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T Cell Differentiation Antigens and Antigenic Lymphocyte Reactivity in Pleural Effusions

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SUMMARY Blood and pleural effusion mononuclear cells from thirteen patients were examined for the expression of T lymphocyte differentiation antigens as well as *in vitro* thymidine incorporation. The ratio of T_4 to T_8 cells was significantly greater among pleural effusion lymphocytes than among blood lymphocytes. Effusion lymphocyte responses to phytohaemagglutinin were less than those of blood lymphocytes. Unstimulated thymidine incorporation was greater in pleural effusion lymphocytes. Antigen-stimulated lymphocyte reactivity was not consistently greater in either blood or effusion lymphocytes. Lymphocytes from tuberculous effusions all reacted to tuberculin. Pleural effusion lymphocytes, regardless of the etiology of the effusion, possessed the same range of antigenic specificities as did blood lymphocytes. Therefore, effusion lymphocyte responsiveness to tuberculin does not prove the presence of tuberculous pleurisy but does indicate sensitisation to tuberculin.

It is known that the cellular immune response may be localized near the site of the antigenic stimulus. Tuberculous pleurisy in man represents an example of such a localised response. While tuberculous pleural effusions have been previously studied and shown to possess lymphocytes which respond to tuberculin with either thymidine incorporation[1-5] or lymphokine production,[5-8] it is not known whether pleural effusions contain lymphocytes which react to other antigens to which the patient has been sensitised. Previous reports reveal that tuberculin-induced reactivity of tuberculous effusion lymphocytes is usually greater than such reactivity in peripheral blood lymphocytes.[1,4,5] The relationship of antigen-induced effusion lymphocyte reactivity to that of blood lymphocytes in patients with non-tuberculous effusions has not been well studied. It is not known whether *in vitro* tuberculin-induced effusion lymphocyte responses can be used diagnostically. Finally, while it is known that pleural effusion lymphocytes are often predominantly T cells,[6,9-13] the ratio of helper to suppressor cells, as determined by surface differentiation antigens in effusions has been previously studied in only three patients.[4,14] Work is presented which addresses these unresolved questions.

MATERIALS AND METHODS

A. Subjects

Thirteen male United States veterans, mean age = 50 ± 17 (s.d.) years, with pleural effusions were studied (Table 1). All had received tetanus toxoid immunisation during their military service. Subjects included one patient with proven tuberculous pleurisy, two with probable tuberculous pleurisy, four with congestive heart failure, one of whom had an IgA myeloma, another of whom also suffered with uraemia, three with malignancy, one with bacterial endocarditis, one with pulmonary emboli and one with an effusion of undetermined etiology. Informed consent was obtained from each subject.

B. Skin tests

Delayed hypersensitivity skin tests were performed by the intradermal injection of 0.1 ml of each test antigen and the subsequent measurement of the diameters of erythema and induration at 24 and 48 hours. Antigens included tuberculin purified protein derivative (PPD) (5 tuberculin units) (Aplisol,

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Table 1 Characteristics of subjects

Subject	Age (yrs)	Diagnosis or clinical characteristics
JA	55	squamous cell carcinoma of lung
JB	49	right upper lobe infiltrate and cavity with non-caseating granuloma on biopsy; negative cultures for ***M. tuberculosis;*** clinical response to antituberculous chemotherapy.
HCl	85	congestive heart failure; IgA myeloma
HCt	63	poorly differentiated adenocarcinoma of prostate with metastases
RH	36	staphylococcal endocarditis, intravenous drug abuse; PPD converter who received chemoprophylaxis with isoniazid for 9 months prior to occurrence of eosinophilic pleural effusion: patient is now well without additional antituberculous treatment.
NH	57	congestive heart failure
JJ	62	squamous cell carcinoma of lung
AL	56	left pleural effusion with weakness, fever, chills, and night sweats; negative cultures for ***M. tuberculosis;*** complete resolution of all signs and symptoms following treatment with isoniazid and rifampin for 18 months. Patient is now well.
HM	30	congestive heart failure, uraemia
AP	57	congestive heart failure
RW	46	pulmonary tuberculosis, diabetes mellitis
CW	28	pulmonary emboli, intravenous drug abuse
FW	24	two week history suggestive of an acute infectious process; no diagnosis made

Parke Davis, Detroit, MI), candidal antigen 1:100 (w/v) and trichophytin 1:100 (w/v) (both from Veterans Administration Allergy Laboratory, Pittsburgh, PA), mumps skin test antigen USP (Eli Lilly and Company, Indianapolis, IN), and fluid tetanus toxoid (0.2 Loeffler units/0.1 ml) (Lederle Laboratories, Pearl River, NY).

C. Lymphocyte thymidine incorporation of pleural fluid and peripheral blood lymphocytes

Antigenic and mitogenic reactivities were determined with the lymphocyte transformation assay using a modification of the method of Oppenheim and Schecter[15] as previously described.[16] For each subject, all assays were performed simultaneously. A lymphocyte-enriched leucocyte suspension was obtained from 50 ml of preservative-free heparinised blood by Ficoll-Hypaque density-gradient centrifugation. Pleural effusion leucocytes were pelleted by centrifugation, washed in Hanks' balanced salt solution, and then sedimented on a Ficoll-Hypaque density-gradient. The lymphocyte-enriched leucocyte suspensions were adjusted to 5×10^5 viable mononuclear cells/ml with RPMI 1640 medium (100 U/ml penicillin, 100 μg/ml streptomycin and 25 mM glutamine) (Gibco, Grand Island, NY) supplemented with 20% autologous plasma.

Aliquots (0.2 ml) containing 1×10^5 mononuclear cells were cultured in microtitre wells (Falcon Plastics, Oxnard, CA.) in quintuplicate. Mitogen-stimulated cultures were incubated with 50 μg/ml of phytohaemagglutinin-P (PHA) (Difco, Detroit, MI) or with 12.5 μg/ml of concanavalin A (Con A) (Sigma Chemical Co., St. Louis, MO). Antigen-stimulated cultures were incubated with 10 μl of antigen solutions. The following antigens were used: 1:100 (w/v) dilutions of dialysed candidal or trichophytin antigen; 1:2 (v/v) dilution of dialysed mumps skin test antigen USP. Additional antigen-stimulated cultures were incubated with tuberculin purified protein derivative (PPD) in final concentrations of 36 or 72 μg/ml; or fluid tetanus toxoid in a final concentration of 0.5 Loeffler u/ml. Negative control cultures contained neither antigen nor mitogen. Mitogen and antigen stimulated cultures were incubated for 3 and 5 days, respectively at 37° C in a 5% CO_2 humidified atmosphere and subsequently pulsed with 1 μCi of ^{3}H-methyl thymidine, (specific activity 20 Ci/mM; New England Nuclear Corp., Boston, MA). Cultures were then

incubated for an additional 6 hours and harvested with a multiple automated sample harvester (Otto Hiller, Madison, WI).

The scintillation fluid consisted of toluene, 2,5-diphenyloxazole (PPO) and *p*-bis-[2-(5-phenyloxazolyl)] benzene (POPOP). Cells were counted in a Packard Tricarb scintillation counter, Model 3255. Stimulation indices were derived by dividing the mean counts per minute of stimulated replicate cultures by the mean of counts obtained per minute with cultures containing no mitogen or antigen.

D. Identification of T lymphocyte differentiation antigens

Indirect immunofluorescence was used to detect cell surface expression of T_4 (helper) and T_8 (suppressor) differentiation antigens.[17] Mixed mononuclear cells from pleural effusions and peripheral blood of each subject were resuspended in Hanks' balanced salt solution with 2% fetal calf serum and 0.1% sodium azide at a final concentration of 10^6 cells per 1.0 ml. Cell suspensions were then incubated (O°C) with OKT_4 and OKT_8 (Ortho Diagnostic Systems, Raritan, NJ) murine monoclonal antibodies, followed by washing and incubation with FITC conjugated goat $F(ab)^2$ anti-mouse IgG (Tago, Burlingame, CA). After 2 additional washes, cells were fixed with 1% paraformaldehyde for 15 minutes (22°C),[18] washed and stored (4°C) for later flow cytometry analysis.[19]

E. flow cytometry

All flow analysis were performed with an Epics IV flow cytometer (Coulter Electronics) equipped with argon laser set at 488 nm, and interfaced with an Apple II Plus computer. The cytometer was standardised before each use for size (forward light scatter) and normalised for fluorescence intensity by commercially prepared microspheres (Coulter Electronics). All fluorescent profiles were based on a minimum of 10,000 cells analysed, and were collected with light scatter windows set to exclude all debris and cell clumps.[19]

F. Statistical analysis

Mean counts per minute ± standard error was determined for each set of quintuplicate cultures. Sets of quintuplicate cultures were compared using the independent t-test. The paired t-test was used to determine the significance of the differences between group means. The one sample t-test was used to determine whether the group mean percent change significantly differed from zero. The Chi square test and McNemar's test,[20] were used to evaluate the differences between proportions in mutually exclusive and non-mututally exclusive groups, respectively.

RESULTS

T lymphocyte differentiation antigens

Blood and pleural effusion lymphocytes from seven subjects were analysed by flow cytometry to determine the proportions of lymphocytes bearing the T_4 and T_8 differentiation antigens (Table 2). For the group it was found that T cells in the peripheral blood were comprised of $53 \pm 4\%$ T_4 and $47 \pm 4\%$ T_8 lymphocytes with a T_4:T_8 ratio of 1.25 ± 0.24 (s.e.). On the other hand, pleural effusion T cells were composed of $71 \pm 4\%$ T_4^+ and $29 \pm 4\%$ T_8^+ cells with a T_4:T_8 ratio of 2.83 ± 0.6 (s.e.). Pleural fluid lymphocytes as a group

Table 2 Differentiation antigens on peripheral blood and pleural effusion T lymphocytes. Values are expressed as percent of total T_4 and T_8 positive cells ($T_4 + T_8 = 100\%$).

Subject	Diagnosis#	Blood			Effusion		
		T_4	T_8	T_4/T_8	T_4	T_8	T_4/T_8
JA	CA	71	29	2.50	73	27	2.65
JJ	CA	59	41	1.43	71	29	2.4
RH	SBE	37	63	0.60	66	34	2.0
HM	CHF	54	46	1.19	49	51	1.0
AP	CHF	44	56	0.78	85	15	5.76
AL##	?TB	44	56	0.78	78	22	3.5
RW	TB	60	40	1.48	72	28	2.53
Mean ± s.e.		53 ± 4*	47 ± 4*	1.25 ± 0.24 (s.e.)	71 ± 4*	29 ± 4* (s.e.)	2.83 ± 0.6 (s.e.)**

*$p < 0.02$ independent t-test; $p < 0.05$ paired and one sample t-test, effusion T_4 vs blood T_4 and effusion T_8 vs blood T_8.
**$p < 0.05$ independent t-test, pleural effusion vs blood.
#CA = cancer; SBE = subacute bacterial endocarditis; TB = tuberculosis
##before treatment

contained significantly more helper cells and fewer suppressor cells than did peripheral blood lymphocytes. Those individuals (RH, AL, and AP) with a T_4:T_8 ratio of less than 1.2 in their peripheral blood did not show a similar finding in their pleural effusion lymphocytes. Thus, peripheral blood findings do not reflect the T_4:T_8 ratios among compartmentalised lymphocytes. One subject (HCt) not included in the analysis, whose differentiation antigen enumeration was performed manually in a clinical pathology laboratory, exhibited anomalous results with markedly decreased helper: suppressor ratios in the pleural effusion.

Mitogenic stimulation of pleural effusion and peripheral blood lymphocytes

Peripheral blood lymphocytes revealed greater PHA (50 μg/ml) induced reactivity than did pleural fluid lymphocytes in seven of ten subjects when counts per minute are compared, and in nine of ten, when the data are evaluated by stimulation indices (Table 3). The group results revealed that blood reactivity to PHA was 41 ± 15 (s.e.)% greater than that of effusions ($p < 0.05$, one sample t-test) (Table 3). Similarly, in five of six subjects, peripheral blood lymphocytes exhibited

Table 3 Phytohaemagglutinin (50 μg/ml)-induced reactivity in 3 day cultures of peripheral blood and pleural effusion lymphocytes.

Subject	Blood		Effusion		% Decrease from blood cpm to effusion cpm
	cpm × 10^3 ± s.e.	Stimulation# index	cpm × 10^3 ± s.e.	Stimulation# index	
HCt	137 ± 3	31	86 ± 3*	7	37
JA	167 ± 11	213	191 ± 33	37	−14
JB	40 ± 2	87	9 ± 1*	3	78
RW	157 ± 16	203	98 ± 5**	98	38
AL	174 ± 7	159	174 ± 30	63	0
JJ	300 ± 46	302	51 ± 3*	80	83
HCl	204 ± 5	408	311 ± 5*	397	−52
NH	257 ± 8	607	36 ± 2*	21	86
CW	414 ± 34	160	67 ± 4*	2.5	84
FW	534 ± 21	156	171 ± 14*	34	68
mean	238 ± 46		119 ± 29##		41 ± 15 (s.e.)+

*$p < 0.001$, **$p < 0.01$ independent t-test.
##$p < 0.05$ paired t-test pleural effusion cpm vs. blood cpm.
+$p < 0.05$, one sample t-test to determine whether the mean percent decrease significantly differs from zero.
#stimulation index = stimulated cpm / unstimulated cpm.

Table 4 Antigen-induced lymphocyte reactivity (cpm × 10^3 ± s.e.) in blood and pleural effusions from patients without tuberculosis.

Subject	Antigen (mm induration)	Unstimulated		Stimulated	
		Blood	Effusion	Blood	Effusion
JA	PPD 72 μg/ml (12 × 15 mm)	0.7 ± 0.2	13 ± 2*	66 ± 4	60 ± 2
HCl	trichophyton 1 : 100 (7 × 8 mm)	0.4 ± 0.2	0.5 ± 0.1	1 ± 0.2	3 ± 1
HCt	PPD 72 μg/ml (15 × 15 mm)	4 ± 0.3	14 ± 1*	10 ± 1	33 ± 2#
NH	trichophyton 1 : 100 (5 × 6 mm)	1 ± 0.1	9 ± 2##	2 ± 0.2	14 ± 1*
	candida 1 : 100 (6 × 6 mm)			26 ± 6	9 ± 0.6+
JJ	PPD 36 μg/ml (10 × 10 mm)	1 ± 0.1	4 ± 0.4*	42 ± 8	8 ± 1#
RH	PPD 72 μg/ml ("positive")	1 ± 0	8 ± 1**	3 ± 1	61 ± 11**

*$p < 0.001$, **$p < 0.005$.
#$p < 0.01$; ##$p < 0.02$.
+$p < 0.05$, independent t-test, pleural effusion vs. blood.

greater reactivity than did such cells from pleural effusions after stimulation by 12.5 μg/ml of concanavalin A.

Antigenic stimulation of pleural effusion and peripheral blood lymphocytes

Six subjects with non-tuberculous pleural effusions were studied using antigens to which positive delayed hypersensitivity skin test reactivity had been elicited (Table 4). In two subjects (HCt and RH), reactivity was greater in the pleural lymphocytes, and in one (JJ) the opposite occurred. In another subject (NH), effusion lymphocyte reactivity was greater than that of blood lymphocytes with trichophytin and less with candidal antigen. In two subjects, there was no statistical difference between the antigen-induced thymidine uptake of lymphocytes derived from blood or effusion, although in one (JA), the stimulation index of PPD-stimulated blood lymphocyte cultures was clearly greater (99 versus 4.6, blood versus effusion). For this group, no predominant effect was present. However spontaneous thymidine incorporation was significantly greater in effusion ($8{,}012 \pm 2{,}085$ cpm) than in blood lymphocytes ($1{,}394 \pm 540$ cpm) and was statistically significant in the group as a whole ($p < 0.02$, paired t-test).

In the single subject (RW) with a proven tuberculous effusion, pleural effusion lymphocytes were highly reactive to tuberculin and clearly less reactive to non-tuberculous antigens (Table 5). Peripheral blood lymphocytes from this person showed greater reactivity in response to tetanus toxoid than to PPD. While effusion lymphocyte reactivity to PPD was much greater than that of blood lymphocytes, reactivity to non-pathogenic antigens was also greater in the pleural fluid lymphocytes in the two subjects (AL and JB) with suspected tuberculous effusions (Table 5). Most importantly, tuberculin-induced reactivity was present in the pleural effusion lymphocytes of the four subjects without tuberculosis who were sensitised to tuberculin (Table 4).

DISCUSSION

It is now appreciated that the cellular response to an antigenic stimulus may be localised at or near the site of the inflammatory response.[21,22] For this reason the immunological characteristics of the T lymphocytes in pleural effusions was studied.

It has been previously reported that pleural effusions contain substantial numbers of T lymphocytes.[6,9-13] However, these studies did not include T lymphocyte subset analysis. T helper cells and T suppressor cells bear the T_4 and T_8 differentiation antigens, respectively.[23-25] Our study of seven patients helps to establish that the ratios of T_4 to T_8 cells that are found in pleural effusions are usually greater than the ratio in the peripheral blood. This is consistent with findings in two previously reported patients with tuberculous pleural effusions[4] and one with a sarcoid pleural effusion.[14] It remains to be determined whether an increased T_4:T_8 ratio is characteristic of T cells that are sequestered in other inflammatory sites. Further experimentation might help to determine whether our finding of increased effusion T_4 cells are functionally related to the higher level of unstimulated thymidine incorporation that was present in pleural effusion lymphocytes as compared with blood lymphocytes (Table 3, as reflected by lower stimulation indices of pleural effusion lymphocytes; Table 4).

Increased spontaneous thymidine uptake has been previously reported in lymphocytes from malignant effusions.[26] This increased spontaneous lymphocyte reactivity may result from *in vivo* stimulation.

In contrast to spontaneous thymidine incorporation, reactivity in response to the T cell mitogens PHA and Con A was generally lower in the effusion lymphocytes than in the blood lymphocytes. However, full dose response studies were not done, and it is possible that these findings would not be present at other mitogen concentrations. Our results contrast with earlier reports that spontaneous and PHA-induced thymidine incorporation are comparable in blood and effusion lymphocytes.[3,27] In five of our six subjects, reactivity of Con A was also greater among peripheral blood lymphocytes. Others have found comparable PHA-and Con A-induced reactivity in blood and effusion lymphocytes from patients with heart failure, while there is diminished reactivity in blood as compared to effusion lymphocytes of cancer patients.[26,28,29] The finding by us and others of lower mitogen-induced effusion lymphocyte reactivity may be because a large proportion of effusion lymphocytes were already committed *in vivo* to undergo blast transformation as indicated by high spontaneous thymidine incorporation.

While mitogenic reactivity was greater in blood lymphocytes, responsiveness to antigenic stimulation was frequently greater in pleural effusion lymphocytes. In the three patients with suspected or proven tuberculous effusions, effusion lymphocytes were more reactive to tuberculin than were blood lymphocytes. In the two subjects with suspected tuberculosis, the pleural fluid lymphocytes were also more reactive to unrelated antigens than were the blood lymphocytes. In the patient with proven tuberculosis, the blood lymphocytes were more reactive to non-tuberculous antigens. Among our control subjects with non-tuberculous effusions, some antigens elicited greater thymidine uptake from peripheral blood lymphocytes and others, greater uptake from the effusion lymphocytes. Subject NH

whose effusion was caused by heart failure illustrates this best (Table 5).

Other workers have also reported tuberculin-induced lymphocyte reactivity in malignant effusions in three of six tuberculin skin test responders.[2] However, reactivity to other antigens has never been previously studied. Our findings uniquely show that pleural effusion lymphocytes may be reactive to antigens such as trichophyton, candida, tetanus toxoid and mumps, which are unrelated to the etiology of the effusion. Our results complement the earlier findings of a study of five neoplastic pleural fluids in which PHA-induced thymidine uptake by effusion lymphocytes approximated that of peripheral blood while PPD-induced responses greatly exceeded that of peripheral blood lymphocytes.[27]

During the early phase of immunisation, circulating antigen-specific lymphocytes become compartmentalised within the stimulated lymphoid organ(s)[30] and are retained.[31,32] Other studies using guinea pig peritoneal exudate cells induced by intraperitoneal injection of mineral oil, an antigenically non-specific inflammatory agent, showed that the peritoneal exudate lymphocyte pool is highly enriched in effector cells with antigenic specificities identical to those of lymph node cells.[33] These murine exudate cells are apparently analogous in their range of antigenic specificities

Table 5 Antigen-induced lymphocyte reactivity (cpm $\times 10^3 \pm$ s.e.) in blood and pleural effusions from patients with proven or suspected tuberculosis

	Antigen	Blood	Effusion	p-value#
RW*	PPD 36 μg/ml	31 ± 2	62 ± 4	<0.001
	tetanus toxoid (35 × 40 mm)+	51 ± 1	9 ± 1	<0.001
	mumps 1 : 2 (v/v) (7 × 7 mm)+	9 ± 2	2 ± 0.3	<0.005
AL**	PPD 36 μg/ml before treatment	5 ± 1	62 ± 8	<0.001
	after treatment for 1 week	62 ± 8	n.d.	
	tetanus toxoid before treatment	1 ± 0.2	19 ± 1	<0.001
	after treatment for 1 week	2 ± 0.2	n.d.	
JB**	PPD 36 μg/ml	0.6 ± 0.2	7 ± 2	<0.001
	mumps 1 : 2 (v/v)("positive"+)	0.7 ± 0.1	13 ± 2	<0.001

*proven tuberculosis; **suspected tuberculosis with clinical response to treatment.
#independent t-test, pleural effusion vs. blood; +skin test reactivity.

Table 6 Summary of PPD-induced lymphocyte thymidine incorporation in lymphocytes from blood and pleural effusions of patients with tuberculous pleurisy.

Number of subjects	Blood reactivity	Effusion reactivity	Effusion >blood	Reference number
3	1	3	2	1
8	n.a.	(7)	n.a.	2
9	n.a.	(5)	n.a.	3
4	2	4	4	4
22	16	22	17	5
1	1	1	1	J. Plouffe, (unpublished)
3	2	3	3	present study
50 (Total)	22 of 33*	33 of 33* (45 of 50)#	27 of 33	

*$p < 0.001$, blood lymphocyte vs. effusion lymphocyte reactivity, McNemar's Test using those subjects on whom data is available for both effusion and blood lymphocyte reactivity.
#includes subjects on whom blood lymphocyte reactivity was not available.
n.a. = data not available.

to the pleural fluid lymphcytes found in our control subjects. This has not been previously demonstrated in humans.

Sequestration of tuberculin-specific lymphocytes in lymph nodes of patients with tuberculosis has been reported.[34] Similarly, tuberculin-reactive lymphocytes are present in the cerebrospinal fluid (CSF) of patients with tuberculous meningitis.[35,36] In patients with tuberculous meningitis, the PPD-induced CSF lymphocyte reactivity was more than twice as intense as that of the peripheral blood lymphocytes. Compartmentalisation of antigen-specific lymphocytes in the CSF of patients with a variety of other infectious meningitides has been found.[37]

Several other investigators have studied pleural effusion lymphocyte reactivity in response to PPD (Table 6). Ellner[1] using a thymidine incorporation assay found tuberculin-induced reactivity in all of three tuberculous effusions but in the blood lymphocytes of only one of the three subjects. Reactivity in the blood lymphocytes of the other two subjects became manifest only after depletion of adherent monocyte suppressor cells. Fujiwara *et al*[5] confirmed Ellner's findings in a study of 27 patients with tuberculous effusions. In 22 of these patients, both blood and effusion lymphocyte reactivity to PPD was studied. In five of the 22, effusion lymphocyte reactivity was lower than blood lymphocyte reactivity. In an additional three subjects, effusion lymphocyte reactivity was only slightly greater than blood lymphocyte reactivity. When adherent cells were removed before culture of peripheral blood lymphocytes from patients with tuberculous effusions, 14 of 16 subjects' lymphocytes exhibited increased thymidine incorporation. Catanzaro and Kalafer[2] reported that pleural effusion lymphocytes from seven of eight patients with pleural tuberculosis responded to PPD by *in vitro* thymidine incorporation. Pettersson *et al*[3] studied nine patients with tuberculous effusions. There was minimal PPD-induced reactivity of pleural effusion lymphocytes in four of their subjects. The group results did not show enhanced PPD-induced thymidine uptake in effusion as compared with blood lymphocytes. Non-tuberculous effusion lymphocytes also possessed tuberculin reactivity although less so than did peripheral blood lymphocytes. Most recently, Okubo *et al*[4] studied the PPD-induced thymidine uptake in four patients with tuberculous pleurisy, two of whom were tuberculin negative by skin testing and had poorly reactive blood lymphocytes similar to our findings in subject JB. Pleural fluid lymphocyte reactivity was present in all four patients and was greater than that of blood lymphocytes in each subject. Finally, J. Plouffe (Ohio State University) studied one patient with proven tuberculous pleurisy. He found that both blood and effusion lymphocytes were reactive to PPD(5 μg/ml) and that the effusion lymphocytes were more reactive than were the blood lymphocytes [3655 $\pm$221 (s.e.) cpm vs 1097 $\pm$141 (s.e.) cpm] (unpublished observations).

Summarising all of our own plus the published data, a total of 50 subjects with tuberculous pleural effusions have been studied. Forty-five of the 50 possessed PPD-induced pleural effusion lymphocyte reactivity. Information regarding pleural fluid and blood lymphocyte reactivity is available on a total of 33 subjects. Twenty-two of the 33 possessed PPD-induced blood lymphocyte reactivity. All possessed PPD-induced pleural fluid lymphocyte reactivity. In 27 of the 33, it is known that PPD-induced pleural effusion lymphocyte reactivity was greater than that of the blood lymphocytes. Patients with tuberculous pleurisy are more likely to possess PPD-induced pleural effusion lymphocyte reactivity than blood lymphocyte reactivity (all of 33 vs. 22 of 33, effusion lymphocyte reactivity vs blood lymphocyte reactivity, $p<0.001$, McNemar's test).

PPD-induced lymphokine production from tuberculous effusion lymphocytes has also been studied. PPD-induced production of leucocyte migration inhibition factor (LIF) has been studied by two groups. One report[6] showed that of twelve patients with tuberculous effusions all exhibited blood lymphocyte production and eight exhibited effusion lymphocyte production of LIF. Two of thirteen patients with malignant effusions also had effusion lymphocytes which produced LIF in response to PPD. LIF production by pleural effusion lymphocytes has also been studied in a second group of five patients with tuberculous pleurisy.[8] Three of the five subjects' effusion lymphocytes produced LIF in response to PPD. Three of nine patients with malignant effusions and four of five with undiagnosed effusions also exhibited PPD-induced LIF production. A study of PPD-induced production of lymphocyte mitogenic factor (LMF) showed that effusion lymphocytes from all of twelve patients with tuberculous effusions clearly produced more LMF than did their blood lymphocytes.[5] In one of three of these patients, removal of adherent peripheral blood cells caused a marked further augmentation of LMF production. Finally, PPD-induced interferon production was studied in eighteen subjects with tuberculous pleural effusions.[7] Of these, effusion lymphocytes from only seven produced interferon in a titre of 16 units/ml or greater. Sixteen units of interferon/ml is two dilutions greater than the essentially negative interferon titres that the authors report were produced by peripheral blood lymphocytes in their patients. PPD-induced production of LMF or interferon when present in effusion lymphocytes was always greater

than that of blood lymphocytes. A review of the literature reveals that PPD-induced thymidine incorporation in lymphocytes from tuberculous pleural fluids is as likely to be positive as is production of LMF (LMF production, 12 of 12, vs thymidine incorporation, 45 of 50, p = n.s., Chi Square test) (Table 6). With the exception of one report,[3] thymidine incorporation in response to PPD was always present in lymphocytes from tuberculous effusions. This response may or may not be greater than that of blood lymphocytes.

Since we have shown that tuberculin reactivity is present in non-butercu-lous effusions of patients with positive tuberculin skin tests, the presence of PPD-induced effusion lymphocyte reactivity cannot be taken to indicate the presence of active tuberculosis. This has not been adequately noted in previous reports. Our work also shows that pleural effusion lymphocytes, regardless of the etiology of the effusion, may be reactive to trichophyton, candida, tetanus toxoid and mumps antigens. Effusion lymphocytes possess the same range of antigenic specificities as do the lymphocytes of peripheral blood. However, tuberculin-induced pleural effusion lymphocyte responses may be present when such responses are absent in blood lymphocytes. Such effusion lymphocyte responsiveness may be useful clinically as an indication of sensitisation to tuberculin.

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