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A histogenesis of malignant lymphoma, small cleaved cell of the B cell type and intermediate lymphocytic lymphoma (mantle zone lymphoma)

An immuno- and enzymehistochemical study*

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Summary. We have studied the histogenesis of malignant lymphoma (ML), small cleaved cell of the B-cell type and intermediate lymphocytic lymphoma (mantle zone lymphoma) by comparing immunophenotypes and ALP-activity of neoplastic cells with those of germinal center cells (follicular center cells) and mantle zone (MZ) cells of secondary follicles in non-neoplastic lymphoid tissues. The neoplastic cells in 3 cases of ML, follicular, small cleaved cell and 1 case of ML, small cleaved cell expressed the phenotypes similar to those of germinal center (GC) B lymphocytes ($SIgM^+$, $B1^+$, $B2^+$, $CALLA^+$, $SigD^-$, $IL\text{-}2R^-$, $Leu\text{-}1^-$ and ALP^-). The neoplastic cells in 2 cases of ML, follicular, small cleaved cell and 12 cases of ML, diffuse, small cleaved cell displayed the characteristic phenotypes of MZ B lymphocytes ($SIgM^+$, $SIgD^+$, $BA\text{-}1^+$, $IL\text{-}2R^+$, $Leu\text{-}1^+$ and ALP^+). The phenotypes of 2 cases of mantle zone lymphoma were closely comparable with those of MZ B lymphocytes. These findings indicate that the histogenesis of ML, small cleaved cell of the B-cell type is heterogeneous and can be divided phenotypically into 2 types (GC B lymphocyte origin and MZ B lymphocyte origin). It is also apparent that intermediate lymphocytic lymphoma (mantle zone lymphoma) is derived from MZ B lymphocytes of secondary follicles.

Key words: B cell lymphoma – Monoclonal antibodies – Immunoenzyme technique – Histogenesis

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Introduction

Malignant lymphoma (ML) small cleaved cell of the B-cell type defined by the International Working Formulation (1982) includes ML, follicular, predominantly small cleaved cell (ML centroblastic-centrocytic (small), follicular by the Kiel classification (Lennert et al. 1978)), and ML, diffuse, small cleaved cell (ML centrocytic (small) by the Kiel classification). Intermediate lymphocytic lymphoma (mantle zone lymphoma) is considered to be a subtype of non-Hodgkin's lymphoma with cytological features between those of small lymphocytic lymphoma and small cleaved cell lymphoma (Weisenburger et al. 1982). In the International Working Formulation, intermediate lymphocytic lymphoma (ILL) has however, been classified as ML, diffuse, small cleaved cell. The cellular origins of these lesions are controversial and not precisely determined. ML, small cleaved cell of the B-cell type is considered to be derived from germinal center cells (follicular center cell, small cleaved cells or centrocytes), ILL, however is thought to be derived from lymphocytes of primary lymphoid follicle and mantle zone of secondary follicles (Nanba et al. 1977; Weisenburger 1984), follicular center cells (Stein et al. 1982) or postfollicular center cells (Cossman et al. 1984). B-cell lymphomas may well represent monoclonal progression arrested at a particular stage of B-cell differentiation (Salmon and Seligmann 1974) and may be distinguished phenotypically by immunological and enzymatic expression that relate to stages of normal B-cell differentiation.

The purpose of this study is to clarify the histo-

Table 1. Monoclonal antibodies used in this study

Monoclonal antibody	Specificity	Cluster designation	Source
Anti-IgM	mu heavy chains		Becton-Dickinson
Anti-IgD	delta heavy chains		Becton-Dickinson
Anti-IgG	gamma heavy chains		Becton-Dickinson
Anti-IgA	alpha heavy chains		Becton-Dickinson
Anti-B1	pan B	CD 20	Coulter Immunology
Anti-B2	C3d-receptor; dendritic reticulum cells subset of B lymphocytes	CD 21	Coulter immunology
NU-B1	unclassified restricted B		Nichirei
BA-1	pan B	CD 24	Hybritech
Anti-CALLA	common ALL antigen	CD 10	Becton-Dickinson
Anti-IL-2R	IL-2R, T-activated	CD 25	Becton-Dickinson
Anti-Leu-1	pan T; small proportion of normal B-cells	CD 5	Becton-Dickinson
Anti-Leu-4	pan T	CD 3	Becton-Dickinson

genesis of ML, small cleaved cell of the B-cell type and ILL (mantle zone lymphoma) by performing immuno- and enzymehistological staining on non-neoplastic and neoplastic lymphoid tissues.

Materials and methods

20 cases of malignant lymphoma (ML), small cleaved cell of the B-cell type and ILL (mantle zone lymphoma) and 7 cases of non-neoplastic lymphoid tissue (5 tonsils and 2 lymph nodes) were used for the present study. ML, small cleaved cell of the B-cell type was classified according to the criteria of the International Working Formulation (1982) and a diagnosis of ILL (mantle zone lymphoma) was made on the basis of the histological criteria by described by Weisenburger et al. (1982). The series included 5 cases of ML, follicular, small cleaved cell (ML, centroblastic-centrocytic, follicular by the Kiel classification), 13 cases of ML, diffuse, small cleaved cell (ML, centrocytic by the Kiel classification) and 2 cases of mantle zone lymphoma. All tissues were fixed in PLP (periodate-lysin-paraformaldehyde) fixative (McLean and Nakane 1974) for 4 h and snap-frozen at −70° C. Five-micron sections were cut and placed on albumin-coated slides before immunostaining.

All mouse monoclonal antibodies used in this study are identified in Table 1. Normal swine serum, swine anti-rabbit immunoglobulin and peroxidase-antiperoxidase complex (PAP) were purchased from Dakopatts (Copenhagen, Denmark). Rabbit anti-mouse IgG and rabbit anti-mouse IgM were obtained from Cappel Products (West Chester, Pennsylvania). Avidin-biotin-peroxidase complex (ABC kit) was from Vector Laboratories Inc., (Burlingame, California).

Immunohistochemical staining was performed by using the PAP method of Sternberger et al. (1970) with some modifications, and the ABC method of Hsu et al. (1981). Immunoelectron microscopy was carried out using a pre-embedding method with certain modifications. The simultaneous staining of different lymphocyte surface antigens was performed by a modification of the double immunoenzymatic labelling technique using two different substrates for horseradish peroxidase conjugated antibodies as described by Si et al. (1983). 3-amino-9-ethylcarbazol (AEC) with a red color reaction and 4-chloro-1-naphthol with a blue one were used as the reaction substrates.

For the alkaline phosphatase (ALP) reaction sections were incubated in ALP medium containing naphthol AS-BI phosphoric acid (10 mg) and fast red violet LB (10 mg) in 0.2 M Tris-HCl buffer, pH 9.3. After 30 min incubation at 37° C, the slides were rinsed in distilled water and subsequently mounted. Double staining with ALP reaction and immunohistochemical staining were performed for identifying ALP^+ $B1^+$ cells.

Controls for method specificity were established by the omission of primary antibody or the replacement of primary antibody with BALB/C mouse serum.

Results

Immuno- and enzymehistochemistry of non-neoplastic lymphoid tissues are shown in Table 2. Distinct staining patterns were clearly discernible after the use of monoclonal antibodies with the PAP or ABC methods as well as after the ALP reaction. The staining patterns of the tonsils and lymph nodes were similar. Anti-IgM reacted with germinal center (GC) cells and mantle zone (MZ) lymphocytes, showing intense membranous staining. In addition, intercellular staining in the GCs was also observed. In contrast with the expression of IgM throughout the lymphatic follicles, expression of IgD was more limited. Anti-IgD reacted intensely with MZ lymphocytes, but not with GC cells. Anti-IgG reacted with small numbers of GC cells, showing membranous and cytoplasmic staining and with intercellular material in the GCs. Anti-IgA reacted with a few GC cells, and there appeared to be some intercellular staining as well.

Table 2. Phenotypic expression of B lymphocytes in lymphoid tissues (tonsils and lymph nodes)

		Germinal center	Mantle zone	Interfollicular area
SIgM		++	++	+
SIgD		−	++	+/−
SIgG		+/−	−	+/−
SIgA		−	−	−
B1	(CD 20)	++	++	+
B2	(CD 21)	+	+	+
NU-B1		+	++	+
BA-1	(CD 24)	−	++	+
CALLA	(CD 10)	++	+/−	−
IL-2R	(CD 25)	−	+/−	−
Leu 1	(CD 5)	−	+/−	+/−
ALPase		−	++	+/−

++ = many cells stained; + = moderate number of cells stained; +/− = cells occasionally stained; − = negative

MZ lymphocytes showed no staining with anti-IgA.

Anti-B1 (CD20) showed prominent membranous staining of GC cells and MZ lymphocytes, and also some intercellular staining in the GCs. Anti-B2 (CD21) reacted with the minority of MZ lymphocytes and GC cells; there was also marked intercellular staining with reticular patterns in some areas of the MZs and in the GCs. BA-1 (CD24) reacted with MZ lymphocytes but not with GC cells. NU-B1 reacted with MZ lymphocytes and stained a minority of GC cells in a hazy, peripheral manner. Anti-CALLA (CD10) showed hazy and diffuse staining of the GCs but did not react with MZs. Immuno-electronmicroscopically, however, the majority of GC cells and a small number of MZ lymphocytes were CALLA positive. Positive reaction products were observed in the cell surface, rough endoplasmic reticulum and perinuclear space in a pointillistic pattern.

IL-2R$^+$ (CD25) B lymphocytes and Leu-1$^+$ (CD5) B lymphocytes were identified by the double immunoenzymatic labelling technique. Only a very limited number of IL-2R$^+$ B lymphocytes were seen in the MZ, mostly adjacent to the interfollicular areas. No IL-2R$^+$ B lymphocytes were seen in the GCs. There were few scattered Leu-1$^+$ B lymphocytes in the MZs and interfollicular areas adjacent to the MZs of secondary follicles and none seemed to be present in the GCs. IL-2R$^+$ B lymphocytes and Leu-1$^+$ B lymphocytes were of small or medium-sized cell type. ALP$^+$ lymphocytes were seen mostly in the MZs, and a smaller number of positive cells were scattered through some interfollicular areas adjacent to the MZs. Fibroblastic reticulum cells in the interfollicular areas also showed ALP-positivity. The double staining

Table 3. Immunologic phenotypes and ALP-activity of malignant lymphoma, small cleaved cell of the B cell type and mantle zone lymphoma

Case No.	Histologic type	Phenotypic expression										
		SIgM	SIgD	B1 (CD 20)	B2 (CD 21)	NU-B1	BA-1 (CD 24)	CALLA (CD 10)	IL-2R (CD 25)	Leu 1 (CD 5)	Leu 4 (CD 3)	ALPase
1	FSC	+	−	+	−	+	−	−	−	−	−	−
2	FSC	+	−	+	−	+	−	+	−	−	−	−
3	FSC	+	+	+	+	+	+	−	+	−	−	−
4	FSC	+	−	+	+	+	+	−	+/−	−	−	+
5	FSC	+	−	+	+	+	−	+	−	−	−	−
6	DSC	+	−	+	−	+	−	−	−	−	−	−
7	DSC	+	+	+	−	+	+	−	−	−	−	−
8	DSC	+	−	+	−	−	−	−	+	−	−	−
9	DSC	+	+/−	+	−	−	−	−	+/−	−	−	−
10	DSC	+	+	+	−	+	+	−	+	−	−	−
11	DSC	+	+	+	−	+	−	+	−	−	−	+
12	DSC	+	−	+	−	+	−	−	−	+	−	−
13	DSC	+	−(SIgA$^+$)	+	−	+	+	−	−	+	−	−
14	DSC	+	+	+	+	−	−	−	−	+	−	−
15	DSC	+	+	+	+	−	+	−	+	−	−	−
16	DSC	+	−	+	+	+	+	−	−	+	−	−
17	DSC	+	+	+	−	+	+	−	+	+	−	+
18	DSC	+	−	+	−	+	+	−	−	−	−	+
19	MZL	+	+	+	−	+	+/−	−	+	−	−	+
20	MZL	+	+	+	−	+	+	−	−	+/−	−	+/−

FSC: Malignant lymphoma, follicular, small cleaved cell; DSC: Malignant lymphoma, diffuse, small cleaved cell; MZL: Mantle zone lymphoma (intermediate lymphocytic lymphoma)

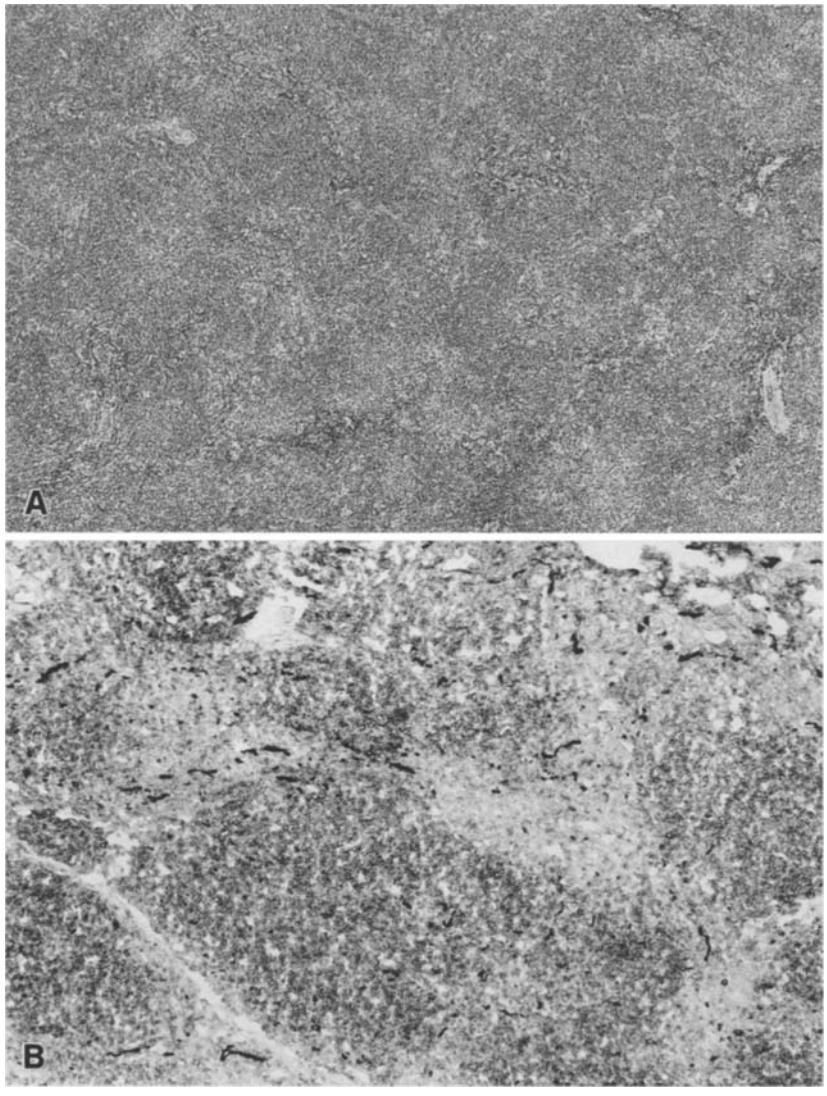

Fig. 1A, B. ML, follicular, small cleaved cell (Case 4).
A Lymph node, showing a follicular (nodular) proliferation of neoplastic cells (Haematoxylin-eosin, ×12).
B The neoplastic follicles are positive for ALP (×40)

technique with ALP reaction and B1 immunostaining confirmed that ALP^+ lymphocytes expressed B1 antigen.

The results of immunohistochemical staining and ALP reaction on 20 cases of ML, small cleaved cell of the B-cell type and ILL (mantle zone lymphoma) are shown in Table 3.

In the 5 cases of ML, follicular, small cleaved cell the neoplastic cells in 3 cases (cases 1, 2 and 5) expressed surface immunoglobulin M (SIgM) and pan B-cell antigens, B1 and NU-B1. Two cases (case 2 and 5) showed CALLA positivity. Cases 3 and 4 expressed SIgM, B1, B2, NU-B1 and BA-1. In addition, case 3 also displayed a positive reaction with anti-IgD and anti-IL-2R, and case 4 expressed IL-2R and ALP-activity (Figs. 1A and B). All 5 cases lacked reactivity to anti-Leu-1 (CD 5) and anti-Leu-4 (CD 3).

In ML, diffuse, small cleaved cell (13 cases) the neoplastic cells were positive with anti-B1 but negative with anti-Leu-4. All cases expressed surface immunoglobulin (SIg); 4 cases (cases 6, 8, 12 and 18) expressed SIgM only, 8 cases (cases 7, 9, 10, 11, 14, 15, 16 and 17) showed the marker expression of both SIgM and SIgD and 1 case (case 13) reacted with anti-IgM and anti-IgA. Six cases (cases 8, 9, 10, 15, 17 and 18) expressed IL-2R and 5 of them showed the marker expression of both SIgM and SIgD. Five cases (cases 12, 13, 14, 16 and 17) expressed Leu-1 antigen (Figs. 2A and B); 2 cases (cases 14 and 16) displayed the expression of both SIgM and SIgD and in 1 case (case 17) SIgM, SIgD and ALP-activity were found on the neoplastic cells. 4 cases (cases 7, 11, 17 and 18) were positive for ALP; 3 cases (cases 7, 11 and 17) expressed both SIgM and SIgD and 2 cases (cases 17 and 18) expressed IL-2R. Case 6 was positive with anti-IgM, anti-B1 and NU-B1. The

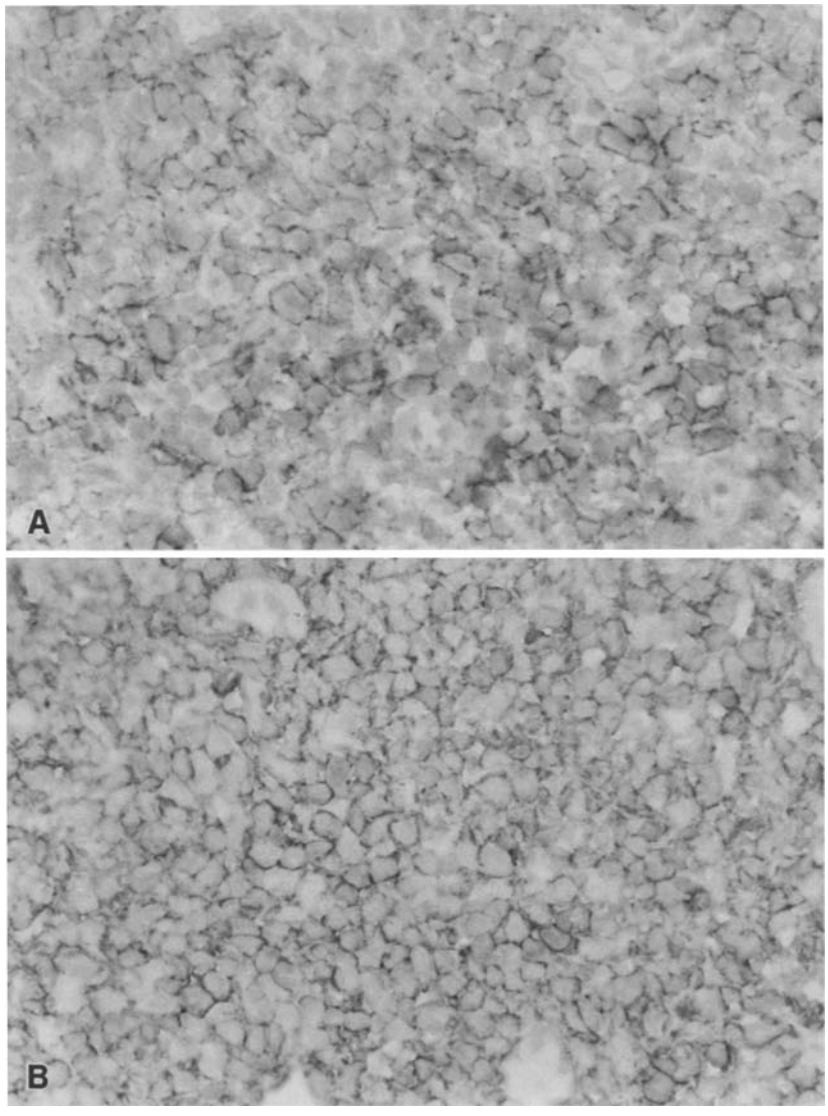

Fig. 2A, B. ML, diffuse, small cleaved cell (Case 14).
A The neoplastic cells react with anti-IgD (× 462) and
B with anti-Leu-1 (CD 5) (× 462)

marker expression was compatible with that of GC cells.

In ILL (mantle zone lymphoma) the neoplastic cells in both cases (cases 19 and 20) showed the marker expression of SIgM, SIgD, B1, NU-B1, BA-1 and ALP (Figs. 3A and B). In addition, cases 19 and 20 expressed IL-2R and Leu-1 respectively. Anti-B2 reacted with dendritic reticulum cells but not with neoplastic cells.

Discussion

Immunohistochemical and histochemical studies on non-neoplastic lymphoid tissues revealed that MZ B lymphocytes were characterized by the marker expression of SIgM, SIgD, BA-1 and ALP. Moreover, it was confirmed by double staining method that only a very limited number of IL-2R$^+$ B lymphocytes and Leu-1$^+$ B lymphocytes were found in the MZs and around the MZs of secondary follicles. However, GC B lymphocytes expressed of SIgM, B1, B2, NU-B1 and CALLA.

The distribution of SIgM$^+$-, SIgD$^+$-, B1$^+$-, BA-1$^+$- and CALLA$^+$-B lymphocytes was roughly comparable with those reported previously by Bhan et al. (1981), Hsu et al. (1984) and Wakasa (1986). Although Weisenburger et al. (1987) reported that mantle and germinal center cells in the reactive lymph nodes were negative for CALLA, immunoelectron microscopic study confirmed the presence of CALLA on only a limited number of MZ lymphocytes, and on the majority of GC cells in non-neoplastic lymphoid tissue. The distribution of IL-2R$^+$ B lymphocytes and ALP$^+$ lymphocytes was different from that found by Hsu et al. (1985)

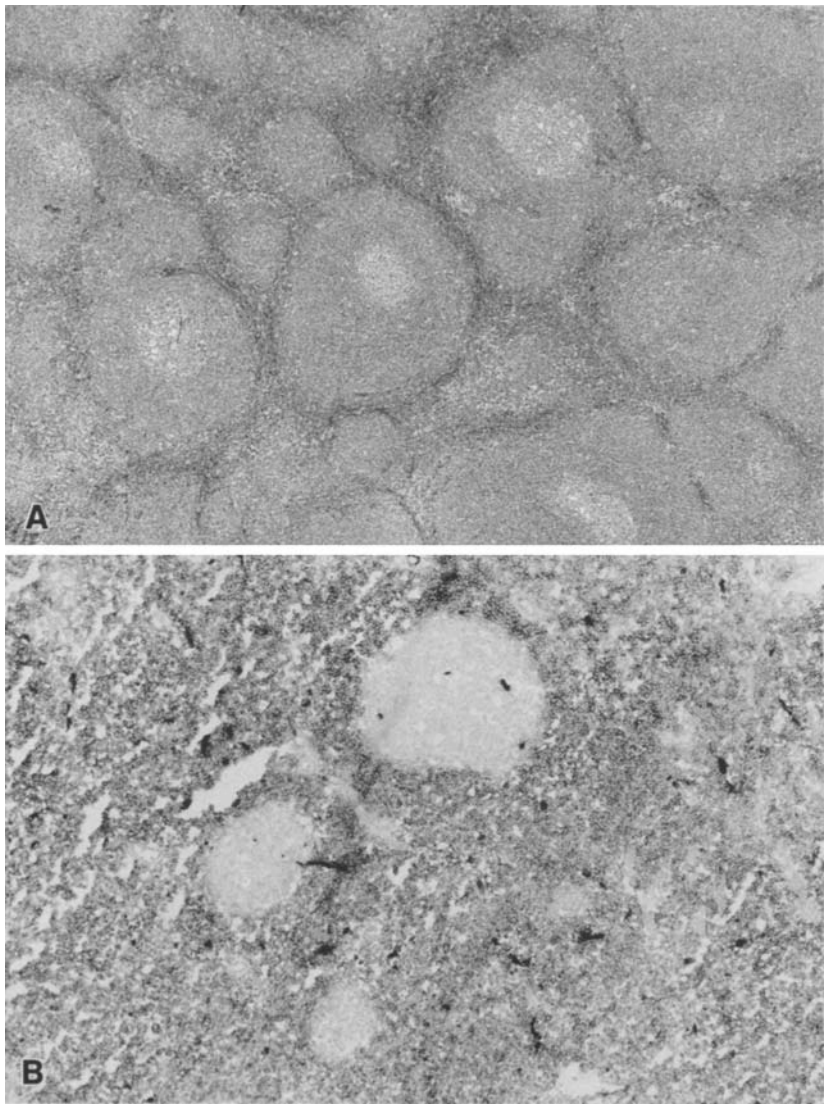

Fig. 3A, B. Mantle zone lymphoma (Case 19). **A** Lymph node, showing an isolated germinal center surrounded by atypical lymphoid cells (Haematoxylin-eosin, ×20).
B ALP activity is found on the neoplastic cells, but not on the benign germinal center (×50)

and Hofman et al. (1985) but that of ALP^+ lymphocytes was similar to the patterns described by Nanba et al. (1979) and Poppema et al. (1980), who found ALP^+ cells in both the primary follicles and the MZ of secondary follicles. Hsu et al. (1985) found that IL-2R and ALP were expressed exclusively on marginal zone B cells in the spleen. In addition, Hofman et al. (1985) described the marker expression of IL-2R on interfollicular B cells and GC cells as well as on MZ B cells. However, in the present study double immunohistochemical staining indicates that $IL\text{-}2R^+$ B lymphocytes are confined to the MZ of secondary follicles and that the majority of ALP^+ lymphocytes are localized in the MZ. Hence, IL-2R and ALP as well as SIgD may be useful markers in determining B cell lymphomas of MZ B lymphocyte origin.

Leu-1 antigen is expressed on certain B lymphocytes, even though this antigen was previously thought to be restricted to T lymphocytes. $Leu\text{-}1^+$ B cells are considered to be part of normal B cell development in the fetus (Bofill et al. 1985; Antin JH 1986) but their role is still unknown. They are found exclusively in large numbers in the fetal follicles of the spleen, lymph nodes and tonsils (Bofill et al. 1985), while in normal adult lymphoid tissues (spleen and lymph node) only a few $Leu\text{-}1^+$ B cells are found (Goffi et al. 1983). However, the distribution of these cells is not clear. The present study using double immunoenzymatic labelling, indicates that almost all of the $Leu\text{-}1^+$ B cells are present in the MZs and in the interfollicular areas adjacent to the MZs of secondary follicles in normal adult lymph nodes and tonsils. However, this finding differs from that of Weisenburger et al. (1987), who reported that Leu-1 antigen was not found

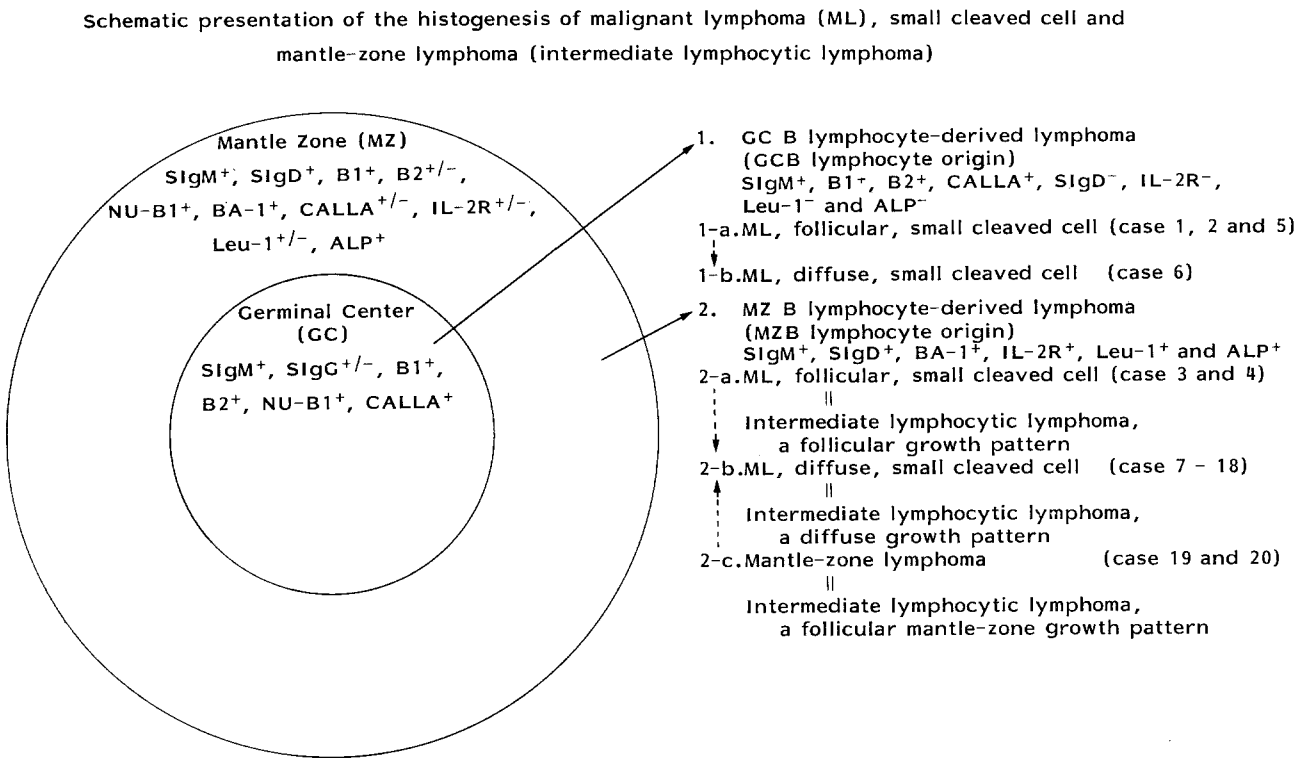

Fig. 4. Schematic presentation of the histogenesis of ML, small cleaved cell of the B cell type and intermediate lymphocytic lymphoma (mantle zone lymphoma). ML, small cleaved cell of the B cell type is derived from distinct populations of B cells (GC B lymphocyte and MZ B lymphocyte). Mantle zone lymphoma is derived from MZ B lymphocytes. ML, follicular, small cleaved cell of MZ B lymphocyte origin corresponds to a follicular variant of intermediate lymphocytic lymphoma (ILL). ML, diffuse, small cleaved cell of MZ B lymphocyte corresponds to a diffuse variant of ILL. Mantle zone lymphoma corresponds to a folicular mantle-zone variant of ILL. A follicular and a follicular mantle-zone growth patterns may progress into a diffuse pattern

on the mantle and germinal center cells of secondary follicles. This difference may be attributable to the technical methods used.

Leu-1 antigen may be a useful marker for distinguishing MZ B lymphocytes from GC B lymphocytes or for separating ILL from the diffuse follicular center cell (FCC) lymphomas (Weisenburger et al. 1987). In any event, it appears imperative that double immunoenzymatic labelling for identifying IL-2R$^+$ B cells and Leu-1$^+$ B cells should be performed, because of the expression of IL-2R and Leu-1 on T cells.

A total of 20 cases with ML, small cleaved cell of the B-cell type and ILL (mantle zone lymphoma) were studied immunohistochemically and histochemically in order to determine the cellular origin of the neoplastic cells, subsequently, it was determined that such lymphomas can be divided into 2 subgroups according to their cellular characteristics (Fig. 4). The first subgroup is derived from GC B lymphocytes. The neoplastic cells display the characteristic phenotypes of GC B lymphocytes (SIgM$^+$, B1$^+$, B2$^+$, NU-B1$^+$, CALLA$^+$, SIgD$^-$, IL-2R$^-$, Leu-1$^-$ and ALP$^-$). The cell type is apparently a small cleaved cell with an irregular nuclear contour. Three follicular lymphomas (cases 1, 2 and 5) and 1 diffuse lymphoma (case 6) belong to this subgroup. ML, diffuse, small cleaved cell type in this subgroup might have evolved from ML, follicular, small cleaved cell type.

Another subgroup is derived from MZ B lymphocytes. The neoplastic cells express the characteristic phenotypes of MZ B lymphocytes (SIgM$^+$, SIgD$^+$, BA-1$^+$, IL-2R$^+$, Leu-1$^+$ and ALP$^+$). The cells are medium-sized similar to MZ lymphocytes, possessing a round or a slightly irregular and indented nucleus. Two follicular lymphomas (cases 3 and 4), 12 diffuse lymphomas (cases 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17 and 18) and 2 mantle zone lymphomas (cases 19 and 20) belong to this subgroup. The neoplastic cells almost always express at least 1 of the 4 phenotypic markers of SIgD, IL-2R, Leu-1 and ALP, and sometimes 2 or even all 4 markers. It is of interest that 2 cases with follicular lymphomas (cases 3 and 4) showed the marker expression of SIgD, IL-2R and ALP characteristic of MZ B lymphocytes. Follicular lymphomas are usually considered to be of follicu-

lar center cell origin (Jaffe et al. 1974; Lukes and Collins 1975). However, our data suggest that follicular lymphomas are not always derived from follicular center cells (GC cells) and some MZ B lymphocyte-derived lymphomas may reveal a follicular pattern.

Mantle zone lymphoma is characterized histologically by a proliferation of small or medium-sized lymphoid cells revealing wide mantle-zone growth around benign-appearing germinal centers. It is a variant of ILL (Weisenburger 1984). In regard to the cellular origin of mantle zone lymphoma, some have reported that the neoplastic cells are derived from the B lymphocytes of primary follicles or mantle zones of secondary follicles (Nanba et al. 1977; Weisenburger 1984), while others have postulated that the neoplastic cells are derived from follicular center cells (Stein et al. 1982) or post-follicular center cells (Cossman 1984). In addition, Van den Oord et al. (1986) have proposed that mantle zone lymphoma probably arises from marginal zone lymphocytes expressing SIgM and ALP, which are located in the outerlayer of the lymphocytic corona or are scattered throughout the corona in the lymph node. The present data indicate that mantle zone lymphoma arises from MZ B lymphocytes of the secondary follicles since the neoplastic cells show the characteristic phenotypes of MZ B lymphocytes ($SIgM^+$, $SIgD^+$, $BA\text{-}1^+$, $IL\text{-}2R^+$, $Leu\text{-}1^+$ and ALP^+). This indicates that mantle zone lymphoma may represent a follicular mantle-zone variant of MZ B lymphocytes-derived lymphomas, as reported by Weisenburger et al. (1982). From the above findings, MZ B lymphocyte-derived lymphomas may express a follicular, a follicular mantle-zone or a diffuse-growth pattern. Follicular and follicular mantle-zone growth patterns may progress to a diffuse pattern (Palutke et al. 1982; Weisenburger 1984). ML, follicular, small cleaved cell of MZ B lymphocyte origin corresponds to a follicular variant of ILL, and ML, diffuse, small cleaved cell of MZ B lymphocyte origin corresponds to a diffuse variant of ILL (Fig. 4).

Five cases of diffuse lymphomas (cases 12, 13, 14, 16 and 17) and 1 case of mantle zone lymphoma (case 20) expressed Leu-1 antigen. Most cases showed a conversion to a CLL-like pattern with neoplastic cells in the peripheral blood. The Leu-1 antigen is expressed on a small number of lymphomas and leukaemias of B-cell origin as well as on certain normal B cells (Kamoun et al. 1981; Foon et al. 1982; Caligaris-Cappio et al. 1982; Foon and Todd 1986), although this antigen was originally defined as pan-T antigen. B-cell type chronic lymphocytic leukaemias (B-CLL) and small lymphocytic lymphomas (or well differentiated lymphocytic lymphomas, WDL) often express Leu-1 antigen. In recent years Van den Oord et al. (1986) and Weisenburger et al. (1987) found the presence of Leu-1 antigen on mantle zone lymphoma or on ILL. Cossman et al. (1983) reported that Leu-1 antigen was found in ILL and WDL but not in follicular center cell lymphomas. In addition, Gobbi et al. (1983) reported that RFA-1 (similar to Leu-1) reacted exclusively with B-CLL but not with centroblastic-centrocytic lymphomas. However, Burns et al. (1983) described that Leu-1 antigen was expressed on follicular lymphomas, ML, diffuse large cell type and large cell immunoblastic type as well as on ML, diffuse small and intermediate lymphocytic type. The present study revealed that the $Leu\text{-}1^+$ B lymphocyte was a small or medium-sized cell type confined to the MZs and interfollicular area adjacent to the MZs of secondary follicles, and $Leu\text{-}1^+$ B cell lymphomas displayed the characteristic phenotypes of the MZ B lymphocyte. In addition Engleman et al. (1981) reported that Leu-1 antigen could not be found on transforming follicular center cells in a number of reactive follicular hyperplasias. $Leu\text{-}1^+$ B cell lymphoma, small cleaved cell type, therefore, may represent the neoplastic counterpart of $Leu\text{-}1^+$ B lymphocytes, especially $Leu\text{-}1^+$ MZ B lymphocytes, and $Leu\text{-}1^+$ B cell lymphoma, large cell type comprising ML, follicular, large cell (ML, centroblastic-centrocytic (large), follicular by the Kiel classification), ML, diffuse, large cell (ML, centrocytic-centrocytic (large), diffuse; centrocytic (large), and centroblastic, diffuse by the Kiel classification) and ML, large cell, immunoblastic (ML, immunoblastic by the Kiel classification) may occur as a result of a transformation of $Leu\text{-}1^+$ small or medium-sized neoplastic B cells or as a result of a malignant transformation of $Leu\text{-}1^+$ small or medium-sized B cells.

It is difficult histologically to distinguish between lymphomas of MZ B lymphocyte origin and lymphomas of GC cell origin, even though there may be present some difference in the morphology of neoplastic cells. Cossman et al. (1984) reported that the major difference in marker expression between ILL and FCC lymphoma was Leu-1 antigen. Weisenburger et al. (1987) reported that the presence of Leu-1 antigen and the absence of CALLA clearly separated ILL from the diffuse FCC lymphomas. However, Cossman et al. (1984) and Jaffe et al. (1987) consider that a variable percentage of cases of ILL express CALLA. In addition, the CALLA-positive neoplastic cells in case 11 simultaneously displayed the characteristic phenotypes of MZ B lymphocytes ($SIgD^+$ and ALP^+).

The present data indicate that CALLA is present not only on the majority of GC cells but on a few MZ lymphocytes in the secondary follicles of reactive lymph nodes and tonsils. These findings would suggest that CALLA$^+$ B cell lymphoma, small cleaved cell type (case 11), may represent a neoplastic proliferation of CALLA$^+$ MZ B lymphocytes. The presence and absence of CALLA is not always useful for distinguishing ILL from the diffuse FCC lymphoma. It is necessary, therefore, to detect the marker expression of SIgD, IL-2R and ALP as well as Leu-1 and CALLA on the neoplastic cells in order to separate ILL from the FCC lymphomas, since Leu-1 is not always expressed on the neoplastic cells of MZ B lymphocyte origin.

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