reaction (DHR) to the target antigen in skin testing and the onset of clinical disease was convincing [24]. The intensity of the DHR predicted the severity of the immunologically induced damage [25]. Such damage could be transferred by sensitized cells but not by serum; see the review by Waksman [24]. The importance of the DHR as an indicator of immunologic reactivity had been clearly shown in the classic paper of Brent et al. in 1958 [26]. These authors showed that soluble antigens, obtained by hypotonic shock following sonication, derived from allogeneic skin grafts, gave a DHR on skin testing the recipient animal. The strength of this DHR reflected the intensity of the graft rejection. Where the graft was strongly antigenic the DHR to the soluble antigen was intense and rejection of the graft was rapid. When weak antigenic barriers were tested the DHR to the target antigen was weak and the tempo of rejection was sluggish.

It was clear that if one were to prepare soluble antigens from the surface of lung cancer cells they would be relevant if they induced delayed hypersensitivity reactions in patients with lung cancer and not in control patients. Furthermore, if one were to use such antigens in order to boost cellular reactions of the host to residual microscopic foci following curative surgery they would have to be free of organ antigen, to avoid the induction of pneumonitis. If allogeneic antigens were to be used, cross reactivity would have to be shown. Such antigens have been identified for squamous cell carcinoma, adenocarcinoma, large and small cell anaplastic carcinoma of the lung [27,28,29]. Since the initiation of the clinical trial in Ottawa in 1973, further evidence has accumulated that lung cancer cells are immunogenic. Tumor-associated antigens have been detected in alveolar cell carcinoma [30], adenocarcinoma [31,32,33,34], and squamous cell carcinoma [31,33,35,36,37,38]. Onco fetal antigens have also been described [31,33,34,36,37,39]. Cross reactivity of common tumor associated antigens is seen in human lung cancers [27,29] and also in animal models of carcinogen-induced lung cancer [40,41,42,43]. Low pH elution techniques have been used to isolate immunoglobulins from lung cancer tissue and pleural effusions [44]. Such antibodies reacted in significant titers with cells of squamous cell carcinoma and adenocar cinomas but not with cells of normal adult or fetal lung or of non-pulmonary tumors [45]. Various techniques in vitro have shown lymphocyte response of cancer patients to lung tumor-associated antigens: lymphoblastogenesis [46,47,48], leucocyte migration inhibition [49,50,51,52], leucocyte
adherence inhibition [53,54], and lymphocytotoxicity to autologous tumor cell suspensions [55].

We chose to use high dose methotrexate (with citrovorum rescue) before immunization in some patients because this drug induces rebound overshoot of *in vitro* lymphocyte performance [28]. For this reason we used as controls patients who were not immunized but who received the drug in an identical fashion. In such patients such a short course of the drug alone was anticipated to have no effect on patient mortality.

METHODS AND MATERIALS

The study was opened in March 1973 and closed in September 1976.

PATIENT SELECTION

Patients were drawn from those having surgical removal of their tumor at the Ottawa Civic Hospital, the National Defence Medical Centre, Ottawa, and the Ottawa General Hospital. Careful staging for TNM [56] was assured by preoperative radiography, scans, mediastinoscopy, and postoperative consideration of the notes of the surgeon and pathologist. Fifty-two patients with Stage I disease are evaluated. Of these, 16 patients acted as a concomitant control group; they were operated upon by the same surgeons. The remaining 36 patients were randomized into one of three treatment groups.

Group I—Eight patients who received methotrexate (MTX) followed by citrovorum rescue once a month, for three months.

Group II—Immunotherapy. Fifteen patients who received allogeneic Tumor Associated Antigen (matched for histology) homogenized with Freund’s complete adjuvant (FCA) once a month for three months.

Group III—Immunochemothecy. Thirteen patients who were immunized seven to nine days after administration of MTX with citrovorum rescue once a month for three months.

In Table I are summarized all the relevant characteristics of the patients with Stage I disease.

*Dose of Antigen*

The preparation and characterization of antigens used for skin testing and immunization have been reported in detail [27].

TUMOR-ASSOCIATED ANTIGENS (TAA)

The mean total quantity of TAA given to patients in the immunotherapy group was 1,495 µg, (range 1,125 µg to 2,200 µg). The mean total quantity of TAA given to the immunochemotherapy groups was 1,610 µg (range 900 to 3,000 µg). An average of 500 µg of antigen in 0.5 ml was homogenized with an equal volume of FCA (Difco Laboratories, Detroit, MI) which contains 10 mg of killed mycobacterium hominis per 10 ml, made up to 8.5 Bayol-F (mineral oil), and 1.5 ml Arlacel A (mannide mono-oleate). The homogenate was given intra-dermally into the deltoid region of the arm, the thigh, and again the arm at monthly intervals. Ulcers developed slowly at the site of injection with erythema and thinning of the skin, ulceration at seven to ten days, and showed gradual healing over months. They looked unsightly yet caused very little discomfort unless they became infected. Such superficial infection responded quickly to oral erythromycin. Occlusive dressings with sterile vaseline
TABLE 1
Data on the Three Groups of Patients: Stage I
Chemotherapy and Concomitant Controls
Immunotherapy and Immunochemotherapy

|                          | MTX and Controls | Immunotherapy | Immunochemotherapy |
|--------------------------|------------------|---------------|--------------------|
| Total patients           | 24               | 15            | 13                 |
| Male                     | 19               | 11            | 9                  |
| Median age               | 57               | 55            | 57                 |
| Age range                | 39 to 74 yr      | 46 to 71 yr   | 45 to 66 yr        |
| Performance status       | 0                | 3             | 5                  |
| At surgery               | 1                | 21            | 10                 |
| Total pneumonectomy      | 6                | 2             | 5                  |
| Right sided              | 14               | 7             | 5                  |
| Hilar node involvement   | 3                | 1             | 2                  |

**Cell Type**

- Epidermoid well differentiated: 9, 7, 5
- Epidermoid poorly differentiated: 3, 1, 4
- Adenocarcinoma: 10, 4, 4
- Anaplastic large cell: 1, 2, 0
- Anaplastic small cell: 1, 0, 1

0, asymptomatic
1, symptomatic ambulant

gauze were useful for the first two months. Healing was completed by seven to 12 months.

Methotrexate (Lederle Laboratories) was given by rapid intravenous infusion, 300 mg and then 700 mg was infused over a period of six hours. A normal creatinine clearance was mandatory and the urine was alkalinized by giving sodium bicarbonate 1.2 gm q6h per os, starting 24 hours before the infusion and continuing during the folinic acid rescue period of 60 hours (12 mg i.m. every six hours). The urine pH was monitored q6h to ensure that the pH remained above 6.

**RESULTS**

Non-Immunized Group. This group consisted of 24 patients; 16 concomitant control patients and eight who received methotrexate. Nine control patients have died of lung cancer at 8, 14, 17, 22, 30, 32, 46, 51, and 65 months. Of those who received methotrexate, five have died of lung cancer at 6, 22, 24, 26, and 64 months.

Immunized Group. This consisted of 15 patients who received allogeneic antigen and Freund's complete adjuvant, and 13 patients who received immunochemotherapy. Of the 15 who were immunized, five have died of lung cancer at 27, 40, 42, and 72 months. This last patient had inoperable recurrence of squamous carcinoma in his trachea in November 1977 and died on June 3, 1980, 31 months later. One patient had a stump recurrence at twenty months of her large cell carcinoma, received cobalt treatment, and died four years later of respiratory failure, free of disease. A third patient had recurrence of his squamous cell carcinoma at 12 months. A total pneumonectomy was performed and he died of respiratory failure and pneumonia 3½ years later, aged 73. At necropsy foci of squamous cell carcinoma were found at the stump of his pneumonectomy, surrounded by a dense desmoplastic reaction with a moderate lymphocytic infiltrate.
Of the 13 patients who received immunochemotherapy, one died of cerebral metastases at 14 months of large cell carcinoma. A second patient developed a stump recurrence of squamous cell carcinoma at three years and died three years later of disseminated cancer. Two patients were free of disease at the time of their death; one of cardiac arrest in bed, 39 months after surgery, the other of staphylococcal septicemia, 51 months after surgery, aged 71.

SURVIVAL

In Table 2 the life-table survival experience is summarized as calculated by the method of Cutler and Ederer [57]. Those patients who died a non-cancer death have been handled as being lost to follow-up. Figure 1 shows the survival curves for the control and treated groups, with the one-sided 95 percent confidence limits for the control and treated groups.

The survival experience of the treated group may be seen to diverge significantly from the control group beyond 24 months. While 96 percent of the immunized group reached the second anniversary, only 72 percent of the controls did so. The corresponding values for the fourth anniversary are 82 percent and 50 percent, respectively. The overall comparison for the treated and control groups using Gehan's generalized Wilcoxon test [58] yielded a statistical significance of $p < .001$.

Late Skin Testing with TAA: Following curative resection of lung, cancer patients were skin tested with soluble tumor-associated antigen, derived in most instances from allogeneic tumors, matched as closely as possible with the histology of the resected tumor. The usual skin test dose was 100 $\mu$g in 0.1 ml. Not all patients could be tested in the later months after surgery; some died, some refused, and for others, distance from Ottawa made such testing impractical.

Non-Immunized Group. Stage I: Twelve patients were tested. Seven patients gave a weak to moderate reaction to tumor antigen; one of these patients died of lung cancer seven months later. Five patients gave no reaction on testing with tumor antigen, yet were able to give a positive reaction to at least one recall bacterial antigen; thus they were not completely anergic. Four of these patients died of lung cancer, 2, 7, 18, and 23 months after testing. Details of these skin tests are given in Table 3.

Immunized Group: Eleven patients were tested at varying intervals following

**TABLE 2**

Life Table Survival Experience of the Non-Immunized and Immunized Groups
The 95 percent one-sided confidence limits for each group are also shown.

|                | MTX and Controls | Immunotherapy and Immunochemotherapy |
|----------------|------------------|---------------------------------------|
|                | 95% One-Sided CL | 95% One-Sided CL                       |
| $P_{0.5}$      | 1.00             | 1.00                                  |
| $P_{1.0}$      | .91              | 1.00                                  |
| $P_{1.5}$      | .86              | .97                                   |
| $P_{3.0}$      | .72              | .82                                   |
| $P_{3.5}$      | .63              | .76                                   |
| $P_{5.0}$      | .54              | .69                                   |
| $P_{5.5}$      | .54              | .69                                   |
| $P_{7.0}$      | .50              | .65                                   |
| $P_{7.5}$      | .46              | .61                                   |
| $P_{10}$       | .46              | .61                                   |
| $P_{10.5}$     | .35              | .51                                   |
surgery, for 13 skin tests. All patients gave a positive reaction and two died of lung cancer 13 and 32 months later. The mean strength of these reactions is three times greater than those in the non-immunized group. Details are given in Table 4.

**Immunochemotherapy Group:** Nine patients were tested following surgery for a total of 16 tests. One patient died of cerebral metastases four months after testing. A second patient died of staphylococcal septicemia 26 months after testing, age 71, clinically free of disease. Much stronger reactions are seen in this group, earlier and maintaining strength with time (Table 5). Patient number 4 was tested 5½ years

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**TABLE 3**

Late Testing of Stage I Patients with Tumor-Associated Antigens (TAA)
Matched for Histology of the Primary Tumor
(Non-Immunized: Control or Methotrexate-treated patients)

| Tested months post-operatively | Recall | TAA          | DHR  | Status     |
|-------------------------------|--------|--------------|------|------------|
| Controls                      |        |              |      |            |
| A, 4 mo                       | +ve    | Squamous     | 5 x 5| Alive 37 mo|
| B, 15 mo                      | +ve    | Squamous     | 10 x 8| Dead 22 mo|
| C, 31 mo                      | +ve    | Squamous     | 8 x 8| Alive 65 mo|
| D, 32 mo +ve                  |        | Squamous     | Neg. | Dead 39 mo|
| E, 42 mo +ve                  |        | Squamous     | 5 x 5| Alive 77 mo|

**MTX**

| Tested months post-operatively | Recall | TAA        | DHR  | Status     |
|-------------------------------|--------|------------|------|------------|
| F, 3 mo                       | +ve    | Squamous   | 16 x 16| Alive 56 mo|
| G, 4 mo                       | +ve    | Alveolar   | Neg. | Dead 27 mo|
| H, 6 mo                       | +ve    | Squamous   | Neg. | Dead 24 mo|
| I, 12 mo +ve                  |        | Squamous   | Neg. | Alive 52 mo|
| J, 12 mo +ve                  |        | Adeno      | 16 x 16| Alive 53 mo|
| K, 20 mo +ve                  |        | Adeno      | Neg. | Dead 22 mo|
| L, 30 mo +ve                  |        | Adeno      | 9 x 9| Alive 70 mo|

Total: 12   Mean, 17.36 mo   DHR: mm of induration at 48 hr   Mean 5.75 mm
DHR positive to soluble cancer antigen 1/7 dead
DHR negative to soluble cancer antigen 4/5 dead
TABLE 4
Late Testing of Stage I Patients with TAA

| Tested months post-operatively | TAA     | DHR     | Status     |
|-------------------------------|---------|---------|------------|
| M, 4 mo                       | Squamous| 14 x 11 | Alive 44 mo* |
| 40 mo                         | Squamous| 40 x 35 | Alive 32 mo |
| N, 5 mo                       | Alveolar | 18 x 11 | Alive 35 mo |
| O, 6 mo                       | Adeno   | 13 x 11 | Alive 41 mo |
| P, 8 mo                       | Squamous| 36 x 27 | Alive 42 mo |
| Q, 10 mo                      | Squamous| 12 x 12 | Dead 42 mo  |
| R, 13 mo                      | Autologous 10 ug | 16 x 15 | Alive 45 mo* |
| 36 mo                         | Autologous 20 ug | 43 x 36 | Alive 55 mo |
| S, 17 mo                      | Adeno (autologous) | 7 x 7  | Alive 57 mo |
| T, 18 mo                      | Squamous| 7 x 5   | Alive 57 mo |
| U, 25 mo                      | Anaplastic | 13 x 13 | Alive 67 mo |
| V, 27 mo                      | Squamous| 16 x 16 | Dead 40 mo  |
| W, 29 mo                      | Squamous| 8 x 7   | Alive 55 mo |

Total: 11  
Mean, 17 mo  
DHR: mm of induration at 48 hr  
Mean 17.9 mm 2/11 positive patients died of lung cancer

*Note the increase of reactivity to antigen over a period of three years.

Following immunization and gave 20 x 20 mm of induration to 100 µg of autologous antigen that had been stored at -80°C. The mean diameter of these delayed hypersensitivity reactions was 32 mm, double that of the immunized group and six times as strong as the mean reaction, in the control non-immunized group.

TABLE 5
Late Testing of Stage I Patients with TAA
(Immunotherapy patients)

| TAA     | DHR     | Status     |
|---------|---------|------------|
| 1. 3 mo | Squamous| 77 x 63    | *          |
| 4 mo    |         | 10 x 10    |            |
| 36 mo   | Squamous| 45 x 31    | Alive 44 mo |
| 2. 3 mo | Squamous| 9 x 8      |            |
| 20 mo   | Adeno   | 22 x 21    | Alive 29 mo** |
| 3. 10 mo| Squamous| 11 x 11    | Dead 14 mo |
| 4. 3 mo | Squamous| 40 x 40    | *          |
| 16 mo   | Anaplastic (autol) | 40 x 40 |            |
| 47 mo   | Anaplastic (autol) | 30 x 30 | Alive 56 mo |
| 5. 18 mo| Adeno (autol) | 14 x 12    | Alive 58 mo |
| 6. 22 mo| Squamous  | 18 x 16    | Alive 62 mo |
| 7. 23 mo| Adeno    | 10 x 10    | Alive 62 mo |
| 8. 24 mo| Squamous  | 25 x 33    | Dead 50 mo (non-cancer death) |
| 9. 25 mo| Adeno    | 55 x 55    |            |
| 56 mo   | Adeno    | 27 x 22    | Alive 65 mo |

Total: 9  
Mean, 19 mo  
autol: Autologous antigen  
1/9 positive patients died of lung cancer

*Note the strong reactivity relatively early following the combination of methotrexate and immunization.

**This patient had a primary squamous adenocarcinoma and was late tested for each antigen.
DISCUSSION

A clear correlation between resistance to tumor growth and a delayed hypersensitivity reaction to soluble tumor-associated antigens has been shown by several workers in experimental animal tumor systems [59,60,61,62]. In general such delayed hypersensitivity reactions last for 28–30 days. We believe that in our patients persistence of such hypersensitivity reactions to tumor antigens for five years or more is a sign of prolonged resistance to tumor growth.

The survival of our non-immunized patients compares almost exactly with the survival of all patients entered into the large multicenter trial of oral B.C.G. in Canada from 1973 to 1976 [10]. The significant improvement in the immunized group's survival may reflect resistance to tumor growth. Four patients had local recurrence of cancer yet survived 31, 36, 42, and 48 months following this recurrence and in three no chemotherapy was given at any time. This long survival is in direct contrast to the study of Green and Kern [63]. Of 1,018 patients followed over a 13-year period, 46 had locally recurrent lung cancer. Median survival for those patients was 11 months following recurrence, and only 5 percent survived three years or more.

In this trial it was decided to give the tumor-associated antigens homogenized with Freund's complete adjuvant since this is the practice in inducing auto-immune target organ damage and delayed hypersensitivity reactions to the target organ antigen. It is recognized that resistance to adenovirus oncogenesis has been shown if the antigen and Freund's complete adjuvant are given separately in hamsters, up to five days after virus inoculation [64]. Strong delayed hypersensitivity reactions can be induced in guinea pigs by using antigen and adjuvant homogenized or separately with a very wide range of antigen, 2 μg to 10 mg [65]. The favourable survival of patients with stage I squamous cell lung cancer [66] receiving Freund's complete adjuvant and separate doses of antigen, as skin tests, from 200 to 400 μg, in the first four months, may indicate that the amount of tumor-associated antigen required is less than had been anticipated in the phase II Ottawa trial. A current multicenter trial in Canada and the United States should shed further light on this, as those patients randomized to receive Freund's complete adjuvant alone will not receive tumor-associated antigens as skin tests during the first year. Whether such nonspecific therapy will be effective in prolonging survival in such patients remains to be seen.

Conclusion: Active immunotherapy using tumor-associated antigens appears effective in prolonging survival of patients with Stage I lung cancer, following curative surgery.

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