

# Immunology of bronchial carcinoma<sup>1</sup>

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In January 1969, Gordon Hamilton Fairley, who met such a tragic and untimely death last year, delivered a Goulstonian Lecture at this College entitled Immunity to Malignant Diseases in Man. In this lecture he reviewed evidence on the topic and gave some indications that the immunological forces of the patient might be influenced so as to confer therapeutic benefit.

In the last seven years there has been a very active interest in this topic so that now it is worth focusing upon the immunological aspects of one particular type of malignant disease, carcinoma of the bronchus.

## ABILITY OF THE PATIENT TO RESIST GROWTH OF HIS OWN CANCER CELLS

**MALIGNANT CELLS CIRCULATING IN THE BLOOD** A variety of abnormal cells, including malignant neoplastic cells, were observed by Kuper (1962) to circulate in the bloodstream of patients with bronchial carcinoma.

In the case of colonic carcinoma, Griffiths *et al.* (1973) have reported that circulating malignant cells are infrequent at times other than at operation. During surgery, however, malignant cells were found in 57% of patients with colonic cancer when blood was taken from the iliac vein during operation. The five-year survival did not correlate with the presence or absence of malignant cells in the blood at operation.

No studies to assess the significance of circulating malignant cells in patients with carcinoma of the bronchus appear to have been published. If the situation in bronchial carcinoma is similar to that shown by Griffiths *et al.* (1973) for colonic carcinoma, then immunological defence mechanisms could be of importance in determining whether a circulating malignant cell does or does not develop into a metastasis.

**REJECTION OF TRANSPLANTED CANCER CELLS** In an attempt to look further into the phenomenon of host resistance, Southam and Brunschwig (1961)

investigated the fate of autotransplants in patients with an extensive recurrence or disseminated cancer. Out of 27 evaluable transplants there were five 'takes'. These 27 patients included two patients with bronchial carcinoma in whom there were no 'takes'. A successful autotransplant probably needs a minimum of 10 000 living cancer cells—even in patients with advanced cancer.

## IMMUNOLOGICAL MECHANISMS INVOLVED IN MALIGNANT DISEASE

The mechanisms which limit the growth of a tumour in the body can be non-specific (that is, not related to the specific antigens on the surface of the tumour cells) and specific. The latter are immune responses.

The non-specific factors relate to such things as vascularization of the tumour, supplies of nutrients, influence of hormones, and, possibly, non-specific cytotoxic activities of macrophages and lymphocytes. Very little is known about such factors in general, and virtually nothing in bronchial carcinoma.

With regard to the specific immunological responses to tumour cells, there is a considerable amount of information. Much of this relates to experimental animal tumours, but there is also a growing body of knowledge concerning human malignant neoplasms. The phenomena can be described under the usual subdivisions of humoral and cellular responses, but it is becoming clear that interactions between these branches of the effector mechanisms are probably of great importance.

It is not at all clear exactly how a cell from the host's body defences kills tumour cells *in vivo*, or indeed which cell is principally involved. However, most of the work done *in vitro* indicates that T lymphocytes and macrophages are involved in tumour cell destruction.

In a recent review of the role of the macrophage in tumour immunity, Lejeune (1975) brings out many of the important points, and it is particularly relevant that some immunothera-

<sup>1</sup>Watson Smith Lecture, Royal College of Physicians of London 1976

peutic procedures have the effect of increasing macrophage activity in the host.

Lymphocytes also exhibit antitumour cell activity *in vitro*, and the way in which the cytotoxicity is effected is the subject of current investigations (Golstein *et al.*, 1973; Ferluga and Allison, 1974). Work on the specific cytotoxicity of human lymphocytes against human cancer cells will be referred to below, together with a discussion as to how humoral factors can modulate this activity.

In this lecture, in order to illustrate certain points, experiments and ideas derived from experimental tumours and from human cancers other than bronchial carcinoma will be quoted.

#### ANTIGENS OF BRONCHIAL CARCINOMA

The application of transplantation techniques in the study of experimental tumours in syngeneic animals has proved quite clearly that some tumour cells have surface antigens which can immunize their hosts.

Searches have been made for antigens different from those of the patient's normal cells in bronchial carcinoma. These seem to fall into two classes: first, those found in both bronchial carcinoma and other cancers (carcinoembryonic antigen (CEA) is the most important) and, secondly, antigens which may be peculiar to bronchial carcinoma.

(a) CARCINOEMBRYONIC ANTIGEN CEA has been found in increased concentrations in the plasma of about 70% of patients with bronchial carcinoma (Lo Gerfo, Krupey, and Hansen, 1971; Reynoso *et al.*, 1972; Laurence *et al.*, 1972). Additional findings of interest were published by Vincent and Chu (1973). Generally speaking, CEA concentrations were lower in low-volume resectable tumours than in extensive and non-resectable tumours. When patients had their tumours resected the CEA concentration rose after operation (even when normal before operation) and then fell to normal at 60 days. A later rise heralds the development of metastases. It may be conjectured that the rise of CEA in the month after operation may be an expression of the temporary survival of malignant cells disseminated at operation.

(b) SURFACE ANTIGENS WHICH MAY BE TUMOUR-SPECIFIC Attempts have been made directly to solubilize the antigens which are likely to exist on the surface of bronchial carcinoma cells and

then to identify and characterize them by various means. Hollinshead, Stewart, and Herberman (1974) showed that the proteins from the tumours differed on polyacrylamide gel electrophoresis from those eluted in a similar way from adjacent normal lung tissue. Frost, Rogers, and Bagshawe (1975) prepared antisera by injecting eluates into rabbits and found that precipitin lines were produced on agar when eluates from other bronchial carcinomata were allowed to react with the antibody so produced. A somewhat similar approach was also used by Watson, Smith, and Levy (1975) and Viza *et al.* (1975).

Inhibition of the migration in agarose of leucocytes from bronchial carcinoma patients, as produced by tissue extracts, was studied by Boddie *et al.* (1975). In general, a greater inhibition was produced by extracts of bronchial carcinoma than by extracts of normal lung. However, there must be some reservations about this study because the extracts were crude, and non-specific factors could have caused the inhibition of migration.

Some cross-reactivity was detected between breast cancer glycoprotein and extract of bronchial carcinoma when assessed by immunodiffusion against an antibody raised by rabbits against the former (Kuo, Rosai, and Tillack, 1973).

(c) There is very suggestive evidence in animals that tumour specific antigens may be produced by a slight change in the structure of a pre-existing antigen resembling HL-A (see Bowen and Baldwin, 1975).

Dellon, Rogentine, and Chretien (1975) investigated the HL-A types of bronchial carcinoma patients. There seemed to be no association between any particular antigens and the actual disorder. However, there was evidence that patients possessing antigens 5 and W19 survived longer than patients who did not possess these antigens. This study will require independent confirmation, and, even if this is forthcoming, its biological meaning will still be a puzzle. However, one possible interpretation is that persons with a certain genetic constitution on chromosome 6 have a superior ability to mount a successful immunological defence against bronchial carcinoma.

#### IN VIVO HUMORAL RESPONSES (MISCELLANEOUS)

It has been shown that the concentrations of IgG and IgA are significantly increased in a population of male patients with carcinoma of the lung (Hughes, 1971), though many individual

patients have values within normal limits (Cardozo and Harting, 1971). Increased IgA concentrations were a feature of long-surviving patients, according to Krant *et al.* (1968).

Complement has been found in increased amounts in the sera of 15 out of 21 patients with squamous carcinoma of the lung. The components particularly increased were C<sub>8</sub> and C<sub>9</sub> (McKenzie, Colsky, and Hetrick, 1967).

Various workers have challenged carcinomatous patients (including bronchial carcinoma patients) with various antigenic stimuli, including plague vaccine and diphtheria toxoid (Krant *et al.*, 1968), tetanus toxoid (Lytton, Hughes and Fulthorpe, 1964), monomeric flagellin from *Salmonella Adelaide* (Lee, Rowley, and Mackay, 1970), and yellow fever vaccine (Levin *et al.*, 1970).<sup>1</sup>

#### HUMORAL RESPONSES OF CANCER PATIENTS (INCLUDING BRONCHIAL CARCINOMA) TO CARCINOMA CELLS

The demonstration of antibody production by cancer patients in response to such antigens as tetanus or plague shows that humoral responsiveness is not seriously impaired, but it does not give information concerning the patient's antibody response to tumour antigens.

Finney, Byers, and Wilson (1960), in the course of a therapeutic experiment, gave eight cancer patients intramuscular injections of their own tumour homogenized in Freund's adjuvant. The antibody titre was measured by a tanned sheep red cell agglutination test. A rise in circulating antibody titre was demonstrated in all the patients (including one with bronchial carcinoma). The initial peak titre was attained in 18 to 36 days after the first injection, and the titre could then be boosted by a further antigenic injection.

Thus the evidence is that the cancer patient can mount a humoral response to antigens on his own tumour cells. Not much seems to be known, however, about the different classes and properties of antibodies against their own cancer cells which are present in patients with bronchial carcinoma—and this could have considerable importance.

#### IN VIVO CELL-MEDIATED DELAYED HYPERSENSITIVITY TYPE IMMUNE RESPONSES

Skin tests provide an easy and useful way of testing cell-mediated immune responses *in vivo*, and a number of workers have used them to study patients with bronchial carcinoma.

The agents used fall into three classes (Table I).

<sup>1</sup>See p. 506 for text omitted from typescript

TABLE I  
AGENTS USED TO TEST THE SKIN FOR DELAYED  
HYPERSENSITIVITY TYPE IMMUNE RESPONSES

'Recall' antigens
Purified protein derivative (PPD) from <i>M. tuberculosis</i> , mumps antigen, trichophyton antigen, Candida antigens
'New' antigens
Dinitrochlorobenzene (DNCB)
Streptokinase/Streptodornase (SK/SD)
Antigens derived from bronchial carcinoma specimens

According to most authors, a population of bronchial carcinoma patients shows a diminished frequency of delayed hypersensitivity responses (DHR).

Krant *et al.* (1968) found that dinitrochlorobenzene (DNCB) negativity correlated with a short survival time, yet some patients with obvious metastases were DNCB positive. Positivity was more frequent in squamous than in more anaplastic tumours. Cerni and Micksche (1975) showed correlations (1) between positive DHR tests and responsiveness to skin testing with antigens from a cultured cell line E 14 derived from a squamous cell carcinoma of lung, and (2) between positive DHR tests and lymphocyte migration inhibition. Lee *et al.* (1975) correlated negative DHR to various agents with a depressed peripheral blood lymphocyte count. Brugarolos and Takita (1972) describe an improvement in DHR following 'curative' therapy with operation, radiotherapy, and chemotherapy. Israel (1973) reports a longer average survival in purified protein derivative (PPD) positive than in PPD negative patients; while Israel *et al.* (1968) claim that, in a group of 31 patients with operable tumours but negative tuberculin reactions, 14 became positive after 15 postoperative days.

Skin tests have been performed using solutions of antigens derived from bronchial carcinoma specimens (Stewart, 1969; Hollinshead *et al.*, 1974; Wells *et al.*, 1973). Patients often have positive DHR skin reactions to antigen preparations from their own tumours, but of course it is to be noted that this test is done only on patients with relatively favourable (that is, resectable) lesions.

It may, therefore, be concluded that there is an impairment of DHR in a significant proportion of a population of bronchial carcinoma patients. Perhaps the most reliable single test is DNCB, and the absence of responsiveness to this test is correlated with a poor prognosis. The DNCB-DHR test can be conveniently used to follow the progress of an individual patient.

#### IN VIVO STUDIES OF CIRCULATING PERIPHERAL LYMPHOCYTES

Krant *et al.* (1968) evaluated the numbers of circulating lymphocytes in patients with bronchial carcinoma. They found that the counts were lower in these patients at the time of diagnosis than in control patients with cerebrovascular accidents or cerebral trauma. The lymphocyte counts became lower as the bronchial carcinoma progressed.

Activity against neoplastic cells is thought to be a function of T lymphocytes which are the principal effectors of cellular immunity. These can be detected and counted by virtue of the fact that they form rosettes with sheep erythrocytes.

The variance of T cell counts was larger among bronchial carcinoma patients (29–89%) than among laboratory personnel constituting healthy controls (mean 77%, range 61 to 87%) in a study by Anthony *et al.* (1975). There was a tendency (though not statistically significant) for low T cell counts to be associated with inoperability and poor survival.

Similar results were obtained by Gross *et al.* (1975). In their study, normal controls gave  $54.0 \pm 4.9\%$  of lymphocytes to be rosette-forming T cells. Their data were criticized by Fudenberg *et al.* (1975) on the grounds that their T cell percentage in normal healthy subjects was rather lower than that usually found. A population of 24 patients with non-malignant pulmonary disease studied by Gross *et al.* (1975) gave a value of  $51.7 \pm 6.7\%$  T cells (which was not significantly different from normals) whereas 29 lung carcinoma patients gave a value of  $34.4 \pm 10.2\%$ , and only five of these patients had T cell percentages greater than 44% (normal mean minus 2 SD). There was a weak statistical association between a low percentage of T cells and the size of the carcinoma, but not with other clinical features, for example, histological type of cancer, duration of symptoms or smoking history.

#### IN VITRO TRANSFORMATION OF LYMPHOCYTES AND MACROPHAGES IN RESPONSE TO VARIOUS STIMULI

Lymphocytes can be transformed *in vitro* by a variety of non-specific stimuli, including periodate ions (Novogrodsky and Katchlaski, 1971), pokeweed mitogen, Concanavalin A, and phytohaemagglutinin (PHA) (Kirchner and Blaese, 1973).

In the process of transformation the lymphocyte develops a larger nucleus and increased volume of cytoplasm in which mitochondria and an endoplasmic reticulum appear.

A considerable number of studies of transformation (or blastogenesis) of lymphocytes from bronchial carcinoma patients have been performed, particularly in response to PHA.

When lymphocytes were thoroughly washed before exposure to PHA blastogenesis was not depressed in lymphocytes from bronchial carcinoma patients (Sutherland, Inch, and McCredie, 1971; Golub, O'Connell, and Morton, 1974; Barnes *et al.*, 1975). When the lymphocytes were not washed transformation was depressed (Ducos, *et al.*, 1970; Al-Sarraf, Sardesai, and Vaitkevicius, 1971; Thomas *et al.*, 1971; Han and Takita, 1972; Braeman and Deeley, 1973). The study of Nelson (1969) found blastogenesis not to be depressed even though he did not wash the lymphocytes thoroughly. The most comprehensive experiment was that of Barnes *et al.* (1975), who took account of age and tumour histology and showed no significant differences between bronchial carcinoma patients and three groups of controls.

#### EFFECTS OF SERUM FROM BRONCHIAL CARCINOMA PATIENTS ON IN VITRO LYMPHOCYTE TRANSFORMATION WITH PHA

Various workers have investigated the influence of serum from patients with bronchial carcinoma on the blastogenesis which can be caused in lymphocytes by various agents including PHA and bronchial carcinoma antigen. Tests conducted have been both autochthonous (that is, the bronchial carcinoma patient's lymphocytes with his own serum) and allogenic (normal person's lymphocytes with bronchial carcinoma serum).

Sample, Gertner, and Chretien (1971) found depression of transformation in response to PHA of normal lymphocytes in bronchial carcinoma serum. Barnes *et al.* (1975), however, in a most carefully conducted experiment, found just the opposite, as had Thomas *et al.* (1971) and Nelson (1969).

So it would appear at present that studying the transformation of lymphocytes from patients and controls *in vitro* in response to non-specific stimuli, and the influence of sera from patients and controls on this process, has not led to any greater understanding of the immunological phenomena in bronchial carcinoma.

#### DEMONSTRATION IN VITRO OF CELL-MEDIATED RESPONSES TO CARCINOMA OF THE BRONCHUS CELLS AND ANTIGENS

**INHIBITION OF LEUCOCYTE MIGRATION** Lymphocytes and other leucocytes have the ability to migrate *in vitro* when placed in a suitable culture

medium. The presence of an antigen to which the cells are immunized in the culture medium inhibits the migration. This serves as a test for cellular immunity.

Cerni and Micksche (1975) eluted antigens from a bronchial carcinoma cultured cell line. They studied lymphocyte migration inhibition in patients with bronchial carcinoma, patients with other malignant conditions, and in patients with non-malignant disease. Lymphocytes from the second and third groups were non-reactive. There was a correlation between migration inhibition results and delayed hypersensitivity reactions in the bronchial carcinoma patients. There was also a significant association between migration inhibition and squamous histology as compared with other histological types of bronchial carcinoma.

An incompatible observation was recorded by Wolberg and Ansfield (1974), who found that patients possessing tumours (including bronchial carcinoma) whose antigens inhibit migration have a poor prognosis. This author points out that non-immunological factors might well be responsible for the inhibition of leucocyte migration.

Most workers would agree with Currie (1974) when he states, 'This assay system has been employed recently in a great many different disease states with variable results. As a technique it is distressingly easy to carry out and extremely difficult to interpret. Quantitation of cell-mediated responses is almost impossible using this type of assay'.

**COLONY INHIBITION AND LYMPHOCYTOTOXICITY TESTS** Since lymphocytes, like other cells in the body, are probably involved in destroying cancer cells, a great deal of effort has been devoted to studying the process *in vitro*.

Hellström *et al.* (1971) studied a large number of patients with different types of cancer by means of two tests—colony inhibition and lymphocytotoxicity. In the former test, a suspension of carcinoma cells growing in culture was placed in a plastic Petri dish in culture medium, and in the absence of any further procedure a certain number of colonies would grow in three to five days. Addition of lymphocytes on the second culture day would on occasion cause a reduction in the number of colonies produced. In the second test, a number of target tumour cells were allowed to adhere to the floor of a well in a plastic plate. After this had happened (usually 24 hours later) a suspension of well washed lymphocytes was introduced. Incubation was continued for a fur-

ther three days and then the numbers of target tumour cells adhering to the plastic were enumerated. The difference between 'test' and 'control' wells (to which lymphocytes were not added) gave a measure of lymphocytotoxicity.

Explanted cells from eight different lung carcinomas were tested with lymphocytes from seven autochthonous and from five allogenic patients with carcinoma of the lung. A clear inhibitory effect of the patients' autochthonous tumour cells was seen in six of the seven cases. The data suggested that there was some cross-reaction between some lung carcinomas, since lymphocytes were generally inhibitory on allogenic lung carcinoma cells.

In a very extensive experiment, Takasugi, Mickey, and Terasaki (1974) used a similar lymphocytotoxicity test to that of Hellström *et al.* (1971). They found that the specificity of the cell-mediated cytotoxicity against cultured tumour cells was not associated with 'histological' types of cancer. For example, lymphocytes from patients with many types of tumours were as cytotoxic to lung cancer-derived target cells as were lymphocytes from patients with lung cancer.

A somewhat similar series of experiments was performed by Pierce and DeVald (1974). They used the same lymphocytotoxicity technique in phase 3 of their study and observed the effect of lymphocytes from cancer patients (including bronchial carcinoma) as well as lymphocytes from healthy persons on primary cultures from a variety of tumours. A greater lymphocytotoxicity was observed using lymphocytes from cancer patients than from normal persons. The latter may have represented a non-specific activity which may have added to it a specific activity in lymphocytes from cancer-bearing subjects. The 'non-specific' activity of lymphocytes from normal donors was variable. The authors conclude that before microcytotoxicity assays can be widely used to monitor specifically the tumour-immune response of patients the factors responsible for the non-specific toxic reactions must be clearly defined and eliminated.

Baldwin (1975), in a review of the problems of lymphocytotoxicity, states, 'Even with reservations about the standardization of the microcytotoxicity assay there are sufficient published reports to show that under "laboratory-defined" conditions the test can yield meaningful data, eg, in characterizing humoral factors interfering with cell-mediated immunity in the tumour-bearing host' (see also Herberman and Oldham (1975)).

INFLUENCE OF SERUM FACTORS ON CELL-MEDIATED  
IMMUNE RESPONSES TO NEOPLASTIC CELLS

(Evidence not derived from human bronchial carcinoma)

**BLOCKING FACTORS** It has been found that the introduction of sera will block lymphocytotoxic activity against neoplastic target cells.

Sjögren *et al.* (1972) subjected various human tumours (but not bronchial carcinoma) to elution at pH 3.1. This eluate was separated by means of molecular filters into two fractions, (a) >100 000 and (b) 10 000 to 100 000 daltons. Lymphocytes from patients with a given type of tumour, for example, carcinoma of the ovary, were shown to be cytotoxic against target cells derived from the same type of tumour in an allogenic system. In some instances the unfractionated eluates obtained from the same type of tumour were shown to possess blocking activity. The two molecular weight fractions derived from such blocking sera, each tested separately, had no such activity, but when they were combined in equal amounts the blocking activity was restored. Blocking was also obtained when the tumour cells were first exposed to the high and then to the low molecular weight fractions, but not when the sequence was reversed.

This work was the extension on human material of work conducted previously on blocking factors on animal systems, particularly in the sera of mice.

It was suggested in consequence that the blocking factor was an antigen-antibody complex though there was no direct proof that the low molecular weight moiety was an antigen. It was considered possible that the blocking factors could coat with the target cells or effector lymphocytes.

As yet there does not seem to have been any systemic study of blocking factors in human bronchial carcinoma.

**UNBLOCKING FACTORS** Serum from mice whose Moloney sarcomas had regressed spontaneously was found to nullify the blocking activity in lymphocytotoxicity tests of serum obtained from mice with growing tumours (Marx, 1974).

Hellström, Hellström, and Warner (1973) demonstrated that sera from certain patients increased the cytotoxicity of carcinoma patients' lymphocytes against the respective type of tumour target cells. Also these sera had the ability to 'arm' (that is, to render cytotoxic) lymphocytes from non-reactive donors.

*Comment* Lymphocytotoxicity tests are difficult to perform reproducibly and, of course, blocking and unblocking activity can be demonstrated only in the presence of lymphocytotoxicity. Nevertheless the fact that several groups of workers have produced somewhat similar results in experimental animals and human patients, and the demonstration in some instances of *in vivo/in vitro* correlations, have at least served to swing attention more towards humoral factors in tumour mechanisms. If these results can be substantiated in human cancers generally, it would appear that the cellular defence mechanisms can be prevented from acting in certain internal environments whereas others enable them to be effective. The soluble factors of importance in this regard are antigens shed from tumour cell surfaces and antibodies produced by the host organism.

NEPHROTIC SYNDROME IN BRONCHIAL CARCINOMA

A clinical suggestion that antigen-antibody complexes are produced in at least some patients with bronchial carcinoma is provided by a study of the uncommon complication of the nephrotic syndrome.

Loughridge and Lewis (1971) and Lewis, Loughridge, and Phillips (1971) reported three patients who had the nephrotic syndrome in association with a bronchial carcinoma. Similar patients have been reported since then by others, eg, Cameron (1975).

These patients showed the histological and electron microscopic appearances characteristic of immune-complex nephritis, viz, diffuse membranous abnormality in the glomeruli with the 'lumpy' distribution of IgG on the glomerular basement membrane with immunofluorescence.

In the patient reported by Lewis *et al.* (1971), an eluate from the kidney contained IgG and IgA. An antigen suspension was made from the bronchial carcinoma and a similar reacting antibody was demonstrated in the patient's glomerular eluate and serum. One point of criticism is that antigen resembling that found in the tumour was not demonstrated in the kidneys.

Both progression (Lewis *et al.*, 1971) and regression (Cameron, 1975) of the renal lesion after successful resection of the primary bronchial carcinoma have been described.

This uncommon complication provides strong clinical evidence of the implication of humoral immunological mechanisms in bronchial carcinoma (see also Ozawa *et al.* (1975)) and fits in with a general background of knowledge concerning immune complex nephropathy associated

with other disorders, such as streptococcal infections, disseminated lupus erythematosus, quartan malaria, and schistosomiasis.

#### CLASSIFICATION OF IMMUNOTHERAPEUTIC PROCEDURES

Classification of immunotherapeutic procedures was given by Currie (1974). Specific or non-specific measures may be aimed at conferring passive, adoptive or active immunity.

Active immunotherapy, both specific and non-specific, has been employed in bronchial carcinoma and will be discussed in more detail.

**NON-SPECIFIC ACTIVE IMMUNOTHERAPY** The two most popular agents in recent times have been Bacille Calmette-Guérin (BCG) and *Corynebacterium parvum* (*C. parvum*), and a recent addition has been levamisole.

(a) *BCG* This agent has the capability of providing non-specific increases in both cell-mediated and humoral immunity to a variety of unrelated antigens. The subject of BCG and cancer has been extensively reviewed by Bast *et al.* (1974).

For therapy of established tumours, there are two ways of using BCG, namely intralesional injection and administration remotely from the lesion. Due to the location of the tumour and the complications of intralesional BCG injection (Sparks *et al.*, 1973), it would appear that this therapy is not appropriate for bronchial carcinoma. Local therapy of neoplastic lesions with BCG and other agents will not be considered further in this lecture.

Israel *et al.* (1967) investigated 130 cancer patients, including 74 with bronchial carcinoma. They were particularly concerned with skin DHR and found that BCG converted the negative Mantoux to positive in about 50% of cases. Later the same group (Israel *et al.*, 1968) established that BCG did not improve survival in advanced cases of bronchial carcinoma.

Khadzhiev and Kavaklieva-Dimitrova (1971) report the use of water-saline extract of BCG in 52 patients with histologically proved cancer of the bronchus, grades III and IV. An improved survival rate was noted in patients treated in this way, and this was particularly seen in patients whose tumours regressed in the radiograph (28.8% of the group). No beneficial effect was observed in patients with metastases.

The effect of BCG in conjunction with 'complete' surgical extirpation of the neoplasm was investigated by Edwards and Whitwell (1974). They gave 0.5 ml BCG Glaxo (500 000 organisms)

subdermally in the deltoid region. In a non-randomized trial in 63 consecutive patients (compared with the previous 64 patients non-BCG treated by the same surgeon), there seemed to be no significant improvement in results associated with BCG when assessed one year after operation.

McKneally, Maver, and Kausel (1976) studied 60 patients in whom bronchial carcinoma had been resected. The patients were randomized into treatment and control groups. The former received intrapleural BCG postoperatively. In stage I patients, the treatment group showed significantly improved survival over one year. Patients with more advanced disease did not show this effect.

Pines (1976) randomized 48 patients with advanced squamous-cell lung cancer, who had exhibited a favourable response to radiotherapy and/or chemotherapy, into treatment and control groups. The former received repeated BCG inoculations at weekly or fortnightly intervals. Patients in the treated group appeared to have an improved short-term survival and eventually succumbed with local recurrence, whereas extrathoracic metastases seemed to occur more commonly in the control group.

(b) *Corynebacterium parvum* This bacterium has been tested in a number of animal systems and has been shown to have some effect when given alone in established tumours.

It has been suggested (Woodruff, 1975) that the main mode of action of *C. parvum* may well be by stimulating the activity of macrophages. Israel and Halpern (1972) randomized 141 patients with advanced inoperable bronchial carcinomas into two groups, a group treated with *C. parvum* and controls. A significantly prolonged survival was noted in the treated group.

In both groups tuberculin positive subjects survived longer than those who were tuberculin negative. In the *C. parvum* treated group the tuberculin positive patients had a mean survival of thirteen months.

Woodruff (1975) reported two trials of *C. parvum*. In the first trial, 30 patients with inoperable bronchial carcinoma, carcinoma of the breast, and melanoma were given an intravenous dose of *C. parvum* strain CN6134 (Wellcome). A dose of 1 mg/per kg body weight was found to produce an unacceptable pyrexia. The protocol was, therefore, modified, and a standard dose of 20 mg *C. parvum* in 100 ml saline was given over one hour. Subsequent injections of 22 mg intramuscularly were given to some patients weekly for 12 weeks and monthly thereafter. These dosages were claimed to provoke only mild

reactions. A marked rise in titre of antibody to *C. parvum* was observed on day 11 and persisted until at least day 39. Arrest of progression of the disease was observed in one man with extensive contralateral pulmonary metastases after a previous lung resection. In the second trial, patients with completely resectable and histologically proven bronchial carcinoma are being randomized into two groups—(a) treated with *C. parvum*, and (b) controls, and this trial is now proceeding.

It would appear that *C. parvum* given alone to patients with bronchial carcinoma may be of value as additional therapy when the carcinoma has been completely resected, but further evidence is required before the point can be taken as proven.

(c) *Levamisole and bronchial carcinoma* Levamisole (the levorotatory isomer of tetramisole) was introduced into therapeutics as an antihelminthic. Later it was discovered that the drug possessed immunostimulant properties (*Lancet*, 1975). Among other reactions, levamisole stimulates macrophage function in man and animals. For example, Hoebeke and Franchi (1973) demonstrated that levamisole, in single doses or in one-week courses, resulted in an increased clearance of intravenously administered carbon particles from the blood of mice, a process known to be subserved by the mononuclear phagocytic system in various organs.

The effect of levamisole on human cancer patients (including 10 with bronchial carcinoma) was studied by Verhaegen *et al.* (1973). They found that skin DHR responses to PPD could be converted from negative to positive much more frequently than in controls, and positive responses to DNCB were boosted. In addition, the clearance of intravenously administered lipid emulsion from the blood (which is due to macrophage activity) was enhanced.

An exemplary trial of levamisole in resectable bronchogenic carcinoma has been published by the Study Group for Bronchogenic Carcinoma (1975). Patients with resectable bronchial carcinoma who had not been exposed to cytostatic drugs, corticosteroids or radiotherapy were randomly assigned to levamisole or placebo groups. Levamisole was given for three days every two weeks, starting three days before surgery. Double-blind follow-up examinations over one year reveal more recurrences in the placebo than in the treatment group. Of course, this study will need to be continued over a longer period of observa-

tion. If the present trend continues, however, it suggests that levamisole may be beneficial for patients with resectable lesions, and if that is so there is a suggestion that it may act by influencing immunological mechanisms.

SPECIFIC ACTIVE IMMUNOTHERAPY OF HUMAN MALIGNANCIES WITH TUMOUR CELL VACCINES This subject has a long and on the whole an inglorious history. The idea that tumours might be antigenic and that their hosts might respond to them in an immunological manner occurred to medical scientists in the nineteenth century. They also embarked upon experiments to try to augment this immunological response using various types of vaccines made from tumour cells. A comprehensive review of the subject has been published by Currie (1972).

It is fair to comment that tumour cell vaccine therapy has generally been given to patients with advanced malignant disease (see Morton *et al.*, 1972). In the present state of knowledge it is difficult to see how immunological processes can be predictably successful in such a situation. This is possibly because of a general impairment of immunological responsiveness in such patients and the presence of blocking factors in the serum which have the effect of protecting the tumour from attack by cellular defence mechanisms.

Despite theoretical considerations and generally disappointing experiences, occasional surprising results have been recorded where doomed patients have exhibited a totally unexpected clinical course after treatment with tumour cell inocula. Examples are the 52-year-old woman with cancer of the cervix (MC) reported by Graham and Graham (1959), the 65-year-old woman with squamous carcinoma of the skin (case 1) reported by Czajkowski *et al.* (1967), and the 38-year-old man with rectal adenocarcinoma (case 3) reported by Taylor and Odili (1972).

The very occasional patients such as these suggest that immunological mechanisms can, under certain special conditions, tip the scales heavily in favour of the host and against the tumour. The snag is that these cases, as studied to date, do not reveal to us the nature of the changes which have taken place in the body's immune mechanisms. However, they do emphasize the fact that it is worth persisting with the logical development of immunological management of patients in whom the chances of success seem much higher, namely, those with minimal residual tumour cell populations.



Few experiments involving the treatment of bronchial carcinoma with tumour cell vaccines have been published.

SOME EXAMPLES OF IMMUNOTHERAPY OF EXPERIMENTAL TUMOURS IN ANIMALS In a comprehensive review of the topic, Old and Boyse (1964) stated, 'Despite the evident antigenicity of a variety of experimental tumors, it is a fact that no immunological maneuver is known that will cause the rejection of an established tumor primary or transplanted regardless of its size.'

The situation has now changed. Regression of animal tumours, varying in extent from partial to complete and in frequency from occasional to commonplace, has been achieved in the five studies listed in Table II. Furthermore, in animals, tumour formation can be inhibited and metastases prevented or reduced in number by the various procedures listed in Table III.

SPECIFIC ACTIVE IMMUNOTHERAPY OF BRONCHIAL CARCINOMA: POSSIBILITIES AND LIMITATIONS

In the present state of understanding, it seems logical to direct immunotherapy at the most pro-

misg situation, namely, the patient who has had primary bronchial carcinoma successfully and 'completely' resected. It is obvious that many such patients already have scattered foci of malignant cells which will develop later into clinical metastases, and therapy is aimed at preventing this process from occurring.

Old and Boyse (1964), summarizing experimental tumour immunology up to that time, point out that 'Another important concept that has arisen from the study of tumor immunity is that tumors induced by any one virus contain the same characteristic cellular antigen whereas tumors induced by chemical carcinogens have individually distinct antigens'. It would seem unwise to assume that the bronchial carcinoma of man does not correspond to the experimental tumours produced by chemical carcinogens (despite the findings of some cross-reacting antigens). Consequently, specific immunological therapy should be planned on an autochthonous basis.

There are obviously several immunotherapeutic possibilities but, in view of the animal models, there is considerable attraction in combining non-

TABLE II  
IMMUNOTHERAPY PRODUCING REGRESSION IN ESTABLISHED EXPERIMENTAL TUMOURS

Author	Tumour	Host Animals	Procedure
Evans <i>et al.</i> (1962)	Shope papilloma	Rabbits	Virus was injected at two sites. One of the two tumours produced was made into vaccine and administered by injection. Regression was never > 25% but in treated animals was always > 50% in controls.
Delorme and Alexander (1964)	3-4 benzpyrene induced fibrosarcoma	Rats	Tumour biopsy. Biopsy irradiated (15 000 r). Syngenic animals immunized. Their lymphocytes collected and injected into original hosts. Temporary regression.  Tumour biopsy. Trypsinized cell suspension injected into random bred albino rats. Their lymphocytes collected and injected into original hosts. Regression observed in 12 of 33 and complete in 1.
Fefer (1971)	Moloney virus sarcoma	Mice	Spleen cells or serum from immunized mice given to host when tumour palpable.
Simmons and Rios (1972)	Methyl-cholanthrene induced fibrosarcoma	Mice	BCG and neuraminidase-treated tumour cells injected at different sites caused regression of 1cm diameter tumours.
Bansal and Sjögren (1974)	Polyoma virus induced tumour	Rats	BCG, splenectomy, and unblocking serum produced regression of established tumours.
Ribi <i>et al.</i> (1975)	Hepatocarcinoma initiated by di-ethylnitrosamine feeding	Guinea pigs	Lipid preparation from BCG on oil-droplets plus oil-treated cell walls of <i>Salmonellae</i> produced regression of 90% of established neoplasms.
Song and Levitt (1975)	3-methyl-cholanthrene induced fibrosarcoma	Mice	Three doses of <i>Vibrio cholerae</i> neuraminidase and mitomycin C-treated tumour cells inoculated after radiotherapy led to regression in 38% of tumours.

TABLE III  
INHIBITION OF METASTASES AND TUMOUR FORMATION BY IMMUNOTHERAPEUTIC MEANS

Author	Tumour	Host Animals	Procedure
Bansal and Sjögren (1971)	Polyoma virus induced tumour	Rats	Antipolyoma serum given to host 1 hour after tumour cell inoculum prevented tumour formation.
Irie and Irie (1971)	Spontaneous mammary tumour	Mice	Immunization with mammary tumour antigen reduced spontaneous incidence.
Fakhri and Hobbs (1973)	Plasma-cytoma	Mice	7S antibodies with peritoneal macrophages prolonged survival after tumour transplantation.
Baldwin and Pimm (1973)	3-methyl-cholanthrene sarcoma	Rats	BCG + irradiated tumour cell at a different site to living tumour cell inoculum prevented tumour development.
Rollinghoff <i>et al.</i> (1974)	Plasma-cytoma	Mice	Inocula of freeze/thawed cells in complete Freund's adjuvant confer immunity against subsequent challenge with living tumour cells.
Fidler (1974)	Melanoma	Mice	Prior immunization with either freeze/thawed or irradiated tumour cells in complete Freund's adjuvant led to a marked decrease in pulmonary tumours after IV injection of living tumour cells.
Baldwin and Pimm (1974)	4-dimethyl aminoazobenzene	Rats	Pulmonary metastases prevented by IV BCG although primary tumour was not affected.
Levy <i>et al.</i> (1974)	Methyl-cholanthrene-rhabdomyo-sarcoma	Mice	BCG plus saline tumour extract following removal of 1 g tumours caused great reduction in incidence of metastases.
Law <i>et al.</i> (1975)	SV 40 virus induced sarcoma	Mice	Injections of antigen preparations protected against the development of neoplasm after challenge with sarcoma cells.
Bomford (1975)	Methyl-cholanthrene fibrosarcoma	Mice	Irradiated tumour cells in saline plus <i>C. parvum</i> injections prevented tumour development when given up to 10 days after living tumour cell inocula.
Ray <i>et al.</i> (1975)	Dimethyl benz-dithio-naphthalene induced fibrosarcoma	Mice	Irradiated tumour cells and neuraminidase treated tumour cells prevented tumour growth after living tumour cells inoculated.
Yamamura <i>et al.</i> (1974)	Sarcoma, mastocytoma and leukaemia	Mice	Mycobacterial fractions associated with oil droplets injected with tumour cells prevented tumour formation.

specific active and specific active immunotherapy in patients with minimal residual neoplastic disease.

The manner of preparation of the tumour cells for active specific immunization requires consideration. One possibility from animal models is to use living cells, but here there are clearly ethical difficulties. Other possibilities for whole cells include irradiation, neuraminidase treatment, mitomycin treatment, and multiple freezing/thawing. Then it is possible to make cell membrane preparations or antigen preparations. It is difficult to know which would be the most appropriate to use for bronchial carcinoma.

How the tumour cell inoculum is to be injected is another problem. Here the main choice is between (a) a simple saline or buffer vehicle, (b) incomplete Freund's adjuvant, and (c) complete

Freund's adjuvant (CFA). Incomplete Freund's adjuvant is a light mineral oil, and when antigens are injected in it the immunological responses are magnified. This enhancement is even greater when dead tubercle bacilli are added giving CFA. The adjuvants particularly promote macrophage proliferation in the neighbourhood as well as making available small amounts of antigen over a long period, and this may well be beneficial in anti-tumour immunity.

The position of the injections of tumour cell inocula may be of importance. Presumably it would be advantageous to have as many lymph nodes as possible actively producing immunized lymphocytes.

The timing of the tumour cell inocula may be of importance. Blocking factors have been observed to disappear from the serum of tumour-

bearing hosts within a few days of tumour excision. Thereafter an unblocking factor may be present. On the supposition that the latter is free antibody it may be augmented in amount by tumour cell inocula, administered when blocking factor has disappeared.

The assessment of results in a clinical immunotherapeutic trial can be in two areas, viz, clinical and laboratory. The choice of laboratory tests will be limited by the expertise locally available among other factors.

In view of the many possibilities, several immunotherapeutic protocols can be constructed, and probably a number will be tried.

**EXPERIMENTS PROCEEDING** It has been stated that Woodruff (1975) is conducting a controlled experiment with *C. parvum* in carcinoma of the bronchus patients following resection.

In Glasgow, Stack (1976), with a group of colleagues is using tumour cell inocula with radiotherapy following resection of bronchial carcinoma.

In Liverpool, an experiment is proceeding with the collaboration of many physicians and the staff of the Regional Thoracic Surgical Centre. Consenting patients are entered into the study if the lesion is 'completely' resectable. They are randomized into 'treatment' and 'control' categories. Immunotherapeutic treatment is given on days 3, 10, 17, 24, and 31 after operation. Each limb receives a BCG injection followed a week later by an injection of irradiated tumour cells in incomplete Freund's adjuvant. In control cases, the irradiated tumour cells are omitted, but their suspending medium containing dimethylsulphoxide is given in incomplete Freund's adjuvant. The tumour cells are irradiated with  $15 \times 10^3$  rads and are kept in liquid nitrogen before injection.

The treatment is given 'blind', that is, without knowledge as to whether the patient is in the treatment or the control category.

Follow-up observations are also performed 'blind' and consist of clinical and laboratory data. The former include DHR (DNCB), and the latter include assessment of T cell percentage. It is hoped to add other laboratory tests later.

Experiments of this type are relatively long-term projects, and time alone will tell if they benefit the cancer patient.

#### CONCLUSION

New antigens are present on the surface of bronchial carcinoma cells. The patient has an impaired

cellular response to these new antigens. This impairment may be due to the soluble neoplastic antigens, or to antibodies produced by the host, or to antigen-antibody complexes.

In certain experimental animals, established tumours can be made to regress by means of various therapeutic measures. In other laboratory models the formation of metastases can be prevented.

There is evidence that non-specific active immunization can improve survival of the bronchial carcinoma patient with a minimal residual tumour load.

It would be of interest to see if the survival of such a patient could be improved further by combining non-specific and specific active immunization procedures.

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<sup>1</sup>A somewhat confused picture emerges, but it is clear that serious depression of ability to produce antibodies is not a standard feature of the carcinoma patient. In addition it has been found (Hodson and Turner-Warwick, *Thorax*, **30**, 367, 1975) that bronchial carcinoma patients have a high incidence of antinuclear antibodies in the adenocarcinomatous type and of antismooth muscle antibody in the undifferentiated type, as compared with controls. The meaning of this observation is obscure.