Aberrant phenotypes in peripheral T cell lymphomas

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SUMMARY Seventy six peripheral T cell lymphomas were examined immunohistologically to test their reactivity with a panel of monoclonal antibodies against 11 T cell associated antigens (CD1-8, CD27, UCHL1, and the T cell antigen receptor). Sixty two (82%) lymphomas showed aberrant phenotypes, and four main categories were distinguished as follows: (i) lack of one or several pan-T cell antigens (49, 64% of the cases); (ii) loss of both the CD4 and CD8 antigens (11, 15% of the cases); (iii) coexpression of the CD4 and CD8 antigens (13, 17% of the cases); and (iv) expression of the CD1 antigen (eight, 11% of the cases). No correlation was seen between the occurrence of aberrant phenotypes and the histological subtype.

It is concluded that the demonstration of an aberrant phenotype is a valuable supplement to histological assessment in the diagnosis of peripheral T cell lymphomas. It is recommended that the panel of monoclonal antibodies against T cell differentiation antigens should be fairly large, as apparently any antigen may be lost in the process of malignant transformation.

Two main groups of T cell tumours may be distinguished: immature (thymic) and peripheral (post-thymic) T cell malignancies. The cells in the former group (T lymphoblastic lymphoma/leukaemia) express antigens characteristic of thymocytes¹ and are fairly homogeneous morphologically. In contrast, peripheral T cell lymphomas are heterogeneous and frequently present diagnostic problems with regard to their distinction from benign T cell infiltrates.

Recent studies have suggested that the expression of aberrant T cell phenotypes—that is, those which differ from phenotypes seen on normal peripheral T cells—mainly, if not exclusively, occurs in malignant T cell proliferations and thus may help to differentiate between benign and malignant disease.^{1,2} As T cell lymphomas are much less common than B cell malignancies few comprehensive studies have been made.

Material and methods

Biopsy samples from 76 peripheral T cell tumours were drawn from the frozen tissue banks at the laboratory of immunohistology, Aarhus (58 cases) and the department of pathology, Rigshospitalet (18 cases). Cases of mycosis fungoides and Sézary's syndrome were excluded, as these have been described in

detail previously.³ Anaplastic large cell lymphomas (Ki-1 lymphomas) were also excluded and will be the subject of a separate report. In all cases routinely processed formalin fixed specimens were also available, and these were classified in accordance with the criteria described by Suchi *et al.*⁴

The biopsy specimens were obtained fresh, snap-frozen, and stored at -70°C until processing. Cryostat sections were fixed in acetone and stained with the anti-T cell antibodies listed in table 1 by a three stage immunoperoxidase method⁵ or a five step alkaline phosphatase-anti-alkaline phosphatase (APAAP) technique.⁶ Sections were also stained with antibodies against B cell associated antigens (CD19, CD22) and macrophage associated markers (EBM11, FMC32, Y1/82a, Ber-MAC3, MAC-387).

Results

In all cases the tumour cells expressed T cell associated antigens and were negative for B cell and macrophage associated markers.

Fourteen (18%) tumours expressed a normal peripheral T cell phenotype (CD1-, CD2+, CD3+, CD4+/CD8- or CD4-/CD8+, CD5+, CD6+, CD7+, CD27+, UCHL1+, F101-01+). Twelve of these resembled T helper/inducer cells (CD4+/CD8-), and two resembled T suppressor/cytotoxic cells (CD4-/CD8+) (table 2). The remaining 62 cases

Table 1 Monoclonal antibodies used in this study

Antigen	Monoclonal antibodies	No of cases examined	Source and reference*
CD1†	Anti-Leu-6, OKT6, Na1/34	74	BD, Ortho, DAKO
CD2	Anti-Leu-5, Lyt3	76	BD, NEN
CD3	Anti-Leu-4, OKT3, UCHT1	75	BD, Ortho, Dr P Beverley
CD4	Anti-Leu-3a, OKT4	75	BD, Ortho
CD5	Anti-Leu-1, Lyt2, Tü71	74	BD, NEN, Dr A Ziegler
CD6	OKT17, Tü33	58	Ortho, Dr A Ziegler
CD7	Anti-Leu-9, Tü14, 3A1	67	BD, Dr A Ziegler, Dr B Haynes
CD8	Anti-Leu-2, OKT8, Tü68, Tü102	75	BD, Ortho, Dr A Ziegler
CD27	S152, VIT14	63	Dr A Bernard, Dr W Knapp
CD45R	UCHL1	54	Dr P Beverley [†]
TCR	F101-01	65	Dr T Plesner [‡]

*BD = Becton Dickinson; Ortho = Ortho Diagnostics; DAKO = Dakopatts; NEN = New England Nuclear.

†CD (cluster of differentiation) designations.

(82%) displayed aberrant T cell phenotypes. About half of the lymphomas with aberrant T cell phenotypes showed abnormal expression of only one antigen; two cases showed as many as eight abnormal immunophenotypic features. No correlation was shown between the occurrence of aberrant T cell phenotypes and the histological subtype (table 2).

Various different aberrant T cell phenotypes were identified, and within different histological subtypes a tremendous heterogeneity in the expression of T cell associated antigens was found. Nevertheless, a distinction could be made between four main categories as follows:

- 1 lack of one or several pan-T cell antigens (CD2, CD3, CD5, CD6, CD7, CD27, UCHL1 or the T cell receptor (TCR));
- 2 loss of both the CD4 and CD8 antigens;
- 3 coexpression of the CD4 and CD8 antigens;
- 4 expression of the CD1 antigen.

The distribution of cases according to these categories is summarised in table 3. Selected examples are illustrated in the figure.

LACK OF PAN-T CELL ANTIGENS

A lack or deficient expression of pan-T cell associated antigens was seen in 49 (64%) tumours (table 3). Twenty two (45%) of these lacked only one antigen.

Eight (16%) cases lacked two antigens, five (10%) cases three antigens, nine (18%) cases four antigens, two (4%) cases five antigens, two (4%) cases six antigens, and one (2%) case seven antigens. The number of missing pan-T cell antigens did not correlate with the histological subtype.

The most common abnormality was (in descending order) a lack of CD7 (45%), CD27 (41%), TCR (25%), CD5 (24%), CD6 (17%), CD2 (15%), UCHL1 (11%) and CD3 (9%).

Staining with antibody F101-01 (against a conformational epitope of the TCR/CD3 complex) as well as CD3 was performed in 63 cases. Forty nine (78%) tumours were positive for F101-01. All but one of these also expressed the CD3 antigen. Among the remaining 14 negative tumours, nine cases were positive for, and five were negative for, CD3.

SIMULTANEOUS LOSS OF THE CD4 AND CD8 ANTIGENS

A lack of both the helper and the suppressor cell associated CD4 and CD8 antigens was seen in 11 (15%) cases. Two of these showed no other immunophenotypic abnormalities. The remaining nine cases lacked either one or several pan-T cell antigens, including, in six cases, the TCR, and in four cases, the CD3 antigen. Three of the CD4 negative, CD8

Table 2 Immunophenotype in relation to histological subtype

Histology	No of cases (%)	T cell antigen phenotype		
		Aberrant	Helper	Suppressor
AILD-like	10 (13)	7 (70)	2	1
T zone	4 (4)	4 (100)	0	0
Lymphoepithelioid	3 (4)	1 (33)	2	0
Pleomorphic, small cell	5 (7)	5 (100)	0	0
Pleomorphic, medium cell	27 (36)	22 (81)	5	0
Pleomorphic, large cell	23 (30)	20 (87)	3	0
Immunoblastic	3 (4)	2 (67)	0	1
Unclassified, high grade	1 (1)	1 (100)	0	0
Total	76 (100)	62 (82)	12 (16)	2 (3)

Figure (a) Peripheral T cell lymphoma of pleomorphic, medium cell type showing (b) negative staining with CD2 (anti-Leu 5) and (c) positive staining with CD5 (anti-Leu 1), and (d) CD3 (anti-Leu 4).

Table 3 Main groups of aberrant immunophenotypes in relation to histological subtype

Histology	Pan-T loss (No/%)	CD4+ /CD8+ (No/%)	CD4- /CD8- (No/%)	CD1+ (No/%)
AILD-like	4/10 (40)	2/10 (20)	1/10 (10)	1/10 (10)
T-zone	3/4 (75)	1/4 (25)	1/4 (25)	0/4
Lymphoepithelioid	0/3	1/3 (33)	0/3	0/3
Pleomorphic, small cell	5/5	0/5	0/5	0/5
Pleomorphic, medium cell	18/27 (67)	6/27 (22)	5/27 (19)	3/26 (12)
Pleomorphic, large cell	16/23 (70)	3/22 (14)	2/23 (9)	3/22 (14)
Immunoblastic	2/3 (67)	0/3	1/3 (33)	1/3 (33)
Unclassified, high grade	1/1	0/1	1/1	0/1
Total	49/76 (64)	13/75 (17)	11/75 (15)	8/74 (11)

negative lymphomas were unreactive with both F101-01 (against the TCR) and CD3.

COEXPRESSION OF THE CD4 AND CD8 ANTIGENS

Thirteen (17%) tumours showed positivity for both the CD4 and CD8 antigens. Eight cases showed no other immunophenotypic abnormalities. Of the remaining five lymphomas, either one or several pan-T cell antigens were missing.

EXPRESSION OF THE CD1 ANTIGEN

Eight (11%) tumours were positive for CD1. Two of these showed no other immunophenotypic abnormalities. The remaining six cases lacked one or several pan-T cell antigens. Coexpression of the CD4 and CD8 antigens was seen in one of the cases.

Discussion

Since monoclonal antibodies became available much progress has been made in the understanding of peripheral T cell tumours. Whereas initial reports emphasised the immunophenotypic similarities between benign and malignant T cell populations, more recent studies have strongly suggested that many peripheral T cell lymphomas express immune phenotypes which differ from those seen on normal peripheral T cells (so-called aberrant phenotypes).^{1,2,10-18}

This is in keeping with the findings of our study in which 62 of 76 (82%) peripheral T cell lymphomas displayed aberrant phenotypes, as shown by examination with monoclonal antibodies against 11 T cell associated antigens.

The prevalence of aberrant phenotypes among peripheral T cell lymphomas clearly depends on the number of antibodies used. Table 4 summarises the results of this and other similar reports. With the use of six or more antibodies, roughly two thirds of peripheral T cell lymphomas show aberrant phenotypes, but even with the same monoclonal antibodies some differences may be seen. This may result partly from differences in the types of lymphomas studied, which is why cases of mycosis fungoides and Sézary's syndrome were excluded from the present study but were included in the study of Borowitz *et al*¹⁰ and Nasu *et al*.¹² This could account for the somewhat lower incidence of aberrant phenotypes seen in these studies, as aberrant phenotypes occur relatively rarely in these conditions.³

In all studies a lack of pan-T cell antigens has been the most common immunophenotypic abnormality (table 4). In 22 (28%) of our cases only one pan-T cell antigen was absent. Similar results were reported by Picker *et al*¹ and Borowitz *et al*,¹⁰ whereas Nasu *et al*¹² reported loss of one pan-T cell antigen in 50% and Weiss *et al*¹¹ in 18% of peripheral T cell lymphomas.

Table 4 Incidence (No/(%)) of aberrant phenotypes in peripheral T cell lymphomas

Studies	No of cases	Monoclonal antibodies	Overall incidence of aberrant phenotypes	Pan cell loss	CD4+ /CD8+ CD4- /CD8- CD1+	Most common abnormality		
Present study	76	CD1, 2, 3, 4, 5, 6, 7, 8, 27, UCHL1, F101-01	62 (82)	49 (64)	13 (17)	11 (15)	8 (11)	CD7
Picker <i>et al</i> , 1987 ¹	88	CD1, 2, 3, 4, 5, 7, 8	70 (80)	67 (76)	1 (1)	15 (17)	0	CD7
Borowitz <i>et al</i> , 1986 ¹⁰	27	CD1, 2, 3, 4, 5, 7, 8	18 (67)	14 (52)	5 (19)	2 (7)	0	CD7
Weiss <i>et al</i> , 1985 ¹¹	51	CD1, 2, 3, 4, 5, 7, 8	38 (75)	27 (53)	3 (6)	8 (16)	0	CD5 and CD7
Nasu <i>et al</i> , 1985 ¹²	26	CD1, 2, 3, 4, 5, 7, 8	19 (73)	19 (73)	0	2 (8)	0	CD7
Doggett <i>et al</i> , 1984 ¹³	13	CD1, 2, 3, 4, 5, 7, 8	11 (85)	11 (85)	0	4 (31)	0	CD5
Grogan <i>et al</i> , 1985 ¹⁴	11	CD1, 2, 3, 4, 5, 8	7 (64)	7 (64)	0	4 (36)	0	CD5
Wood <i>et al</i> , 1983 ¹⁵	7	CD1, 2, 3, 4, 5, 8	6 (86)	5 (71)	0	3 (43)	0	CD3 and CD5
van der Valk <i>et al</i> , 1986 ¹⁶	10	CD1, 3, 4, 5, 8	4 (40)	4 (40)	0	0	0	CD5
Jack <i>et al</i> , 1986 ¹⁷	17	CD3, 4, 7, 8	7 (41)	3 (18)	4 (24)	0	0	CD4+ /CD8+
Weis <i>et al</i> , 1986 ¹⁸	40	CD4, 5, 8	9 (23)	4 (10)	2 (5)	3 (8)	0	CD5

In most studies the most common abnormality was an absence of CD7, followed by an absence of CD5.

Pickar *et al*¹⁹ used the monoclonal antibody β -F1 (against a framework epitope of the TCR β chain) in their survey of T lineage lymphomas and reported that expression of this epitope was independent of CD3 in one third of 33 peripheral T cell lymphomas. With the use of monoclonal F101-01 (against a conformational epitope of the TCR/CD3 complex,⁹ we found that this antigen is coexpressed with CD3 in 48 of 63 lymphomas. Five (8%) cases lacked both antigens, and the antigens were expressed independently of each other in 10 (16%) cases. In all but one of these the F101-01 antigen was lost. This suggests that monoclonal antibody F101-01 can help detect aberrant phenotypes.

The phenotype CD4⁺/CD8⁺/TCR⁺ is displayed by fetal thymocytes and also by a minor fraction of adult cortical thymocytes and peripheral T cells.²⁰ Among 11 lymphomas with simultaneous loss of the CD4 and CD8 antigens, eight cases were examined for expression of both CD3 and the TCR. Six of these were TCR negative and half of them also lacked CD3.

In contrast to the findings of Pickar *et al* and others (table 4), who observed coexpression of the CD4 and CD8 antigens only rarely, this phenotype was expressed by 17% of the present cases. Similar results were reported by Borowitz *et al*¹⁰ and Jack *et al*¹⁷ and do not support the view that coexpression of CD4 and CD8 is indicative of lymphoblastic lymphomas.

Eleven per cent of our peripheral T cell lymphomas expressed the CD1 antigen; this antigen has been identified only rarely in other studies.¹⁰⁻¹⁸ The morphology of the CD1 positive lymphomas varied, but none resembled lymphoblastic lymphoma.

It is concluded that the demonstration of aberrant T cell phenotypes is a useful supplement to traditional histological methods for distinguishing between benign and malignant T cell infiltrates. It is recommended that the panel of monoclonal antibodies against T cell differentiation antigens should be fairly large, as apparently any antigen may be lost in the process of malignant transformation.

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