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Diagnostic Differentiation between Reactive and Malignant Lymphoid Cells in Serous Effusions

E.J. JOHNSON,* C.S. SCOTT,† L.A. PARAPIA* and A.N. STARK†

**Department of Haematology, Bradford Royal Infirmary, Duckworth Lane, Bradford, BD9 6RJ and †Department of Haematology, Cookridge Hospital, Leeds LS16 6QB, U.K.*

Abstract—*Lymphoid cell components in a total of 34 pleural and ascitic aspirates were investigated immunologically. The results indicate that reactive lymphocytes predominate in effusions from non-haemopoietic malignancies and benign conditions, while a significant proportion of fluids from patients with non-Hodgkins lymphoma show unequivocal evidence of lymphomatous involvement. Immunological typing of lymphocytes in serous effusions is a valuable adjunct to conventional methods of diagnosis particularly in those patients in whom invasive procedures are undesirable.*

INTRODUCTION

CONVENTIONAL morphological assessments of cellular components in serous fluids have in the past been limited by an inability to differentiate between inflammatory, or non-specific processes, and malignant infiltration. In neoplastic lymphopoietic tumours, the presence of lymphocytes in ascitic or pleural fluids is often taken to infer lymphomatous involvement even though, in many cases, their morphological features may be indistinguishable from reactive or normal lymphoid elements. However immunophenotypic investigations, which allow the identification of lymphocyte subpopulations have provided a means whereby the nature of lymphocytic infiltrates can be interpreted precisely. In this study, we report the immunological characteristics of ascitic and pleural fluid lymphocytes in 16 cases of lymphoid neoplasia, 12 cases of non-haemopoietic tumours and 6 effusions not associated with malignancy.

PATIENTS AND METHODS

A total of 34 uninfected pleural effusions and ascitic fluids were examined in this study. Disease stages and relevant clinical details for these patients are summarised in Table 1 and included 12 cases of non-Hodgkin's lymphoma (NHL), 2 cases of chronic lymphocytic leukaemia (CLL) and 2 cases of Hodgkin's disease (HD). Of the non-haemopoietic tumours, there were 6 cases of breast carcinoma, 2 of bronchus, 3 ovarian and 1 liposarcoma. The majority of cases of NHL were widespread with involvement of multiple nodal sites although two cases of T lymphoblastic lymphoma were confined to the mediastinum and one case of low grade NHL was limited to the abdomen. Of the patients with non-haemopoietic malignancy, 10/12 had metastatic disease when examined. The 6 cases of non-malignant effusion comprised 2 of Meigs syndrome, 3 of liver disease and 1 case of congestive cardiac failure (CCF).

Morphology was assessed independently, without knowledge of clinical or immunological findings, on May–Grunwald–Giemsa stained cytocentrifuge preparations of pleural and ascitic fluid. For immunological studies, cells were collected from serous fluids by centrifugation and, after washing three times in phosphate-buffered saline, resuspended to 4.0×10^6/ml. Immunophenotypic analyses were carried out by conventional indirect immunofluorescent or rosetting techniques as previously described [1, 2]. Leu12 (CD19: Becton-Dickinson); and T11 (CD2: SRBC receptor: Coulter Electronics) monoclonal antibodies were employed as 'pan-B' and 'pan-T' markers; clonality of B cell surface immunoglobulin (SIg) was determined with antibodies (Unipath) to kappa and lambda light chains and T helper and suppressor cell subpopulations defined by anti-T4 and T8 (CD4 and CD8: Ortho Diagnostics) respectively. Additional antibodies used to further characterise lymphoid cells in low-grade NHL cases included TU1 (CD23: Biotest-Folex) and FMC7 (Sera-Labs), which are expressed at early and late stages

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Correspondence to be addressed to: Dr. C.S. Scott, Department of Haematology, Cookridge Hospital, Leeds LS16 6QB, U.K.

Table 1. Diagnostic categories and relevant clinical details of patients examined in this study

Patient	Diagnosis	Stage	Effusion cell count*
1	NHL	Stage 4-widespread	nt
2	NHL	Stage 4-widespread	8.6
3	NHL	Stage 4-widespread	nt
4	NHL	Stage 4-widespread	nt
5	NHL	Stage 4-widespread	2.5
6	NHL	Stage 4-widespread	nt
7	NHL	Intrabdominal only	1.0
8	NHL	Stage 4-widespread	2.4
9	NHL	Intrabdominal and inguinal	nt
10	NHL	Mediastinum only	nt
11	NHL	Stage 4-widespread	26.5
12	NHL	Mediastinum only	nt
13	CLL	Blood and marrow involved: No nodes	1.6
14	CLL	Marrow but not blood involved: Skin infiltrate	11.3
15	HD	Not known	nt
16	HD	Stage 4-widespread	nt
17	Ca Breast	End stage-widespread metastases	1.7
18	Ca Breast	End stage-widespread metastases	nt
19	Ca Breast	End stage-widespread metastases	1.0
20	Ca Breast	End stage-widespread metastases	0.5
21	Ca Breast	End stage-widespread metastases	1.3
22	Ca Breast	End stage-widespread metastases	1.1
23	Ca Ovary	Eng stage-widespread metastases	nt
24	Ca Ovary	End stage-widespread metastases	nt
25	Ca Ovary	Newly diagnosed case-no disease outside abdomen	0.3
26	Ca Bronchus	Local nodal involvement with metastases	nt
27	Ca Bronchus	No known metastases	1.5
28	Liposarcoma	Primary on thigh and intrabdominal	8.1
29	Meigs syndrome	Not applicable	nt
30	Meigs syndrome	Not applicable	1.3
31	Liver disease	Not applicable	2.3
32	Liver disease	Not applicable	1.0
3	Liver disease	Not applicable	0.5
34	CCF	Not applicable	2.6

nt: not tested
*Effusion cell count $\times 10^9$/l

of B cell differentiation respectively, and CALLA (CD10: common-acute lymphoblastic leukaemia antigen: Becton-Dickinson); which stains malignant cells in a proportion of NHL cases. High-grade lymphomas were also examined for the presence of nuclear TdT (Pharmacia) and the thymocyte-associated membrane marker T6 (CD1: Coulter Electronics). T200 (Hybritech) was employed as a leucocyte common antigen.

RESULTS

Morphological studies

A total of 12 serous effusions from patients with NHL were examined (Table 2). Of these, 9 (cases 1–9) were histologically of low-grade and 3 (cases 10–12) of high-grade type. Cell counts in the effusions examined ranged from 1.0 to 26.5 $\times 10^6$/ml. Morphological features in the 9 low-grade NHL cases were variable. Lymphoid cells were prominent in all cases but atypical (cases 1, 2 and 9) or pleomorphic (case 8) lymphocytic components were present in 4. All 3 cases of high-grade NHL were morphologically characterised by the presence of immature 'blasts'. Cells in the CLL and HD effusions were of lymphoid appearance with atypical features (nucleolation and nuclear convolution) in case 14.

Cellular components in fluids from patients with non-haemopoietic malignancies (cases 17–28: Table 3) were predominantly lymphocytic (> 60% of cells) in all but 2 cases (patients 25 and 26). In these latter effusions, a minor lymphoid element was seen together with larger cells which morphologically were suggestive of macrophages or mesothelial origin. None of the effusions examined contained malignant cells. Cell counts in these cases ranged from 0.3 to 8.1 $\times 10^6$/ml.

Table 2. Immunological characteristics of lymphocytic components in serous effusions from patients with non-Hodgkins lymphomas (NHL, cases 1–12: CLL, cases 13 and 14) and Hodgkins disease (HD, cases 15 and 16)

Patient	Immunological studies* Pan-T	T-helper	T-suppressor	Pan-B	Others	Interpretation
1	9	nt	nt	55	SIg^- $CALLA^-$ $FMC7^-$ $TU1^-$	High-grade (?centroblastic) lymphomatous involvement
2	4	nt	nt	69	$SIg\text{-}k^+$ $CALLA^+$	Monoclonal neoplastic B cell component
3	46	nt	nt	54	$SIg\text{-}\lambda^+$	Monoclonal neoplastic B cell component
4	21	nt	nt	62	$SIg\text{-}\lambda^+$	Monoclonal neoplastic B cell component
5	7	nt	nt	37	$SIg\text{-}k^+$ $CALLA^+$	Monoclonal neoplastic B cell component
6	11	nt	nt	61	$SIg\text{-}\lambda^+$ $CALLA^-$ $TU1^-$	Monoclonal neoplastic B cell component
7	81	50	33	20	$SIg\text{-}k^+$ $CALLA^-$ $FMC7^+$ $TU1^-$	Minor but monoclonal B cell component: T-helper:suppressor ratio 1.5:1
8	68	53	19	12		Reactive (polyclonal) lymphoid elements: T-helper:suppressor ratio 2.8:1
9	81	82	16	2		Reactive lymphoid elements: T-helper:suppressor ratio 5.1:1
10	56	90	14	2	TdT^+ $T6^+$	Lymphoblastic lymphoma/leukaemia of T cell type
11	80	80	49	3	TdT^+ $T6^+$	Lymphoblastic lymphoma/leukaemia of T cell type
12	95	91	90	0	TdT^+ $T6^+$ $CALLA^+$	Lymphoblastic lymphoma/leukaemia of T cell type
13	54	29	20	2		Reactive T cell elements: T-helper: suppressor ratio 1.4:1
14	3	nt	nt	2	$T200^+$	'Lymphoid' cells lack T/B cell markers: T200 expression indicates their haemopoietic origin
15	91	45	28	5		Reactive T cell components
16	54	nt	nt	26		Majority of cells are T lineage. Subpopulations not investigated

*SRBC receptor or T11 expression used as 'Pan-T' markers. T-helper and T-suppressor cells defined by T4 and T8 monoclonal antibodies respectively. Leu12 expression used as a 'Pan-B' marker.
nt: not tested.

Table 3. Immunological characteristics of lymphocytic components in serous effusions from patients with non-haemopoietic malignancies (n=12) and benign non-malignant disorders (n=6)

	Patient	Immunological studies* Pan-T	T-helper	T-suppressor	Pan-B	Th:Ts ratio†	Interpretation
Non-haemopoietic tumours:	17	75	63	12	15	5.25	Reactive T cells
	18	54	29	19	12	1.50	Reactive T cells
	19	77	54	18	5	3.00	Reactive T cells
	20	49	35	17	24	2.00	Reactive T cells
	21	77	58	15	4	3.80	Reactive T cells
	22	68	56	18	8	3.10	Reactive T cells
	23	56	43	10	28	4.30	Reactive T cells
	24	55	35	17	6	2.00	Reactive T cells
	25	26	56	7	1	na	Reactive T cells
	26	16	6	0	0	na	Reactive T cells
	27	68	48	17	9	2.00	Reactive T cells
	28	61	51	12	20	4.25	Reactive T cells
							Reactive T cells
Non-malignant disorders:	29	63	40	27	7	1.50	Reactive T cells
	30	55	32	19	6	1.70	Reactive T cells
	31	13	9	6	0	1.50	Reactive T cells
	32	52	35	15	3	2.20	Reactive T cells
	33	64	49	15	5	3.30	Reactive T cells
	34	68	50	17	13	2.90	Reactive T cells

*Results expressed as percentages of positive cells
†Helper–suppressor T cell ratio
na: not applicable

Effusions from patients with non-malignant disorders showed a predominance of lymphocytes in 5/6 cases with the other (case 31: Table 3) showing a high proportion of mesothelial cells. Cell counts in this group ranged from 0.5 to 2.6 × 10^6/ml.

Immunological studies

Of the 9 low-grade NHL cases examined, immunological investigations revealed B lymphocyte SIg light chain restriction in 5 (patients 2–7) even though the proportions of B cells in these showed considerable variation (range 20–69%). Three of these also showed increased CALLA-positive (patients 2 and 5) or FMC7-positive components. A further case (patient 1) had a predominance of B cells, defined by the presence of membrane Leu12, but did not express SIg or other B cell differentiation markers. Together with morphological impressions, it was considered that this case represented B cell lymphomatous involvement of probable high-grade (?centroblastic) type. The remaining 2 low-grade NHL cases (8 and 9) showed minor B cell components only (2 and 12%) with a predominance of reactive T cells (T-helper : suppressor ratios of 2.8 and 5.1). Effusions from the 3 cases of high-grade NHL all contained high proportions of T-lymphoblastic (TdT^+ $T6^+$ $T11^+$ $Leu12^-$) leukaemia/lymphoma cells.

Reactive T cell components were found in 1 of the CLL effusions (case 13: T-helper : suppressor ratio 1.4 : 1) and both cases of Hodgkins disease. In the other CLL (case 14), which showed a relatively high cell count (11.3 × 10^6/ml), occasional cells only expressed B or T cell markers even though their morphological appearance was lymphoid and T200 expression indicated their haemopoietic nature.

Immunological studies of effusions from patients with non-haemopoietic malignancies indicated most lymphoid cells to be of T lineage (mean 55.3%) with minor proportions of B cells (mean 9.2%). Analysis of relative T-helper : suppressor distributions indicated a preponderance of helper cells with consequently increased helper : suppressor ratios (mean ratio 3.1 : 1 with 6 of the 10 cases tested showing ratios in excess of 3.0 : 1). One interesting observation in case 25 was the presence of higher numbers of cells stained for T4 (56%) than for T11 (26%). Cytocentrifuge preparations in which bound monoclonal antibodies were 'visualised' by indirect rosetting revealed a high proportion of non-lymphoid cells with an apparent $T11^-$ $T4^+$ $T8^-$ phenotype. These cells were not however morphologically or cytochemically suggestive of macrophages and further studies to establish their nature are in progress.

Lymphocyte populations in the non-malignant disorders also showed a predominance of T cells although it was notable that T-helper : suppressor ratios in these cases were generally lower (mean 2.2 : 1) than in the carcinoma group.

DISCUSSION

Malignant lymphomas may infiltrate serous cavities causing the development of effusions [3–6]. Although lymphomatous involvement is often assumed by the presence of atypical lymphoid forms with distinctive morphological features, such as nuclear clefting, 'reactive' lymphocytes may have cytological appearances indistinguishable from neoplastic lymphoid cells. Additionally, interpretation of cellular components in serous effusions can be complicated by the coexistence of liver disease in some patients [7]. In these cases, a study of T and B cell subpopulations may be of great value in determining the nature of the lymphoid cells present.

The results of this study indicate that the most useful single immunological parameter for assessment of B-cell lymphomatous involvement of serous fluids is SIg light chain monoclonality. A previous study [8], which did not examine SIg determinants, concluded that a relative increase in B cells alone was consistent with malignant infiltration. However, it was clearly shown in this present study that malignant B cells, when present, do not always constitute the major cell population and that demonstration of SIg light chain restriction is a far more accurate indicator of lymphamatous involvement.

All patients with detectable lymphomatous involvement of pleural or ascitic effusions, had disease present elsewhere although not necessarily in an accessible site. For example, in two children presenting with isolated mediastinal masses and pleural effusions, immunological studies of pleural cells confirmed the suspected diagnosis of T-lymphoblastic lymphoma without recourse to mediastinoscopy and consequent general anaesthetic. In addition, a further case (adult: patient 7) showed no superficial lymph node infiltration or involvement of peripheral blood or bone marrow and was thought clinically to be a pancreatic carcinoma. However, immunological studies of the ascitic fluid provided the diagnosis of NHL, a potentially treatable disease. Thus, examinations of body fluids, which are relatively simple and atraumatic procedures, may lead to a firm diagnosis in patients in whom invasive techniques are undesirable or impossible.

In accord with previous observations [7, 9, 10], effusions from cases of lymphoid malignancy in which no malignant cells were detected generally

showed a predominance of T lymphocytes. A further study [11] reported a case of B-CLL in which the pleural effusion showed a high proportion of T cells and it was suggested that this was possibly due to central lymphatic blockage by mediastinal lymphadenopathy. In this context, it is perhaps relevant that a study [12] of skin lesions in a series of CLL patients also showed T cell predominance, most of which were of helper cell type. Whether these observations indicate defects in lymphatic recirculation or secondary responses to malignancy are not clear although T cell predominance was also a feature of effusions obtained from both patients with non-haemopoietic malignancies and those with non-malignant disorders.

The results of this present investigation further indicate a relative increase in the proportion of T-helper cells in patients with carcinoma. Blood helper : suppressor cell ratios are generally normal in localised cancers although reductions in circulating helper cell numbers are found in advanced forms [13, 14].

However, when cancer patients with normal blood T subset distributions were examined, the mean helper : suppressor ratio in effusions was significantly higher than in peripheral blood. A marked excess of helper cells in has also been reported in an effusion due to sarcoidosis [15] whilst helper : suppressor T cells ratios in other non-lymphoid diseases, malignant and non-malignant, have been reported in the order of 3.5 : 1 [8]. Our results confirm previous impressions [10, 16] that B cell populations in carcinoma and non-malignant groups are minor. It is also notable tht although absolute counts and distributions of lymphocyte subpopulations in carcinoma patients and non-malignant effusions were similar, there was an apparent relative increase in T-helper cells in the malignant cases. Whether these observations indicate an active host immune response against tumour cells, as suggested by Robinson *et al.* [17], is speculative although further immunological studies (in progress) which examine T-cell activation markers such as interleukin-2 (IL-2) receptors, HLADr (Ia-like) ad TQ1 determinants may resolve this.

In conclusion it is evident that differentiation of malignant from reactive lymphoid cells in infiltration of serous cavities can be established by immunological studies and this may obviously be of particular value in patients who do not have significant lymphoma present in marrow or peripheral blood and in whom invasive procedures are undesirable. Immunological typing is able to provide direct evidence of lymphomatous involvement in serous effusions, but this can only be conclusive when SIg is examined and light chain restriction demonstrated, proving clonal origin.

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