Virchows Arch [Pathol Anat] (1983) 401:289–305

Virchows Archiv A

Characterization of B-cell Non-Hodgkin's lymphomas

A study using a panel of monoclonal and heterologous antibodies

P. van der Valk [1], J. Jansen [2], M.R. Daha [3], and C.J.L.M. Meijer [4, 5]

[1] University Medical Centre, Leiden, Departments of Pathology, [2] Haematology and [3] Nephrology, [4] Stichting Samenwerking Delftse Ziekenhuizen, Department of Pathology Delft and [5] Department of Pathology, Free University Amsterdam, Amsterdam, The Netherlands

Summary. A panel of monoclonal and heterologous antibodies directed against clearly defined antigens was used to characterize the cellular composition of 57 non-Hodgkin's lymphomas, classified according to the Kiel classification, with a slight modification. The antisera were directed against T-lymphocytes and their subsets (Leu1, Leu2a, Leu3a, TA1), B-lymphocytes and their subsets (BA1, BA2, HLA-DR, CR1, sIg), macrophages (TA1, OKM1, anti-human monocyte 1, HLA-DR, CR1), dendritic reticulum cells (CR1, BA2, HLA-DR), interdigitating reticulum cells (HLA-DR, BA1) and Langerhans cells (OKT6, NA1/34). On the basis of the staining pattern of the neoplastic cells with the antibodies used and the nature and number of admixed cells, in particular T-cell subsets, dendritic reticulum cells and macrophages, the NHL could be divided into groups which correspond to the different diagnostic categories of the Kiel classification. Furthermore, the results underlined the existence of intermediate lymphocytic lymphoma as a separate diagnostic category. Histogenetically, the marker pattern of the neoplastic cells and the number and arrangement of the admixed cells are consistent with the view that at least two different lines of B-cell lymphomas can be recognized. One is related to the germinal centre cell reaction (to which B-lymphoblastic (Burkitt type), centroblastic, centroblastic/centrocytic, centrocytic, and intermediate lymphocytic lymphoma, and polymorphic immunocytoma belong) and the other is related to the plasma cell reaction (including chronic lymphocytic leucaemia and lymphoplasmacytoid immunocytoma), whereas B-immunoblastic lymphoma can originate from either line. Thus, polymorphic immunocytoma is a follicle centre cell lymphoma with differentiation into plasma cells rather than a lymphoplasmacytoid immunocytoma with blastic cells.

Key words: B-cell – Non-Hodgkin's lymphomas – Monoclonal antibodies – Dendritic reticulum cells

Offprint requests to: C.J.L.M. Meijer, Department of Pathology, Free University, De Boelelaan 1117, NL-1007 MB Amsterdam, The Netherlands

The recent development of monoclonal antibodies and the introduction of immunohistochemical methods in diagnostic cyto/histopathology have created the possibility of detecting precisely defined antigens on cells in tissue sections. Characterization of the cellular content of the tumour by antibodies with defined specificity can contribute to a better classification of the non-Hodgkin's lymphomas in particular. Furthermore, as it is generally accepted that non-Hodgkin's lymphomas represent neoplastic counterparts of reactions that normally take place in particular compartments of the lymphatic tissue after antigenic stimulation, one may expect that a precise cellular inventory will result in a better understanding of the histogenesis of lymphomas.

To achieve a better insight into the lymphomas it is important to study not only the neoplastic cells, but the number and nature of the admixed cells as well, since it can be expected that certain types of non-Hodgkin's lymphomas will have their own characteristic pattern of admixture of non-neoplastic cells. Most studies with monoclonal antibodies have focused on either the tumour cells (Warnke et al. 1980; Foon et al. 1982; Gajl-Peczalska et al. 1982) or the admixed cells (Dvoretsky et al. 1982). Some studies have evaluated both, but in a restricted number of types of lymphoma (Stein et al. 1981a; Van der Valk et al. 1983), or with a restricted number of monoclonal antibodies (Janossy et al. 1980).

In the present study we have characterized the neoplastic as well as the admixed cells of 57 Non-Hodgkin's lymphomas of B-cell origin by immunohistochemistry on frozen sections using a panel of monoclonal and heterologous antibodies.

The histopathological diagnosis of each lymphoma was supported by the presence of classical markers, such as membrane-bound and/or intracytoplasmic immunoglobulins, receptors for the Fc portion of IgG (Fc) or activated third component of complement (C3), and acid phosphatase and α-naphtyl acetate esterase.

Analysis of the marker profile of the neoplastic cells, as it was found with the antisera used, revealed differences between most types of non-Hodgkin's lymphomas. When the nature and number of admixed cells were also taken into account the differences were even more pronounced. The results support the concept that intermediate lymphocytic lymphoma is a seperate diagnostic entity, and that B-lymphoblastic and centroblastic lymphomas are histogenetically closely related. Moreover, the results indicate that histogenetically polymorphic immunocytoma is a follicle centre cell lymphoma with plasma cell differentiation rather than a subtype of lymphoplasmacytoid immunocytoma.

Material and methods

57 cases of Non-Hodgkin lymphomas were studied. The diagnosis was based on the morphological criteria as described by Lennert (1978). In addition, we distinguished intermediate lymphocytic lymphoma according to the criteria reported by Weisenburger et al. (1981). Since this lymphoma probably originates from the mantle zone of the germinal centre (Nanba et al.

Table 1. Antibodies and their specificity

Antibody	Monoclonal (M) Hetero-logous (H)	Specificity
Leu1[a]	M	95% human thymocytes, ≧95% peripheral T lymphocytes, some sIg^+ B-CLL cells, no normal B-lymphocytes (Wang et al. 1980)
Leu2a[a]	M	suppressor/cytotoxic T-subset (Ledbetter et al. 1981)
Leu3a[a]	M	helper/inducer T-subset (Evans et al. 1981)
TA1[f]	M	100% peripheral blood T-lymphocytes, 70% thymocytes ≧95% peripheral blood monocytes (LeBien and Kersey 1980)
BA1[f]	M	peripheral blood B-lymphocytes, pre-B-ALL and B-CLL cells, granulocytes (Abramson et al. 1981), interdigitating cells (van der Valk et al. submitted)
BA2[f]	M	<1% peripheral blood lymphocytes, lymphohemopoietic progenitor cells in bone marrow, non T-non B-ALL cells (Kersey et al. 1981), dendritic reticulum cells (van der Valk et al. submitted)
HLA-DR[a]	M	B-lymphocytes, monocytes/macrophages, activated T-lymphocytes (Warnke and Levy 1980)
CR1	H	cells having a C3b receptor, i.e. macrophages, dendritic reticulum cells, a subset of B-lymphocytes (Daha et al. 1982)
OKM1[b]	M	peripheral blood monocytes, granulocytes, acute myelomonocytic leukemia cells (Breard et al. 1980)
anti-human[c] monocyte 1	M	peripheral blood monocytes <25% of granulocytes, <25% of platelets (Ugolini et al. 1980)
OKT6[b]	M	70% thymocytes (Reinherz et al. 1980), epidermal Langerhans cells (Murphy et al. 1981)
NA1/34[d]	M	cortical thymocytes, epidermal Langerhans cells, (McMichael et al. 1979)
sIg[e]	H	cells carrying immunoglobulins on their surface, i.e. mainly B-lymphocytes

[a] Purchased from Becton and Dickinson (Sunny Vale, California)
[b] Purchased from Ortho (Raritan, New Jersey)
[c] Purchased from Bethesda Research Laboratories (Bethesda, USA)
[d] Purchased from Seralab (Tilburg, the Netherlands)
[e] Purchased from Dakopatts (Copenhagen, Denmark)
[f] Gifts from Drs. J.H. Kersey and T.W. LeBien (University of Minnesota, Minneapolis, MN, USA)

1977; Weisenburger 1982) a better name would be "mantle zone lymphoma" (Weisenburger 1982).

The histopathological diagnosis was supported by the results in the following techniques:

Immunoperoxidase for the demonstration of intracytoplasmic immunoglobulins.

Immunofluorescence for demonstration of membrane-bound immunoglobulins.

Rosette Assays for demonstration of C3 and Fc receptor.

Enzyme histochemistry for localization of acid phosphatase and α-naphtyl acetate esterase.

Table 2. Summarized staining results of non-Hodgkin's lymphomas

Diagnosis			Tumour					
Type	F/D	*n*	BA1	BA2	HLA-DR	Leu1	CR1	sIg/cIg
CLL	D	5	70–100%	−(4)[a] 30–40%(1)	− 80–100%	(100%)(4) −(1)	−(4) 90%	K(3) $\mu\delta$(5) λ2
LP	D	5	50–90%	few(4) (10%)(1)	60–80%	(100%)	−(4) 5%(1)	K(3) μ(5) λ(2) (δ)1
B-IB	D	7	−/few(4) 50–90%(3)	few/20%(3) 50/100%(4)	60–100%	−	−(3) few/10%(3) (80%)(1)	K(3) μ(5) λ(4) $\alpha\mu$(1) γ(1)
B-LB Burkitt type	D	5	−/few(2) 60–80%(3)	−	(100%)	−	(20–50%)(3) (60–90%)(2)	K(4) μ(3) λ(1) $\mu\delta$(1) α(1)
CB	F D	2 5	−/few(6) 70%(1)	−/few(6) 90%(1)	−/25%(3) 100%	−	(30–70%)(7)	K(5) μ(2) λ(2) γ(5)
CB/CC	F	11	N:70–100%(10) few(1)	few	70–100%	−	−/<5%	K(3) μ(6) λ(8) α(1) γ(1) $\alpha\mu\delta$(2)
			IF: few–10%(3) 10–30%(8)	−	5–20%(3) 30–60%(8)	−	<5(7) 5–10(4)	
CB/CC	D	3	40–80%	few	80–90%	−	5–10%	K(1) γ(1) λ(2) μ(2)
CC	F	1	70%	few	100%	−	(60%)	$K\mu$
CC	D	2	90–100%	few	100%	−(1) (100%)(1)	(40–60%)	$K\mu$
ILL	F	1	90%	60%	70%	−	(90%)	$\lambda\mu$
ILL	D	3	70–80%	60–80%	70–80%	(90–100%)	(70–80%)	K(2) $\mu\delta$(3) λ(1)
PI	D	7	60–80%	few	50–70%	−(6) 50–60%(1)	−(3) 30–60%(4)	K(2) μ(3) γ(2) λ(5) (α) μ(1) μ(δ)(1)

sIg/cIg = membrane-bound and/or cytoplasmic immunoglobulines; F = follicular; D = diffuse; N = noduli; IF = interfollicular area; *n* = number of lymphomas; few = <1%; (%) = weak staining. No tumour cells stained with Leu2a, Leu3a, TA1, OKT6 and NA1/34

[a] Number of lymphomas involved

These methods and materials used for these techniques have been described before (Van der Valk et al. 1981).

The distribution of the lymphomas over the different diagnostic categories was as follows:

- Chronic lymphocytic leukaemia, B-cell type (CLL): 5 cases.
- Lymphoplasmacytoid immunocytoma (LP): 5 cases.
- B-immunoblastic lymphoma (B-IB): 7 cases.
- B-lymphoblastic lymphoma, Burkitt type (B-LB): 5 cases.
- Centroblastic lymphoma (CB): 7 cases.

Admixed cells					
T-cells				Macrophages	
Leu1	Leu2a	Leu3a	Leu3a/Leu2a	aHM1	OKM
5–15%	≦5%	10–15%	2–3:1	few	few
15–25%	5–15%	15–30%	2–3:1(3) 3:1(2)	few	few
few(2) 5–20%(5)	few(5) 5–10%(2)	few(3) 5–15%(4)	1:1(3) 2:1(4)	5%(4) 10–15%(3)	few(6) 5%(1)
≦5%(4) 10%(1)	<5(4) 5–10%(1)	<5%(4) 5–10%(1)	1:1(3) 1:1–2(2)	<5%(3) 5–10%(2)	<5%(4) 5–10%(1)
≦5%(5) 10–15%(2)	≦5%(6) 10%(1)	≦5%(5) 5–10%(2)	1:1(5) 1–2:1(2)	5%(5) 10–20%(2)	≦5(5) 10–20%(2)
N:5–30%	few	5–30%	3:1(4) 6–15:1(6)	few	few
IF:35–70%	10–30%	30–70%	2–7:1	few	few
25–50%	10–20%	25–40%	2–4:1	few	few
10% 10–15%	<5% <5%	<10% 10%	2:1 2:1	few few	few few
15% 25–40%	5% 10–20%	10% 20–30%	2:1 2:1	few few	few few
20–40%	5–20%	15–30%	2–3:1	few	few

- Centroblastic/centrocytic lymphoma (CB/CC): 14 cases (11 nodular, 3 diffuse).
- Centrocytic lymphoma (CC): 3 cases (1 nodular, 2 diffuse).
- Intermediate lymphocytic lymphoma (ILL) or "mantle zone lymphoma": 4 cases.
- Polymorphic immunocytoma (PI): 7 cases.

A lymphoma was only called follicular if nodules were observed in the routinely stained histological sections. These stains included haematoxylin-eosin, Giemsa, PAS, a reticulin stain (Gomori) and an elastin stain (von Gieson).

Demonstration of antigens in frozen sections

Tissue specimens were snapfrozen in liquid nitrogen. Cryostat sections were cut 6 μm thick. The sections were air-dried, fixed for 10 min in acetone, dried again and stored for at least 30 min at −20° C degrees. Subsequently they were dried again, fixed for another 10 min in acetone, and dried. Then an indirect immunoperoxidase procedure was used. The antibodies employed are listed in Table 1. The monoclonal antibodies were raised in mice, the heterologous

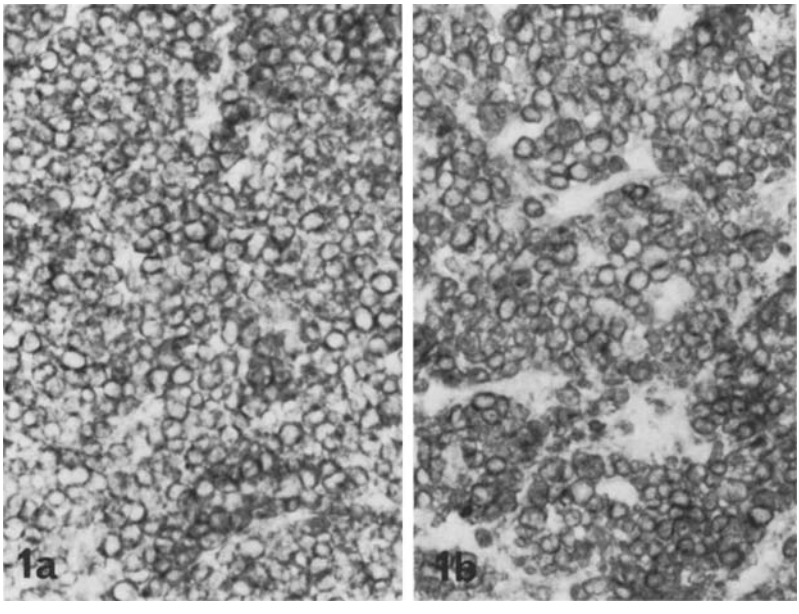

Fig. 1 a, b. General B-cell markers. **a** BA-1 (B-lymphoblastic lymphoma, Burkitt type) ×350. **b** HLA-DR (B-lymphoblastic lymphoma, Burkitt type) ×350

antibodies in rabbits. The specificity of the antisera in tissue sections was tested on normal lymphoid tissue, i.e. lymph node, tonsil, spleen and thymus. For OKT6 and NA $^{1}/_{34}$ normal skin sections were also used. The second step for the mouse antibodies was a rabbit anti-mouse IgG labelled with horse-radish peroxidase (Dakopatts, Copenhagen, Denmark). For the heterologous antibodies a horse-radish peroxidase labelled goat anti-rabbit IgG was used as a second step (Miles Laboratories Inc., Elkhard, IN, USA). The heterologous CR1 antiserum is directed against the C3b receptor (Daha et al. 1982). Human erythrocyte C3b receptor was purified according to Fearon (1979), an antibody was raised in rabbits, and the IgG fraction of this antiserum was used. The surface immunoglobulins were demonstrated with indirect immunofluorescence. The antisera against α, γ, μ, heavy chains and κ and λ light chains were obtained from Dakopatts (Denmark), the anti-δ and the fluorescein-labeled horse anti rabbit IgG, the second step, were purchased from the Central Laboratory of the Blood Transfusion Service (Amsterdam, The Netherlands).

Scoring of the staining results

The percentages of both neoplastic and reactive cells were semiquantitatively estimated in proportion to the total number of cells. In follicular lymphomas the neoplastic follicles and the inter-follicular area were both evaluated.

At least 5 high-power fields (×400) were scored for the estimation of both neoplastic and reactive cells. Two pathologists independently estimated the percentages. Their data, which correlated well, were averaged.

Results

The results of the staining procedures of all cases are summarized in Table 2. The tumour cells of the B-cell lymphomas usually stained with BA1 except

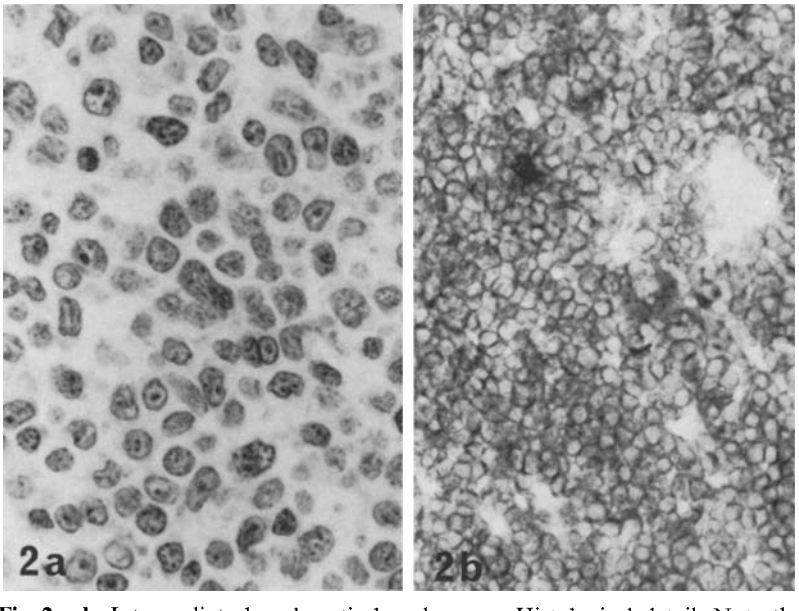

Fig. 2a, b. Intermediate lymphocytic lymphoma. **a** Histological detail. Note the irregularity of the nuclei. Giemsa-stained, methyl-methacrylate embedded ×540. **b** BA-2 staining of the same case. Approximately 70% of the cells stain positive. Staining intensity varies ×350

for 6 out of 7 centroblastic and 1 B-immunoblastic lymphoma and HLA-DR (except one case of centroblastic lymphoma) (Fig. 1). BA2 staining was less common: all the intermediate lymphocytic lymphomas, 5 our of 7 B-immunoblastic and 1 centroblastic lymphomas were positive (Fig. 2). Table 2 also shows the variability of the staining.

In 15 cases Leu1 stained the tumour cells of these B-cell lymphomas, i.e. 4 out of 5 CLL all lymphoplasmacytoid immunocytomas one case of centroblastic/centrocytic lymphoma one centrocytic lymphoma 3 out of 4 intermediate lymphocytic lymphomas and one polymorphic immunocytoma. There was a clear difference between the staining of the tumour cells and reactive T-cells, the latter reacting much stronger. In none of the B-cell lymphomas staining of the tumour cells with Leu2a, Leu3a or TA1 was observed.

With CR1 staining of part of the tumour cells was found in B-lymphoblastic, centroblastic, centrocytic and intermediate lymphocytic lymphoma (Fig. 3). CR1 staining was also seen in 2 cases of CLL in 4 polymorphic immunocytomas (nos. 53, 55, 56, 57) and in 2 B-immunoblastic lymphomas.

The number of T-lymphocytes in the B-cell lymphomas varied (Fig. 4); most large-cell lymphomas and B-lymphoblastic lymphomas (Burkitt type) had small numbers of T-cells (<10%). Moderate numbers (10–15%) of T-cells were found in CLL, centrocytic lymphomas and within the nodules of the centroblastic/centrocytic lymphomas. Abundant T-lymphocytes (>15%, up to 50% in one case) were present in lymphoplasmacytoid and

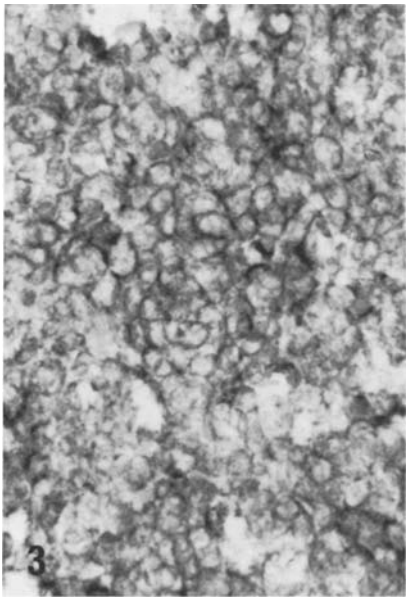

Fig. 3. C_{3b} receptors present on a centroblastic lymphoma as demonstrated with the antibody CR-1. ×350

polymorphic immunocytomas, intermediate lymphocytic and diffuse centroblastic/centrocytic lymphomas. Even higher percentages (over 50%) were found in the interfollicular areas of follicular lymphomas. The ratio of Leu3a and Leu2a positive cells varied slightly, and was approximately 2–3:1 for the small cell lymphomas. The large-cell and Burkitt lymphomas showed a lower ratio, approximately 1:1. Though no double-labeling experiments were done, the results pointed to the presence of HLA-DR positive T-cells in the diffuse centroblastic/centrocytic lymphomas. Polymorphic immunocytoma also appeared to contain HLA-DR positive T-lymphocytes, though not so many.

Macrophages were relatively frequent in the large-cell and Burkitt lymphomas (approx. 5%), the small-cell lymphomas showed few macrophages.

Presence of DRC's was assessed with CR1, BA2 and HLA-DR antisera (Fig. 5). Table 3 lists the results. Many DRC's were present in centroblastic/centrocytic lymphomas, both nodular and diffuse, in centrocytic and intermediate lymphocytic lymphomas, and in 2 centroblastic lymphomas, 2 polymorphic immunocytomas (Fig. 6) and 1 B-immunoblastic lymphoma. The staining revealed a follicular pattern; in the centroblastic/centrocytic and centrocytic lymphomas the pattern showed mostly round or oval follicles, whereas in the other lymphomas, a distorted pattern was seen, showing less, but mostly larger follicles and of irregular shapes, elongated, dumb-bell shaped or with irregular contours. Additionally, some lymphomas had DRC's scattered among the tumour cells, without a clearly follicular structure (Table 3 and 4). Interdigitating cells, identified by HLA-DR and BA1

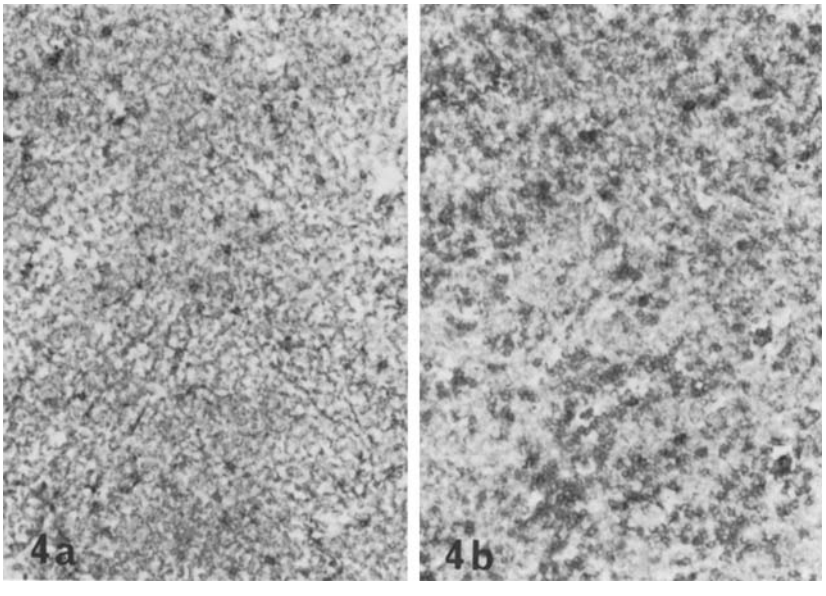

Fig. 4a, b. T-Lymphocytes in B-cell Non-Hodgkin's lymphomas. **a** Moderate number in a centrocytic lymphoma. TA-1 staining × 135. **b** High number in a lymphoplasmacytoid immunocytoma. TA-1 staining × 135

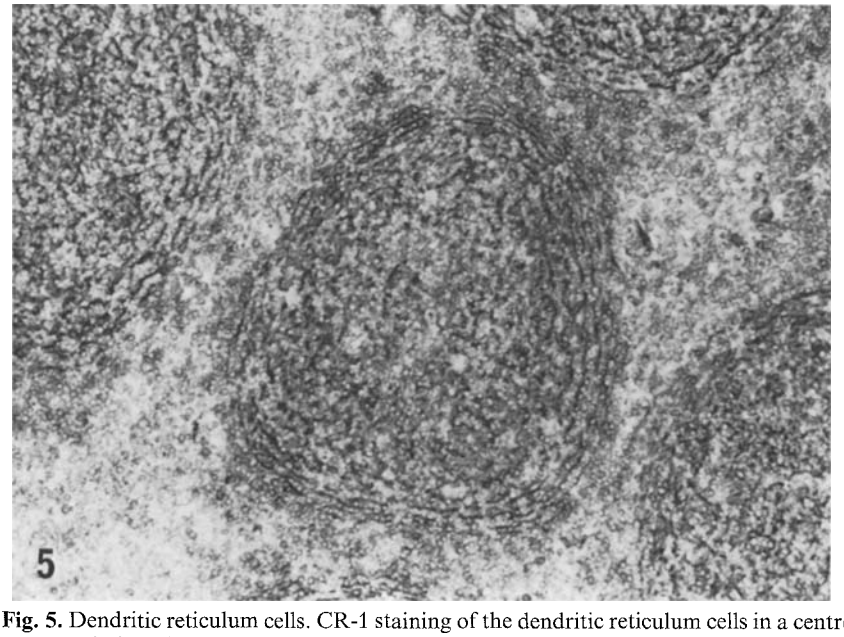

Fig. 5. Dendritic reticulum cells. CR-1 staining of the dendritic reticulum cells in a centroblastic/centrocytic lymphoma. Note the strong, thread-like positivity. The lymphoid cells are largely negative. × 135

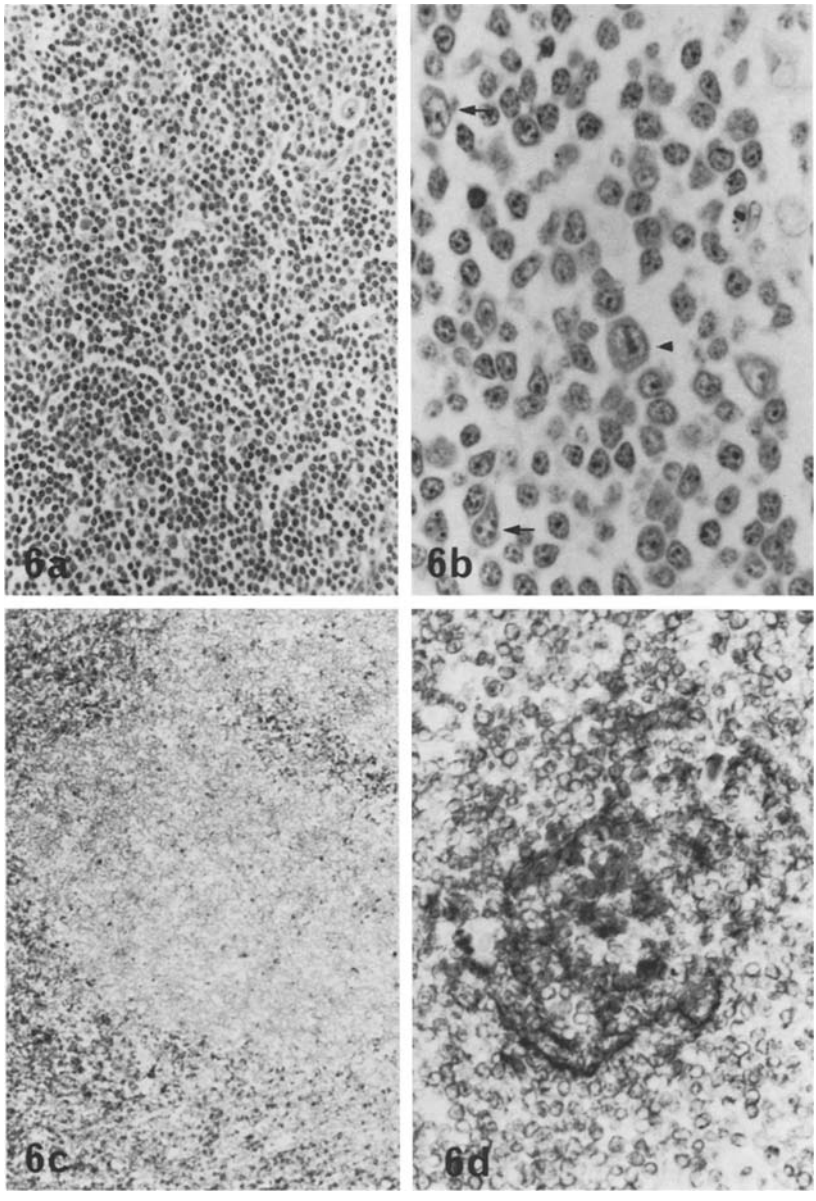

Fig. 6a–d. Polymorphic immunocytoma. **a** *Low-power view*. A diffuse proliferation is seen. Giemsa-stained, methyl-methacrylate-embedded ×135. **b** Histological detail. Centroblasts (*arrows*) and an immunoblast (*arrowhead*) are present. Giemsa-stained, methyl-methacrylate-embedded ×540. **c** *Leu-1 staining*. The distribution of the T-lymphocytes is suggestive for follicularity ×75. **d** *CR-1 staining*. The staining shows follicularly arranged dendritic reticulum cells in this histologically diffuse lymphoma ×300

Table 3. Presence of dendritic reticulum cells in Non-Hodgkin's lymphomas

Lymphoma		Pattern of growth (Histological)	DRC-s		Staining pattern
CLL	($n=5$)	diffuse	$-/+$		$-$
LP	($n=5$)	diffuse	$-$		$-$
B-IB	($n=7$)	diffuse	$-$	($n=3$)	$-$
			$\pm$	($n=3$)	scattered
			$+$	($n=1$)	follicular pattern
LB (Burkitt type)	($n=5$)	diffuse	$\pm$	($n=2$)	scattered
			$+$	($n=3$)	scattered
CB	($n=7$)	nodular ($n=1$)	$++$	($n=1$)	follicular pattern
		diffuse ($n=6$)	$-/\pm$	($n=2$)	$-$
			$\pm$	($n=3$)	scattered
			$+/++$	($n=1$)	follicular pattern
CB/CC	($n=14$)	nodular ($n=11$)	$++$	($n=11$)	follicular pattern
		diffuse ($n=3$)	$+/++$	($n=3$)	distorted follicular pattern
CC	($n=3$)	nodular ($n=2$)	$++$	($n=2$)	follicular pattern
		diffuse ($n=1$)	$++$	($n=1$)	follicular pattern
ILL	($n=4$)	diffuse ($n=4$)	$++$	($n=4$)	distorted follicular pattern
PI	($n=7$)	diffuse ($n=7$)	$-/\pm$	($n=2$)	$-$
			$\pm$	($n=3$)	scattered
			$+$	($n=2$)	follicular pattern

Table 4. Staining pattern of the different Non-Hodgkin's lymphomas

	CLL	LP	IB	LB*	CB	CB/CC		CC	ILL	PI
	$n=5$	$n=5$	$n=7$	$n=5$	$n=7$	$n=11$	$d=3$	$n=3$	$n=4$	$n=7$
HLA-DR	$+++$	$++$	$+++$	$+++$	$-/++$	$+++$	$+++$	$+++$	$+++$	$++$
BA1	$+++$	$+++$	$-/++^{\cdot}$	$-/++^{\cdot}$	$-$	$+++$	$++$	$+++$	$+++$	$+++$
BA2	$-$	$-$	$+$	$-$	$-/++^{\cdot\cdot}$	$-$	$-$	$-$	$++/+++$	$-$
CR1	$-/+++$	$-$	$-$	$-/++^{\cdot}$	$+$	$-$	$-$	$++$	$+++$	$-/+$
LEU1	$-/+++^{\cdot}$	$+++$	$-$	$-$	$-$	$-/+^{\cdot\cdot}$	$-$	$-/++$	$-/+++^{\cdot}$	$-/+^{\cdot\cdot}$
TA1	$-$	$-$	$-$	$-$	$-$	$-$	$-$	$-$	$-$	$-$
T-cells	2	3	$\frac{1}{2}$	1	$\frac{1}{2}$	2	4	2	3	3
Macro-phages	1	1	2	2	2	2	1	1	1	1
DRC	none	none	none/F	few	few/F	F	F	F	F	few/F

$-$ = <25%; $+$ = 25–50%; $++$ = 50–75%; $+++$ = >75%
1 = <5%; 2 = 5–15%; 3 = 15–30%; 4 = >30%
$^{\cdot}$ = usually positive; $^{\cdot\cdot}$ = usually negative; F = follicular pattern; N = nodular; D = diffuse; LB* = LB (Burkitt type)

staining were seen in the interfollicular areas of nodular centroblastic/centrocytic lymphomas only.

Discussion

As shown by others (Janossy et al. 1980; Warnke et al. 1980; Stein et al. 1981a; Foon et al. 1982; Gajl-Peczalska et al. 1982) most B-cell lymphomas stain positively with HLA-DR and BA1. Furthermore, BA2 staining was found on intermediate lymphocytic lymphomas and some large-cell lymphomas, especially B-immunoblastic lymphomas. Gajl-Peczalka et al. (1982) found BA2 on Burkitt-like undifferentiated lymphomas (Rappaport classification), but in our study the Burkitt type lymphomas were all negative for BA2, Leu1 was also found on part of the lymphomas; all lymphoplasmacytoid immunocytomas, all cases but one of CLL and intermediate lymphocytic lymphoma and one case each of polymorphic immunocytoma and centrocytic and centroblastic/centrocytic lymphoma were positive. We never found Leu1 on centroblastic, B-immunoblastic or B-lymphoblastic lymphoma. That some B-cell lymphomas react with the antibody Leu1 has been reported before (Stein et al. 1981a; Foon et al. 1982). Additionally, weak CR1 staining was found on B-lymphoblastic, centroblastic, centrocytic and intermediate lymphocytic lymphomas and some polymorphic immunocytomas; not all cells were stained, except in the intermediate lymphocytic lymphomas, where the majority of the tumour cells were positive. This partial staining of the germinal centre cell lymphomas is in accordance with earlier observations (Stein et al. 1981b), that in these lymphomas approximately half the cells carrying a complement receptor have a receptor for C3b (CR1 only stains cells with a C3b receptor). The only difference from that study is that we did not find substantial percentages of CR1 positive cells in centroblastic/centrocytic lymphomas. It is not easy to assess the number of positive lymphoid cells in these lymphomas, due to the staining of the DRC's with their dendrites between the lymphoid cells. Nevertheless, we found most tumour cells to be negative for CR1. In rosette assays the cells of these lymphomas form EAC rosettes and as this demonstrates both C3b and C3d receptors (Stein 1978) we therefore assume that the majority of the neoplastic cells in centroblastic/centrocytic lymphomas carry other types of C3 receptors, for instance a C3d receptor.

The number of T-lymphocytes in the lymphomas studied was variable. In general, it was low (<5%) in the B-cell large-cell and B-lymphoblastic (Burkitt type) lymphomas, with a ratio of Leu3a to Leu2a positive cells of 1:1. In the small-cell lymphomas the number of T-lymphocytes was moderate to high (10–50%) with a Leu 3a/Leu2a ratio of 2–4:1, which is more similar to the ratio found in peripheral blood (Evans et al. 1981). In the nodular lymphomas the nodules mostly contained few T-lymphocytes. The interfollicular areas, however, contained many T-cells, the numbers comparable to those found in the diffuse centroblastic/centrocytic lymphomas, or even higher. The numbers of T-lymphocytes we found in the nodular lymphomas correspond with those mentioned by Dvoretsky et al. (1982).

Although no double-labeling experiments were done, comparison of adjacent sections stained with HLA-DR, TA1 and Leu1 or Leu3a showed that in some cases of centroblastic/centrocytic lymphoma, especially in the diffuse ones, and in some polymorphic immunocytomas, at least part of the T-lymphomas were HLA-DR positive, pointing to activated T cells. In the diffuse centroblastic/centrocytic lymphomas more than half the T cells were positive for HLA-DR. In the follicular lymphomas this percentage was much lower or no HLA-DR positive cells were seen at all; if they were present they were located in the interfollicular area. Perhaps these activated T-cells represent an immunological reaction against the neoplastic B-lymphocytes, or maybe they exert a modulating influence on the neoplastic B-cells. The finding that lymphomas with many T-cells originate from compartments of the lymphatic tissue that normally contain many T-cells (intermediate lymphocytic lymphoma and the follicle mantle zone, lymphoplasmacytoid immunocytoma and the medullary cords) supports the last-mentioned hypothesis.

Macrophages, as demonstrated by the monoclonal antibodies OKM1, anti-human monocyte 1, and TA1, were scarce in the small-cell lymphomas and frequent in the B-cell large-cell and B-lymphoblastic lymphomas. Their number seems to correlate with a high rate of decay in these rapidly growing, fast dividing lymphomas.

The marker pattern of the tumour cells in conjunction with the numbers, nature and arrangement of the T-lymphocytes and DRC's made it possible to distinguish each diagnostic category (Table 4), underlining the usefulness of monoclonal antibodies in the differential diagnosis of non-Hodgkin's lymphomas, next to classical markers. For instance, not only is the presence of intracytoplasmic immunoglobulins (cIg) useful for the differentiation between CLL (cIg-) and lymphoplasmacytoid immunocytoma (cIg+), but the latter also has more T-cells and as these T-cells are negative for HLA-DR and BA1, has patchy HLA-DR and BA1 staining. Intermediate lymphocytic or mantle zone lymphoma is often difficult to differentiate histologically from centrocytic lymphoma and polymorphic immunocytoma. The presence of BA2 antigens on intermediate lymphocytic lymphoma is very helpful for diagnosis. This is especially important since intermediate lymphocytic or mantle zone lymphoma has a clinical behaviour different from centrocytic lymphoma and polymorphic immunocytoma (Weisenburger 1982). Furthermore these antibodies can help to distinguish centroblastic lymphoma from either B-immunoblastic or B-lymphoblastic lymphoma. Generally, the centroblastic lymphomas are HLA-DR$^+$, BA1$^-$, BA2$^-$ and CR1$^+$. The B-immunoblastic lymphomas usually are HLA-DR$^+$, BA1$^+$, BA2$^+$ and CR1$^-$, whereas the B-lymphoblastic (Burkitt type) lymphomas are mostly HLA-DR$^+$, BA1$^+$, BA2$^-$ and CR1$^+$. These differences are not absolute; actually the similarities, both morphologically and in marker studies have been stressed before (Lennert 1978; Stein et al. 1981b). Nevertheless this panel of monoclonal antibodies can be an additional support in these sometimes very difficult differential diagnoses.

The results of our investigations also hold some histogenetic implica-

tions. Recent insights in non-Hodgkin's lymphomas have led to the assumption that non-Hodgkin's lymphomas are the neoplastic counterparts of reactions which normally take place in certain compartments of the lymphatic tissue after antigenic stimulation (Mann et al. 1979). Apart from the morphology and the marker pattern of the lymphoid cells, the presence and arrangement of certain types of non-neoplastic cells can give additional information about the compartment and the reaction to which a certain type of lymphoma is related. At least two reactions are important for the B-cell lymphomas, i.e. the germinal-centre cell reaction (Wakefield and Thorbecke 1968), taking place in the follicles, and the plasma cell reaction (Veldman 1970), taking place in the non-follicular B-cell areas (Mann et al. 1979; Stein and Tolksdorf 1981). The lymphomas of follicular origin can not only be recognized by their morphology and the marker pattern of the tumour cells, but also by the presence of a nodular growth pattern and by the presence of follicularly arranged DRC's. A nodular growth pattern can be identified in routine histology, especially in the reticulin stain, but in some lymphomas the distribution of T-lymphocytes and/or the arrangement of DRC's show a follicular pattern where the routine histology does not. Using these additional criteria in combination with data from the literature the following lymphomas are likely to be of follicular origin: B-lymphoblastic, Burkitt type lymphomas (mainly data from literature (Mann et al. 1976); DRC's are present, but not in follicular arrangement), centroblastic-, centroblastic/centrocytic-, centrocytic-, (Jaffe et al. 1974) and intermediate lymphocytic lymphoma (Nanba et al. 1977 and Weisenburger et al. 1982). The fact that all above mentioned lymphomas have C3 receptors is an addition argument supporting the view that they are of follicular origin. On the basis of our results (Table 3 and 4) polymorphic immunocytoma should also be considered a follicle centre cell lymphoma, as these tumours contain a varying number of DRC's, sometimes in follicular arrangement, and in Leu1 or TA1 staining the distribution of T-cells suggested follicularity quite often (Fig. 6). The plasmacytoid features must then be interpreted as differentiation of the follicle centre cells into plasmacytoid cells. Thus polymorphic immunocytoma is a follicle centre cell tumour rather than a blastic transformation of lymphoplasmacytoid immunocytoma. This was already suggested by Wright (1982). In spite of the similar terminology lymphoplasmacytoid and polymorphic immunocytoma have to be considered as essentially different tumours. Further studies of clinical behaviour are needed to find out if these histogenetic differences have clinical relevance.

The lymphomas related to the plasma cell reaction are CLL and lymphoplasmacytoid immunocytoma (Mann et al. 1979). These two tumours are closely related morphologically as well as in marker studies (with classical markers, as well as with monoclonal antibodies), as was stressed by several investigators (Lennert 1978; Stein et al. 1981b). The absence of DRC's in both lymphomas only underlines this further.

B-immunoblastic lymphomas may belong to either line. In fact, Lennert (1978) and Lukes and Collins (1975) already mention the existence of a pure B-immunoblastic lymphoma and a B-immunoblastic lymphoma evolv-

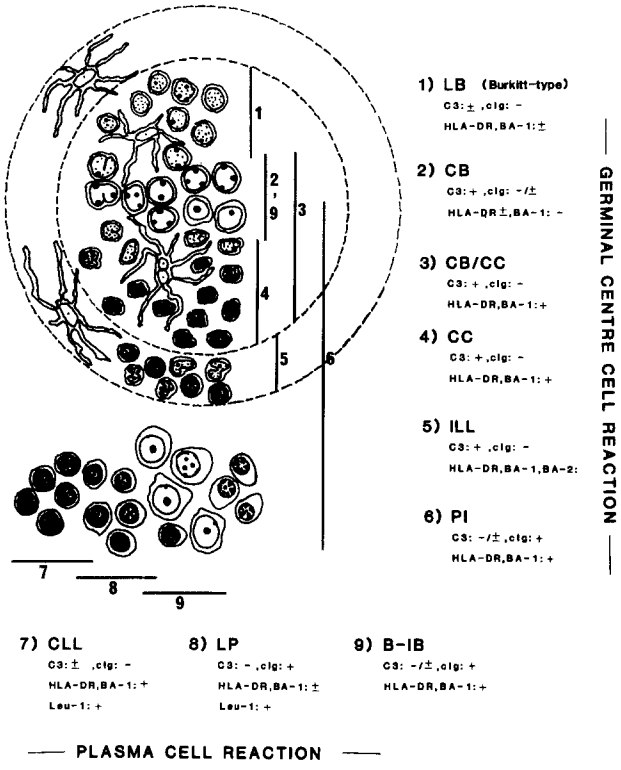

Fig. 7. Scheme of the presumed relationship between B-cell non-Hodgkin lymphomas and the follicular compartment in lymphoid tissues. In this compartment follicular dendritic reticulum cells are found. The outer dotted circle represents a secondary follicle with a mantle zone (area between the outer and inner circles) and a germinal center (area within the inner circle). Lymphoma types 1–6 and 9 are related to the germinal center cell reaction, which takes place in the germinal center after antigenic challenge and are related to certain transformation stages during this reaction. The plasmacell reaction takes place outside the follicular compartment in areas with few or no follicular dendritic cells. Lymphomas 7–9 are related to this reaction. Note that B-immunoblastic lymphoma is related to both types of reactions and that polymorphic immunocytoma and lympho-plasmacytoid immunocytoma are related to different reactions in different compartments

ing from a large-cell follicle centre cell lymphoma. When a centroblastic lymphoma contains a sizeable percentage of immunoblasts, Lennert calls it polymorphic centroblastic. We believe that the pure B-immunoblastic lymphoma is related to the plasma cell reaction as these lymphomas lack any indication of follicularity (i.e. presence of DRC's or T-lymphocytes in a follicular distribution), in contrast to the second type of B-immunoblastic lymphoma which is related to the germinal centre cell reaction. To designate a polymorphic large-cell lymphoma as either centroblastic or B-immunoblastic can be extremely difficult, since it depends on an accurate estimation of the number of immunoblasts. For such a determination morphometry is indicated (van der Valk et al. 1982).

The discussed hypothetical relation of the different types of non-Hodgkin's lymphomas to the germinal centre cell and plasma cell reactions is depicted in Fig. 7.

Acknowledgements. We would like to thank Drs. J.H. Kersey and T.W. LeBien (University of Minnesota) for the gift of the BA1, BA2 and TA1 antisera and we acknowledge the excellent technical assistence of Mrs. G. van den Besselaar-Dingjan, Mrs. J. Cnossen, Mrs. C. de Graaf-Reitsma and Mrs. A. Meeuwissen. Mrs. R.J.J.R. Scholte provided secretarial assistence.

References

Abramson CS, Kersey JH, LeBien TW (1981) A monoclonal antibody (BA1) reactive with cells of the human B lymphocyte lineage. J Immunol 126:83–88

Breard J, Reinherz EL, Kung PC, Goldstein G, Schlossman SF (1980) A monoclonal antibody reactive with human peripheral blood monocytes. J Immunol 124:1943–1948

Daha MR, Kok DJ, Van Es LA (1982) Regulation of the C3 nephritic factor stabilized C3/C5 convertase of complement by purified human erythrocyte C3b receptor. Clin Exp Immunol 50:209–214

Dvoretsky P, Wood GS, Levy R, Warnke RA (1982) T lymphocyte subsets in follicular lymphomas compared with those in non-neoplastic lymph nodes and tonsils. Human Pathol 13:618–625

Evans RL, Wall SW, Platsoucas CO, Siegal FP, Fikrig SM, Testa CM, Good RA (1981) Thymus-dependent membrane antigens in man: inhibition of cell-mediated lympholysis by monoclonal antibodies to TH2 antigen. Proc Natl Acad Sci USA 78:544–548

Fearon DT (1979) Regulation of the amplification C3 convertase of human complement by an inhibitory protein isolated from human erythrocyte membrane. Proc Natl Acad Sci USA 76:5867–5871

Foon KA, Schroff RW, Gale RP (1982) Surface markers on leukemia and lymphoma cells: Recent advances. Blood 60:1–19

Gajl-Peczalska KJ, Bloomfield CD, Frizzera G, Kersey JH, LeBien TW (1982) Diversity of phenotypes of non-Hodgkin's malignant lymphomas. From: B- and T-cell Tumours. UCLA Symposia on Molecular and Cellular Biology Vol 24. Eds. E Vittetta, CF Fox. Acad Press New York, pp 63–67

Janossy G, Thomas JA, Pizzolo G, Granger SM, McLaughlin J, Habeshaw JA, Stansfeld AG, Sloane J (1980). Immuno-histological diagnosis of lymphoproliferative diseases by selected combinations of antisera and monoclonal antibodies. Br J Cancer 42:224–242

Kersey JH, LeBien TW, Abramson CS, Newman R, Sutherland R, Greaves M (1981) p 24. A human leukemia associated and lymphopoietic progenitor cell surface structure identified with monoclonal antibody. J Exp Med 153:726–731

LeBien TW, Kersey JH (1980) A monoclonal antibody (TA1) reactive with human T-lymphocytes and monocytes. J Immunol 125:2208–2214

Ledbetter JA, Evans RL, Lipinski M, Rundles C, Herzenberg LA (1981) Evolution conservation of surface molecules that distinguish T-lymphocyte helper/inducer and cytotoxic/suppressor subpopulations in mouse and man. J Exp Med 153:310–323

Lennert K (1978) Malignant lymphomas, other than Hodgkin's disease. Handbuch der speziellen pathologischen Anatomie und Histologie I/3/B Springer, Berlin Heidelberg New York

Loo EM van der, Valk P van der, Jansen J, Daha MR, Meijer CJLM (1983) Distribution of lymphoid and dendritic cells in lymphatic tissue. Analysis by monoclonal antibodies. Virchows Arch [Cell Pathol]

Lukes RJ, Collins RD (1975) New approaches to the classification of the lymphomata. Br J Cancer 31 suppl II 1–28

Mann RB, Jaffe ES, Braylon RC, Nanba K, Frank MM, Ziegler JL, Berard CW (1976) Non-endemic Burkitt's lymphoma A B-cell tumor related to the germinal centers. N Engl J Med 295:685–691

Mann RB, Jaffe ES, Berard CW (1979) Malignant lymphomas – A conceptual understanding of morphologic diversity. Am J Pathol 94:104–191

McMichael AJ, Pilch JR, Galfre G, Mason DY, Fabre JW, Milstein C (1979) A human thymocyte antigen defined by hybrid myeloma monoclonal antibody. Eur J Immunol 9:205–210

Murphy GF, Ban AR, Sato S, Mihm jr MC, Harrist TJ (1981) A new immunologic marker for human Langerhans cells. N Engl J Med 304:791–792

Nanba K, Jaffe ES, Braylan RC, Soban EJ, Berard CW (1977) Alkaline phosphatase positive malignant lymphomas. A subtype of B-cell lymphomas. Am J Clin Pathol 68:535–542

Reinherz EL, Kung PC, Goldstein G, Levey RH, Schlossman SF (1980) Discrete stages of human intrathymic differentiation: analysis of normal thymocytes and leukemic lymphoblasts of T-lineage. Proc Natl Acad Sci USA 77:1588–1592

Stein H (1978) The immunologic and immunochemical basis for the Kiel classification. In: Lenner K Malignant lymphomas, other than Hodgkin's disease. Springer, Berlin Heidelberg New York

Stein H, Tolksdorf G (1980) Development and differentiation of the T-cell and B-cell systems: a perspective. In: van den Tweel JG, Taylor C, Bosman FT (eds) Malignant lymphoproliferative diseases. Leiden University Press, The Hague/Boston/New York, pp 13–29

Stein H, Mason DY, Gerdes J, Ziegler A, Naiem M, Wernet P, Lennert K (1981) Immunohistology of B-cell lymphomas. In: Knapp W (ed) Leukemia markers, Academic Press, London, pp 99–108

Stein H, Staudinger M, Tolksdorf G, Lennert K (1981) Immunologic markers in the differential diagnosis of non-Hodgkin's Lymphomas. J Cancer Res Clin Oncol 101:29–42

Ugolini V, Nunez G, Smith GR, Stastny P, Capra JD (1980) Initial characterization of monoclonal antibodies against human monocytes. Proc Natl Acad Sci USA 77:6764–6768

Valk P van der, Velde J te, Jansen J, Ruiter DJ, Spaander PJ, Cornelisse CJ, Meijer CJLM (1981) Malignant lymphoma of true histiocytic origin: Histiocytic sarcoma. A morphological, ultrastructural, immunological, cytochemical and clinical study of 10 cases. Virchows Arch [Pathol Anat] 391:249–265

Valk P van der, Hermans J, Brand R, Cornelisse CJ, Spaander PJ, Meijer CJLM (1982) Morphometric characterization of diffuse large-cell (Histiocytic) lymphomas. Am J Pathol 107:327–335

Valk P van der, Mosch A, Kurver PHJ, Meijer CJLM (1983) Morphometric characterization of 52 B-cell Non-Hodgkin's lymphomas. J Clin Pathol 36:289–297

Veldman JE (1970) Histophysiology and electron microscopy of the Immune Ressponse. Thesis Groningen. N.V. Boekdrukkerij Dijkstra Niemeyer

Wakefield JD, Thorbecke GJ (1968) Relationship of germinal centers in lymphoid tissue to immunological memory. J Exp Med 128:153–187

Wang CY, Good RA, Ammirati P, Dymbort G, Evans RL (1980) Identification of p 69, 71 complex expressed on human T-cells sharing determinants with B type chronic lymphocytic leukemic cells. J Exp Med 151:1539–1544

Warnke R, Levy R (1980) Detection of T- and B-cell antigens with hybridoma monoclonal antibodies: a biotin-avidin-horseradish peroxidase method. J Histochem Cytochem 28:771–776

Warnke R, Miller R, Grogan R, Pederson M, Dilley J, Levy R (1980) Immunological phenotype in 30 patients with diffuse large-cell lymphoma. N Engl J Med 303:293–300

Weisenburger DD, Nathwani BN, Diamond LW, Winberg CD, Rappaport H (1981) Malignant lymphoma, intermediate lymphocytic type. A clinicopathologic study of 42 cases. Cancer 48:1415–1425

Weisenburger DD, Kim H, Rappaport H (1982) Mantle-zone lymphoma. A follicular variant of intermediate lymphocytic lymphoma. Cancer 49:1429–1438

Wright DH (1982) The identification and classification of non-Hodgkin's lymphomas: a review. Diagnostic Histopathology 5:73–11

Accepted June 10, 1983