Carcinomatous neuromyopathy:

2. Immunological studies

An electrophysiological and immunological study of patients with carcinoma of the lung

D. W. PATY¹, M. J. CAMPBELL², AND D. HUGHES

From the M.R.C. Unit on Demyelinating Diseases and the Muscular Dystrophy Group Research Laboratories, Newcastle General Hospital, Newcastle upon Tyne

SYNOPSIS Significant cellular sensitivity to peripheral nerve antigens was found by MIF assay in 36% of patients with lung cancer ($P < 0.005$) as compared with 62% of patients with peripheral neuropathy without neoplasia. No sensitivity was found in controls or the cancer sub-group without neuromuscular abnormality. The significance of these findings for an immune mechanism is discussed.

Non-metastatic remote complications in the nervous system of neoplastic diseases are now well recognized (Brain and Norris, 1965). The occurrence of peripheral neuropathy, proximal myopathy, or cerebellar ataxia in a middle-aged or elderly patient in the absence of known metabolic or toxic cause has a reasonable possibility of being secondary to an occult neoplasm. The modern era of characterization of these syndromes began with the report by Denny-Brown (1948) of two cases of sensory neuropathy associated with carcinoma of the lung. The largest clinical experience with these syndromes has been reported from the London Hospital (Brain and Henson, 1958; Croft and Wilkinson, 1965, 1969; Brain and Wilkinson, 1965). These authors have suggested an overall incidence of neuromuscular disease associated with carcinoma of 6% with the highest incidence at 16% in males with carcinoma of the lung (Croft and Wilkinson, 1965). This high incidence of non-metastatic complications provides the clinical investigator with a situation which lends itself easily to prospective study.

A possible role for the immune system in the pathogenesis of the carcinomatous neuropathies

was mentioned by Brain and Henson (1958) and developed further by Prehn (1965), and later by Burnet (1969). Impetus was given to this idea by Wilkinson (1964) and Wilkinson and Zeromska (1965), who found serum antibrain antibodies in four cases of sensory neuropathy associated with carcinoma. Interestingly enough, no reaction against peripheral nerve extracts could be found. Field and Caspary (1970) have recently found cellular sensitivity to central nervous system (CNS) basic protein in many patients with neoplastic disease, supporting the suggestion that there may be a sharing of antigenic sites by CNS basic protein and malignant tumour proteins.

In this study of patients with early carcinoma of the lung, we have performed both electrophysiological and immunological studies. Details of the clinical and electrophysiological findings are reported by Campbell and Paty (1974). The immunological studies reported here consist of assay of macrophage inhibition factor (MIF) (Rocklin *et al.*, 1970) in the presence of a peripheral nerve extract, as an indicator of cellular sensitivity to peripheral nerve antigens.

METHODS

PATIENTS The following four groups of patients have been studied: (1) 30 otherwise unselected

¹ Present address: University Hospital, London, Ontario, Canada.

² Present address: Frenchay Hospital, Bristol, BS16 1LE.

patients with early lung cancer in whom neurological disease had not been suspected (lung cancer group); (2) 11 patients with known cancer and well-recognized neurological complications who had been referred for a neurological opinion because of the latter (carcinomatous neuropathy group); (3) 13 patients with a chronic non-specific neuropathy but without evidence of neoplastic disease (non-cancer group); and (4) 15 subjects without cancer or peripheral nervous system disease who served as a control group.

The lung cancer group was formed from routine admissions for bronchoscopy to the chest medicine wards of two hospitals over a six month period. At the time of the clinical, electrophysiological (EMG), and immunological studies, the final diagnosis was not known. Patients had not been on medication known to produce EMG or immunological changes, and had blood taken for lymphocyte culture before bronchoscopy or other surgical procedures. Some patients have been seen subsequently in follow-up assessments, and the studies were repeated at three and six monthly intervals (Table 1). Patients with carcinomatous neuropathy and with non-carcinomatous chronic neuropathy were studied in the same way as the prospective group of lung cancer patients (Table 2, A and B). The non-cancer group included eight patients with chronic sensorimotor polyneuropathy of unknown cause. Control patients were selected from neurology wards, and included patients with minimal structural disease limited to the central nervous system (Table 3). Care was taken throughout to exclude subjects who had been given any drugs, such as phenothiazines (Knowles *et al.*, 1970) or corticosteroids (McIntyre, 1970) known to inhibit lymphocyte function.

ANTIGENS Human peripheral nerve extract (PN) was prepared after the method of Waksman and Adams (1955) and Rocklin *et al.* (1971). It consisted of an initial homogenization of 33% w/v fresh normal human peripheral nerve in normal saline. Particulate matter was removed by centrifugation ($1,000 \times 15$ min). The supernatant was then diluted 1:100 in normal saline, filtered in a bacteriological glass filter; aliquots were prepared and stored frozen at -20°C until used.

LYMPHOCYTE TISSUE CULTURE AND MACROPHAGE INHIBITION FACTOR (MIF) ASSAY This was performed in an adaptation of the method of Rocklin *et al.* (1970) after the original work of Thor *et al.* (1968). Forty millilitres of blood were defibrinated in siliconed universal bottles using 2–3 mm diameter glass beads. The lymphocytes were separated following the method of Coulson and Chalmers (1967). The defibrinated blood was mixed with one-third of its

volume of 0.5% methyl cellulose and 125 mg carbonyl iron powder (G.A.F. (G.B.) Ltd., Manchester, England) for 40 minutes. Sedimentation was allowed to proceed at 37°C in siliconed vessels for 20 to 40 minutes. The lymphocyte-rich supernatant was taken off, centrifuged (250×10 min), and the cell pellet washed three times in 10 ml. TC199 (Gibco, Long Island, U.S.A.).

Cultures of 3×10^6 lymphocytes in 1 ml. TC199 (including 200,000 u/l. penicillin and 100,000 $\mu\text{g/l}$. streptomycin) were incubated at 35°C in conical tightly-stoppered centrifuge tubes (M.S.E., London). The medium overlaying the cell pellet was changed and collected daily for three days with the old medium being stored at 4°C and pooled on completion of the collection. The pooled supernatant was usually collected from multiple cultures containing a total of $9\text{--}15 \times 10^5$ lymphocytes in each set of control and experimental replicate cultures.

The experimental cultures were exposed to the saline extract of human peripheral nerve (see section on antigens) at a concentration of 1% in TC199 alone without serum supplement. Control cultures were set up in TC199 without antigen, and, after collecting and pooling, the supernatants were reconstituted to 1% with the same peripheral nerve extract.

The pooled supernatants were centrifuged ($1,000 \times 10$ min) to remove particular matter and placed in dialysis tubing that had been well boiled to eliminate chemical toxicity. Dialysis was carried out in the cold successively against 60 volumes of 0.15 M sodium chloride, 60 volumes of glass distilled water, and another 30 volumes of glass distilled water, each for 24 hours. Control and experimental dialysis bags were always dialysed in the same vessel. The contents of the dialysis bags were then freeze dried and the dry residue stored at -20°C . Within three days the freeze dried material was quantitatively dissolved in 2.3 ml. TC199 with 10% inactivated guinea-pig serum and 5% foetal calf serum. These were then designated as 'NIL' for the control-reconstituted medium and 'PN' for the experimental medium. Normal culture medium (Absolute control) consisted of TC199 with 10% inactivated guinea pig serum and 5% foetal calf serum.

Normal guinea pig macrophages were harvested from the peritoneum five to seven days after intraperitoneal injection of 20 ml. liquid paraffin (B.P.), and were washed three times in TC199. Following the method of Hughes (1972), these macrophages were loaded as a concentrated suspension ($60 \times 10^6/\text{ml.}$) into 5 cm lengths of polyethylene capillary tubing (Portex PP30), one end heat sealed, and centrifuged (550×10 min). The tubing was cut at the cell/fluid interface and six capillaries mounted radially on a

TABLE 1
LUNG CANCER STUDY

Patient	Age (yr)	Sex	Histology	Lympho- cyte trans- formation to PPD IR*	MIF assay (% migration)				Electro- physio- logical findings†	
					Nil cf Abs.†	PN cf Abs.‡	PN cf Nil§	P value		
1	R.R.	49	M	Oat cell	× 18.0	67.3	67.9	100.8	—	C + P
2	W.T.	64	M	Oat cell		59.8	54.4	91.2	0.025-0.02	C + P
3a	T.T.	49	M	Squamous	× 12.7	100.9	96.8	96.0	—	N
3b	T.T.	49	M	Squamous (post-lobectomy)		92.6	66.5	71.7	< 0.001	C
4	G.B.	63	M	Undifferentiated		96.2	79.6	82.7	< 0.001	C
5	N.F.	47	M	Squamous						C
6a	J.M.	64	M	No biopsy	× 23.4	97.4	49.8	51.1	< 0.001	C
6b	J.M.	64	M	No biopsy (post-DXR)		99.9	88.5	88.5	< 0.001	C
7	D.R.	48	M	Undifferentiated						C
8a	W.S.	61	M	Squamous	× 26.6	100.9	98.7	97.9	—	C
8b	W.S.	61	M	Squamous (post-lobectomy)		86.6	81.3	93.9	0.05-0.025	C
9	J.T.	44	M	Squamous	× 37.7	99.2	102.8	103.5	—	C
10a	G.Mc.	60	M	No biopsy	× 9.0	101.9	79.3	77.8	< 0.001	C
10b	G.Mc.	60	M	No biopsy		86.6	84.2	97.2	—	C
11a	T.J.	64	M	Unproven		88.4	69.2	78.1	< 0.001	C
11b	T.J.	64	M	Unproven		82.3	82.9	100.7	—	C
12	J.A.	62	M	Squamous	< 1	95.4	89.2	93.5	0.005-0.001	M
13	M.E.	69	M	Squamous	< 1	96.6	75.2	77.9	< 0.001	M
14	F.M.	69	M	No biopsy	× 11.4	80.9	83.2	102.8		M
15	O.Mc.	42	F	Undifferentiated	× 19.3					M
16	H.R.	49	M	Squamous		104.1	75.2	72.2	< 0.001	M + LE syn
17	S.B.	66	M	Squamous		85.9	82.6	96.1	—	D + P
18	F.F.	70	M	Adenocarcinoma		83.5	81.9	98.3	—	D + P
19	J.H.	52	F	Squamous		88.7	84.7	95.5	—	D
20	J.P.	66	M	Squamous		98.2	103.9	105.7	—	D
21	L.T.	63	M	Undifferentiated	× 7.8	92.4	86.6	93.6	—	D
22	G.C.	48	F	Oat cell		98.5	90.2	91.6	< 0.001	N
23	A.F.	49	M	Undifferentiated		82.5	84.5	102.0	—	N
24	W.N.	57	M	Oat cell	× 16.1	88.0	95.5	108.4	—	N
25	C.S.	67	F	Undifferentiated		87.0	87.5	100.5	—	N
26	T.W.	52	M	No biopsy	× 12.0	94.3	87.6	92.9	0.02	N
27	W.C.	74	M	Oat cell	< 1	99.1	98.8	99.7	—	—
28	W.J.	50	M	No biopsy		86.8	88.9	102.3	—	—
29	M.N.	43	F	Oat cell	× 12.0	85.4	74.5	87.2	0.005-0.001	—
30	M.P.	57	F	Undiagnosed haemothorax	× 8.5	89.4	82.6	92.4	0.02-0.01	—

* IR: Index of response to 5 µg/ml. PPD in lymphocyte transformation: = $\frac{\text{cpm in stimulated cultures}}{\text{cpm in unstimulated cultures}}$

† NIL cf Abs = $\frac{\text{Migration in NIL medium reconstituted with PN antigen}}{\text{Migration in Absolute control medium}} \times 100.$

‡ PN cf Abs = $\frac{\text{Migration in PN medium}}{\text{Migration in Absolute control medium}} \times 100.$

§ PN cf NIL = $\frac{\text{Migration in PN medium}}{\text{Migration in NIL medium + PN antigen}} \times 100.$

¶ Electrophysiological findings: C = combined myopathic and denervation changes, P = slowed nerve conduction; N = normal findings; M = myopathic changes; LE syn = myasthenic (Lambert-Eaton) syndrome; D = chronic denervation changes.

cover slip in a 1 ml. volume Sykes-Moore (modified) tissue culture chamber. The chambers were sealed and at least two chambers (12 capillaries) were filled with each 'NIL', 'PN', or absolute control culture medium (preparation described above). After 18 hours' incubation at 35° C, the migration area was traced using a camera lucida and the relative migration areas assayed by the weight of the traced area.

Results of the macrophage migration inhibition factor (MIF) assay are expressed thus:

% migration in the presence of PN culture medium = $\frac{\text{mean migration area in PN medium}}{\text{mean migration area in Absolute control medium}} \times 100.$

% migration in the presence of NIL culture

medium (Control)=(mean migration area in NIL medium/mean migration area in Absolute control medium) × 100.

% migration due to lymphocyte sensitivity to peripheral nerve antigens (PN cf NIL)=(mean migration area in PN medium/mean migration area in NIL medium) × 100.

The significance of these individual migration values, and also that of the patient groupings, has been estimated by Student's *t* test using the 2P values.

LYMPHOCYTE TRANSFORMATION STUDIES The whole blood lymphocyte transformation method of Paty and Hughes (1972) was utilized to provide a positive

control in 16 of the lung cancer patients. The antigen used was tuberculin purified protein derivative (PPD) at a final concentration of 5 µg/ml. The technique consisted of culturing blood cells washed four times with TC199, together with normal human group AB serum and the appropriate antigen in TC199 culture medium (stimulated cultures). The antigen was omitted in control (unstimulated) cultures. After five days incubation at 37° C the cultures were pulsed with tritiated-thymidine (1 µC/ml.) for four hours and the cells were then harvested. The lymphocyte radioactivity was measured, after red cell lysis with 3% acetic acid, trichloroacetic acid precipitation, and Hyamine solubilization in a Tri-carb liquid scintillation spectrometer (Packard

TABLE 2
PATIENTS WITH CHRONIC NEUROMUSCULAR DISEASE

Patient	Age (yr)	Sex	Clinical diagnosis	% Migration				Electro-physio-logical findings§	
				Nil cf Abs*	PN cf Abs†	PN cf Nil‡	P value		
A. Carcinomatous neuropathy group									
31	J.R.	70	M	Adenocarcinoma colon	79.3	71.9	90.6	0.005-0.001	P
32	W.R.	56	M	Undifferentiated carcinoma	69.2	59.6	86.2	< 0.001	P
33	M.B.	70	F	Carcinoma breast	78.0	69.7	88.5	< 0.001	D
34	G.H.	62	M	Adenocarcinoma lung	80.9	85.2	105.2	—	C + P
35a	M.C.	68	F	Carcinoma lung (no biopsy)	86.1	73.9	85.6	< 0.001	C
35b	M.C.	68	F	Carcinoma lung (no biopsy)	80.7	62.9	77.9	< 0.001	C
36	E.K.	41	F	Carcinoma stomach (undifferentiated)	98.0	89.5	91.3	0.05	M
37	D.H.	52	M	Carcinoma lung (undifferentiated)	110.5	105.9	95.9	—	M + LE syn
38	S.H.	55	M	Carcinoma lung (undifferentiated)— Radiation myelitis	82.8	89.1	107.0	—	D
39	J.L.	66	M	Carcinoma lung					C
40	J.R.	55	M	? Carcinoma lung					M + LE syn
41	I.W.	56	F	Carcinoma lung (oat cell)					M + LE syn
B. Non-cancer group									
42	R.K.	69	M	Idiopathic polyneuritis	90.5	79.4	87.9	< 0.001	P
43	A.B.	63	M	Idiopathic polyneuritis	59.9	42.2	70.5	< 0.001	P
44	C.W.	68	M	Idiopathic polyneuritis	64.3	50.4	78.4	< 0.001	P
45	N.T.	56	M	Idiopathic polyneuritis	87.6	71.2	81.2	< 0.001	?P
46	J.E.	68	M	Idiopathic polyneuritis	100.5	89.5	88.9	< 0.001	P
47	M.E.	67	F	Idiopathic polyneuritis	87.7	71.4	81.5	< 0.001	P
48	R.R.	68	M	Idiopathic polyneuritis	88.8	74.2	83.5	0.005-0.001	P
49	G.R.	58	M	Idiopathic polyneuritis	87.7	84.7	96.5	—	P
50	C.Ph.	63	M	Vitamin B12 deficiency	76.3	66.2	86.7	0.005-0.001	C
51	S.P.	60	F	Bilateral carpal tunnel syndrome					
				?myxoedema	89.5	83.0	92.8	—	D
52	W.W.	59	M	Tabes dorsalis	94.1	92.8	98.5	—	D
53	L.P.	14	F	Peroneal muscular atrophy (axonal type)	90.8	100.9	111.2	—	D
54	J.B.	67	F	Cerebellar degeneration and myopathy	94.5	69.8	73.9	< 0.001	M

* NIL cf Abs = $\frac{\text{Migration in NIL medium reconstituted with PN antigen}}{\text{Migration in Absolute control medium}} \times 100.$

† PN cf Abs = $\frac{\text{Migration in PN medium}}{\text{Migration in Absolute control medium}} \times 100.$

‡ PN cf NIL = $\frac{\text{Migration in PN medium}}{\text{Migration in NIL medium + PN antigen}} \times 100.$

§ Electrophysiological findings: C=combined myopathic and denervation changes; P=slowed nerve conduction; M=myopathic changes; LE syn=myasthenic (Lambert-Eaton) syndrome; D=chronic denervation changes.

Instruments). Results are expressed as counts per minute (cpm) per 100,000 lymphocytes in each culture tube. An index of response to each antigen can be obtained thus:

$$IR = \frac{\text{cpm stimulated cultures}}{\text{cpm unstimulated cultures}}$$

RESULTS

LUNG CANCER GROUP Table 1 gives the clinical details and immunological findings in the 30 patients with carcinoma of the lung. Lung cancer had been satisfactorily proven in 28 patients, and a histological diagnosis was obtained in 23 cases. Only two patients presented with symptoms of neuromuscular disease (NMD), but proximal limb weakness was found in 16 patients, and was severe in seven. The electrophysiological studies in 26 patients are summarized in the Table and are given more fully by Campbell and Paty (1973). These demonstrated a high incidence of subclinical neuromuscular disease. With full electrophysiological studies, only five patients could be considered to be entirely normal.

Thirty-two MIF assays were performed on 27 patients of the lung cancer group (Table 1). In 12 studies significant inhibition of migration was

found with the supernatants from unstimulated cell cultures (NIL) compared with Absolute controls (see Discussion section). This had considerable influence on the migration results with supernatants from cultures exposed to peripheral nerve antigen (PN). The specific *in vitro* response due to the latter was best seen in direct comparison with the NIL findings. These results are shown in the third column from the right of Table 1 together with their levels of significance. Significant inhibition of migration ($P < 0.005$) due to cellular sensitivity to the peripheral nerve extract was found in 11 studies of the lung cancer group, including nine patients (36%) with proven cancer. A lesser degree of cellular sensitivity ($P < 0.05$) was shown in a further four patients, but the percentage migration overlapped that found in control subjects. The sensitivity corresponded roughly with the severity of subclinical neuromuscular disease, particularly with neuromyopathy where eight of the 16 patients gave a significant response. Of some interest was the absence of cellular sensitivity in five patients with chronic denervation alone, even when severe and associated with peripheral neuropathy in two cases. One patient, who had both normal EMG and MIF assay

TABLE 3
CONTROL PATIENTS

Patient	Age (yr)	Sex	Clinical diagnosis	% Migration			
				Nil cf Abs*	PN cf Abs†	PN cf Nil‡	P value
55 W.B.	47	M	Pulmonary inflammation	67.0	69.9	104.5	—
56 W.D.	50	M	Cervical spondylosis	91.1	97.6	107.8	—
57 J.J.	41	M	Depression	98.9	95.6	96.7	—
58 J.C.	66	M	Pulmonary inflammation	93.1	93.4	100.3	—
59 J.W.L.	38	M	Lumbar disc lesion	81.6	81.3	99.5	—
60 O.M.	52	F	Spontaneous hypoglycaemia	86.8	85.6	98.5	—
61 P.B.	36	F	Epilepsy ?hypoglycaemia	66.3	63.1	95.2	0.4
62 E.P.	57	F	Mild CVA	90.0	82.8	92.0	0.1-0.05
63 P.F.	24	M	Subarachnoid haemorrhage	82.8	90.3	109.1	—
64 J.W.	44	F	Labyrinthitis	112.2	101.9	90.8	0.1
65 D.J.	74	F	Depression	91.6	97.1	105.9	—
66 E.G.	73	F	Orbital pain	111.7	116.4	104.2	—
67 D.P.	52	F	Amaurosis fugax	101.7	108.5	106.6	—
68 A.A.	67	F	Pulmonary inflammation	82.8	83.1	100.4	—
69 K.C.	24	M	Migraine	100.8	98.7	97.9	—

* NIL cf Abs = $\frac{\text{Migration in NIL medium reconstituted with PN antigen}}{\text{Migration in Absolute control medium}} \times 100.$

† PN cf Abs = $\frac{\text{Migration in PN medium}}{\text{Migration in Absolute control medium}} \times 100.$

‡ PN cf NIL = $\frac{\text{Migration in PN medium}}{\text{Migration in NIL medium + PN antigen}} \times 100.$

FIGURE Percent migration of guinea-pig macrophages as an assay for MIF production by human lymphocytes after exposure to a 1% suspension of a human peripheral nerve extract (PN). Basic medium is TC-199 with 15% inactivated guinea-pig serum and 5% foetal calf serum (see text for details).

initial evaluation, developed electrophysiological changes of a neuromyopathy and a positive MIF assay (71.7% migration) three months later (Figure). When those patients with lung cancer but without clinical or electrophysiological evidence of neuromuscular disease were considered separately (patients 3a, 22-30), only two out of 10 had significant evidence of cellular sensitivity ($P < 0.005$) to the peripheral nerve antigen. Moreover, the mean migration (97.3%) for this sub-group was not significantly different from 15 control patients.

Sixteen of the lung cancer patients had parallel testing with lymphocyte transformation, looking for responsiveness to PPD. Thirteen of the 16 responded to the PPD antigen, with at least a seven-fold increase in thymidine uptake as compared with control cultures (Table 1), as evidence that the most of these cancer patients had at least some cellular immune responsiveness.

CARCINOMATOUS NEUROPATHY GROUP Eight of the 11 patients with known carcinoma and

secondary neuromuscular syndromes were tested with the MIF assay (Table 2, A). Evidence of significant cellular sensitivity to the peripheral nerve antigen (PN cf NIL) was found in four patients (50%), but this did not correlate with the clinical or electrophysiological type of neurological disease. Negative results were obtained in a patient with subacute severe neuromyopathy, and in another with a myopathic-myasthenic (Lambert-Eaton) syndrome.

NON-CANCER GROUP Cellular sensitivity to the PN antigen (PN cf NIL) was found in nine of the 13 patients in this group with chronic neuropathic disease (Table 2, B). Seven of the eight patients with idiopathic sensorimotor polyneuropathy showed a significant response. The negative results included a girl with long-standing severe peroneal muscular atrophy of axonal type.

CONTROL SUBJECTS In only one of the 15 control subjects (Table 3), a man with a mild cere-

TABLE 4
MACROPHAGE MIGRATION INHIBITION TEST: SUMMARY OF GROUPED DATA

	% Migration ($\pm$ standard deviation)		
	Nil of absolute control	PN antigen of Absolute	PN antigen of NIL
A Controls (N=15)	90.56 $\pm$ 13.58	92.96 $\pm$ 13.36	100.60 $\pm$ 5.59
B Lung cancer (N=32)	90.52 $\pm$ 9.72	82.95 $\pm$ 12.34	91.99 $\pm$ 12.11
C Lung cancer without neuromuscular disease (NMD) (N=10)	91.19 $\pm$ 6.49	88.69 $\pm$ 7.27	97.30 $\pm$ 6.38
D Cancer NMD (N=9)	85.06 $\pm$ 12.21	78.63 $\pm$ 14.88	92.02 $\pm$ 9.37
E NMD alone (N=13)	85.55 $\pm$ 11.77	75.05 $\pm$ 16.30	87.04 $\pm$ 10.96
Comparisons	Student's t test (2P values)		
A to B	Not significant (NS)	0.02-0.01	0.02-0.01
A to C	NS	NS	NS
A to D	NS	0.025-0.02	0.005-0.001
A to E	NS	0.005-0.001	<0.001
C to D	NS	0.1-0.05	NS
C to E	NS	0.025-0.02	0.02-0.01
D to E	NS	NS	NS

bral infarction, was there any indication of cellular sensitivity to the PN antigen ($P=0.1-0.05$). The lowest migration obtained after exposure of cells to the PN extract in the cultures, compared with NIL, was 90.8% ($P=0.1$). It was noted, however, that significant MIF was obtained from unstimulated cells (NIL) compared with Absolute control cultures in nine of the 15 control subjects.

The results of all the MIF assays of supernatants obtained from lymphocyte cultures after exposure to peripheral nerve antigen are shown in Figure. The lung cancer patients without neuromuscular disease are shown as a separate subgroup alongside the control patients. From this, the cellular sensitivity to peripheral nerve antigen can be seen to be clearly related to the presence of neuromuscular disease, with the maximum inhibition occurring in those patients with longstanding neurological disease alone.

A statistical analysis of these results is shown in Table 4. The ten lung cancer patients without clinical or electrophysiological evidence of neuromuscular disease (3a, 22-30) are considered separately (group C). As mentioned earlier, a degree of inhibition with supernatants obtained from unstimulated cell cultures (NIL) compared with culture medium alone (Absolute control) was found in all groups, including control patients (Table 4, column 1). However, no significant difference was found between the groups,

although a 5% greater inhibition was present in those patients with neuromuscular disease. Mean macrophage migration in the presence of supernatants from stimulated lymphocytes (PN Absolute, column 2) was depressed in all experimental groups but significantly so only in those patients with chronic neuromuscular disease alone (group E, $P=0.005-0.001$). These results were clearly influenced by the non-specific inhibitory effect of unstimulated cell culture (NIL), and the specific *in vitro* cellular response to peripheral nerve antigen was best seen in individual comparisons of these migrations (PN of NIL).

These results (column 3) revealed that in comparison with the control group (A: mean migration 100.6%) the mean migration of the group of lung cancer patients (B: 92.0%) just failed to achieve statistical significance ($P=0.02-0.01$), while that of the lung cancer sub-group without neuromuscular disease (C: 97.3%) was clearly not significantly different. Significant inhibition compared with controls was found in the two groups with established neuromuscular disease, both with cancer (D: 92.0%; $P=0.005-0.001$) and without neoplastic disease (E: 87.0%; $P<0.001$). However, no significant difference was found between these two groups. Similarly, no significant difference was found between the groups of cancer patients either with (D: 92.0%) or without (C: 97.3%) neuromuscular disease.

The largest inhibitions were obtained from the non-cancer patients with chronic neuromuscular disease alone (E: 87.0%), but these did not quite achieve a significant difference ($P=0.02-0.01$) when compared with the lung cancer group without neuromuscular disease (C: 97.3%), possibly because of the small numbers involved.

DISCUSSION

The MIF assay results show clearly that most patients with carcinomatous neuromuscular disease, and also patients with chronic non-specific neuropathy, have detectable lymphocyte sensitivity to the peripheral nerve (PN) extract. Nine (36%) of the 25 patients studied with proven carcinoma of the lung also had significant cellular sensitivity to the PN antigen. This cellular sensitivity could not be strictly correlated with the presence or absence of subclinical or electrophysiological signs of neuromuscular disease in the lung cancer group. Although eight of the 16 patients with a neuromyopathic picture had significant sensitivity, positive MIF assays were found in two cancer patients without neuromuscular disease.

The evidence of cellular sensitivity to PN antigens in nine of 25 (36%) patients with carcinoma of the lung and 12 of 21 (62%) patients with chronic neuromuscular disease, may or may not have pathogenic significance. There were many lung cancer patients (Figure) with subclinical electrophysiological evidence for a loss of motor neurones or muscle denervation in whom cellular sensitivity to the PN extract was not detected. Conversely, there were two cancer patients who were normal clinically and electrophysiologically but in whom lymphocyte sensitivity was found.

Recent studies by Rocklin *et al.* (1971) and Caspary and Field (1970) have suggested that cellular sensitivity to central nervous system (CNS) antigens may be only a secondary phenomenon following the release of sequestered antigens from damaged neural tissues. The presence, in this study, of cellular sensitivity in most cases of established neuropathy is in agreement with this suggestion. Of the patients studied with neuropathy of unknown aetiology, a certain percentage could be expected to be associated with occult neoplasia, but it would be indeed

fortuitous if all these cases of obscure aetiology had an occult neoplasm and/or an immune pathogenesis.

In contrast with this view of predominant secondary sensitization are the suggestions of Prehn (1965) and Burnet (1969). Immunological studies of experimental tumours and tumour-bearing patients have revealed that many subjects with neoplastic disease have detectable sensitivity to either tumour antigens (Gold, 1970) or to CNS basic protein (Field and Caspary, 1970). This lends support to the theory of Prehn (1965) that some tumours might share antigenic sites with nervous system proteins. Hence, development of sensitivity to the tumour antigens could result in a cross-reaction with nervous tissue proteins and subsequent immunologically mediated damage to nervous tissues.

If such a primary immunological mechanism were operative in the non-metastatic carcinomatous neuromyopathies, then one would expect the early appearance of cellular sensitivity to peripheral nerve antigens, and particularly in a significant proportion of cancer patients without neuromuscular disease. Thirty-six per cent of the early lung cancer patients did show cellular sensitivity in the MIF test, but this was not quite significant in the group as a whole ($P=0.2-0.1$) and was clearly insignificant in the sub-group with lung cancer and no neuromuscular involvement, although sensitivity was present in two individual patients. Patients with established neural damage did show cellular sensitivity, but no significant difference was found between the groups with and without associated cancer. This lends no support for a primary immunological pathogenesis in carcinomatous neuromyopathy.

We would like to comment on the detection of cellular sensitivity by the MIF assay. Rocklin and co-workers (1971), in their report of a study of MIF production to peripheral nerve extract in a variety of peripheral nerve and central nervous tissue diseases, found significant cellular responses in only four of 25 patients with peripheral neuropathy, all of whom had persisting damage from the Guillain-Barré syndrome, a predominantly demyelinating disorder affecting peripheral nerves. They also found cellular sensitivity to peripheral nerve extract in five of 34 patients with central nervous system (CNS) disease, in two following cerebrovascular acci-

dents and in three with multiple sclerosis. Five patients with amyotrophic lateral sclerosis (motor neurone disease) did not respond to either peripheral nerve or CNS (basic protein) antigens. In this study we have found a much higher incidence of cellular sensitivity in patients with chronic neuromuscular disease, none of whom had the Guillain-Barré syndrome, and only three of whom had clear electrophysiological evidence of demyelinating neuropathy (Campbell and Paty, 1974). Evidence is discussed elsewhere (Campbell and Paty, 1974) suggesting that the majority of these cancer patients have a primary neuronal disorder, possibly within the central nervous system.

The high incidence of MIF production by unstimulated lymphocytes (NIL) also merits discussion. Such an inhibitory effect might be due to the toxic effect of the antigen which is added as a control after incubation, or alternatively it might represent a range of non-specific factors, including treatment and drugs. It is possible, however, that in certain cases of neuromuscular disease, the lymphocytes have already been stimulated *in vivo* to produce MIF, and that further *in vitro* stimulation during incubation will produce little significant increase in production. This effect would be present in the NIL cultures and mask that in the final analysis (PN cf NIL). Such an explanation might account for the failure to detect cellular sensitivity in certain severe neuromuscular diseases cases, where a high production of MIF was already present in the NIL cultures—for example, 1, 2, 17, 18, and 34). However, when all the groups are compared, including controls, there was no significant difference in the mean MIF production with unstimulated (NIL) cultures.

SUMMARY

Immunological studies were performed on four groups of patients: (1) 30 patients with early lung cancer; (2) 11 patients with established carcinomatous neuromuscular disease; (3) 13 patients with non-specific neuropathy; and (4) 15 control patients.

The immunological method used was peripheral blood lymphocyte culture utilizing an assay of macrophage inhibition factor (MIF) with a 0.33% saline extract of human peripheral

nerve (PN) as antigen. A significant degree of cellular sensitivity to the PN extract was found in 36% of patients with proven lung cancer ($P < 0.005$), but no direct correlation was found between the electrophysiological findings of associated sub-clinical neuromuscular disease and this cellular sensitivity. In the patients with established neuromuscular disease, 62% showed significant cellular sensitivity, with no significant difference in sensitivity between the groups with or without associated neoplasia. No significant cellular sensitivity was found in a group of 10 cancer patients without neuromuscular disease, although two individual subjects gave a positive response. Of 15 control subjects, a slight degree of sensitivity ($P = 0.1-0.05$) was detected in only one patient who had a minimal recent stroke.

These results are interpreted as showing no evidence for a primary immunological mechanism in the non-metastatic carcinomatous neuromuscular syndromes. The demonstrable cellular sensitivity probably arises as a result of a secondary reaction to previously sequestered neural antigens.

We would like to thank Mr. H. K. Cousin for his excellent technical assistance. We are also much in debt to the physicians acknowledged in the companion paper who allowed us to see their patients. One of us (D.W.P.) was in receipt of a travelling fellowship from the University of Western Ontario, London, Canada, another (M.J.C.) was supported by the Medical Research Council.

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