Virchows Arch. A Path. Anat. and Histol. 384, 149–158 (1979)

Virchows Archiv A

Histologic and Immunohistochemical Findings in the Differential Diagnosis of Chronic Lymphocytic Leukemia of B-Cell Type and Lymphoplasmacytic/Lymphoplasmacytoid Lymphoma

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Summary. 114 cases of malignant lymphoma consisting chiefly of lymphocytes were classified by histology as chronic lymphocytic leukemia of the B-cell type (B-CLL) or lymphoplasmacytic/lymphoplasmacytoid lymphoma (LP immunocytoma) and investigated with the immunoperoxidase-bridge (PAP) method for the presence of heavy and light immunoglobulin chains. Fifteen cases were excluded because they showed a completely negative reaction, which might have been an artifact. Of the remaining 99 cases, 46 revealed polyclonal immunoglobulin-positive plasma cells only and could be clearly classified as B-CLL. In 33 cases there were a moderate or large number of plasma cells or plasmacytoid cells with monoclonal intracytoplasmic positivity. Two heavy chain classes were demonstrated in three other cases, and both light chain types were detected in one case. These 37 cases were finally classified as LP immunocytoma. Ten cases contained only a few monoclonal plasmacytoid cells and were interpreted as borderline cases between B-CLL and LP immunocytoma. Six cases have not yet been clarified – there was an inexplicable discrepancy between their histology and immunostaining.

In LP immunocytoma, the heavy chain class demonstrated most often was the μ chain (27 cases). Light chains of the κ type were about 2.5 times as common as λ chains.

The differential diagnostic criteria for distinguishing B-CLL from LP immunocytoma are discussed and compared. PAS-positive tumor cells are an almost definite criterion of LP immunocytoma. At present, a critical evaluation of the results of PAP immunostaining is the most reliable way to clearly distinguish B-CLL from LP immunocytoma.

Key words: Chronic lymphocytic leukemia – LP immunocytoma – Histology – Immunohistochemistry.

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** Supported by the Kind-Philipp-Stiftung

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0340-1227/79/0384/0149/$02.00

Malignant lymphoma, lymphoplasmacytic/lymphoplasmacytoid is defined as a low-grade malignant B-cell neoplasm in which the cells show differentiation toward immunoglobulin (Ig)-secreting cells (plasma cells or plasmacytoid cells) (Lennert, 1973; Stein et al., 1973; Lennert et al., 1975). Because the predominant cells are lymphocytes and the diagnostically essential cells are elements of the plasma cell series, the term "lymphoplasmacytoid (LP) immunocytoma" was proposed (Lennert, 1973; Lennert et al., 1975). LP immunocytoma has now been subdivided into three subtypes (Lennert and Mohri, 1978): (1) the lymphoplasmacytic subtype, which consists of lymphocytes and plasma cells of the Marschalkó type, (2) the lymphoplasmacytoid subtype, which contains plasmacytoid cells instead of typical plasma cells, and (3) the polymorphic subtype, which contains numerous immunoblasts and often centroblasts and centrocytes, in addition to lymphocytes, plasma cells, and plasmacytoid cells. All three subtypes may be associated with IgM, IgG, IgA, or IgE paraproteinemia. Waldenström's disease (Waldenström, 1958, 1968) is identical with a number of the cases of LP immunocytoma (Lennert, 1973). In many cases, however, paraproteinemia is not found (Lennert, 1973; Stein et al., 1973; Stacher et al., 1976), as first demonstrated by Diebold et al. (1971) and Hurez et al. (1972).

LP immunocytoma is closely related to chronic lymphocytic leukemia of the B-cell type (B-CLL) in both morphology and origin. It is important to make a distinction between these two neoplasms because of their somewhat different clinical manifestations. This is sometimes difficult, especially in cases that show the typical histologic picture of B-CLL but also contain plasma cells. What has to be clarified is whether these plasma cells are part of the tumor proliferation or are independent reactive plasma cells.

With this diagnostic problem in mind, we have tried to correlate the results of immunohistochemical staining for intracytoplasmic immunoglobulin (CIg) with various histologic features in 99 cases of malignant lymphoma diagnosed as B-CLL or LP immunocytoma.

Material and Methods

114 cases with the histologic picture of B-CLL or LP immunocytoma (lymphoplasmacytic and lymphoplasmacytoid subtypes, excluding the polymorphic subtype) were studied with the immunoperoxidase-bridge (PAP) method. Fifteen cases in this series were not evaluated because the immunostaining was completely negative, which in most cases probably resulted from over-fixation of the biopsies. In the other 99 cases, at least a few positively stained cells could be found and thus we could be sure that the reaction worked.

The PAP method for immunoglobulin (Sternberger, 1974; Taylor, 1974) was applied on well deparaffinized 4 μm sections. Monospecific rabbit anti-human Ig sera were obtained from Nordic (Tilburg, The Netherlands). The following working dilutions were used: 1:50 for anti-μ, anti-γ, and anti-α, and 1:100 for anti-κ and anti-λ. A sheep anti-rabbit IgG serum produced in our laboratory by proper immunization and exhaustive absorption of the obtained serum was used as the second serum at a dilution of 1:10. The peroxidase-antiperoxidase (PAP) complex was purchased from Dakopatts (Copenhagen, Denmark) and was diluted 1:20 before use. The peroxidase activity was demonstrated with a solution containing 60 mg diaminobenzidine and 0.003% H_2O_2 in 100 ml Tris buffer, pH 7.6. The sections were counterstained with hematoxylin, dehydrated, and covered with Eukitt (Kindler, Freiburg, Germany). Control sections were stained in the same manner, except that monospecific serum was omitted.

Table 1. Immunoglobulin classes in reactive plasma cells in 46 cases of chronic lymphocytic leukemia of the B-cell type (group I)

Ig class	No. of cases
Heavy chains	
μ	25
γ	40
α	10
Light chains	
κ+λ	46

Table 2. Immunoglobulin classes in 10 borderline cases (group II) between chronic lymphocytic leukemia of the B-cell type and LP immunocytoma

Heavy chains	Light chains		Total
	κ	λ	
μ	6	1	7
γ	1	–	1
α	–	–	–
None	1	1	2
Total	8	2	10

For histologic examination, sections from all cases were stained with hematoxylin-eosin, Giemsa, Gomori (silver impregnation), and periodic acid Schiff (PAS).

Results

The 99 cases of B-CLL or LP immunocytoma were classified into four groups according to the results of the immunostaining:

Group I. 46 cases with polyclonal plasma cells, interpreted as B-CLL with reactive plasma cells.

Group II. 10 cases with only a few monoclonal plasmacytoid cells or plasma cells, interpreted as borderline cases between B-CLL and LP immunocytoma.

Group III. 37 cases with numerous monoclonal (or biclonal) plasmacytoid cells or plasma cells, interpreted as LP immunocytoma.

Group IV. Six cases with polyclonal Marschalkó plasma cells, but with a number of plasmacytoid cells in Giemsa-stained sections that were considered to be suspicious for LP immunocytoma.

The cases in the *first* group contained a variable number of immunopositive plasma cells, which revealed predominantly γ chains, sometimes μ chains, and rarely α chains. Both light chain types (κ and λ) were always demonstrated (Table 1).

In the *second* group, the number of monotypically CIg-positive cells was usually lower than 10 cells per histologic section and never exceeded one Ig-positive cell per high-power field. The frequency of the Ig classes found in the cases in this group is shown in Table 2.

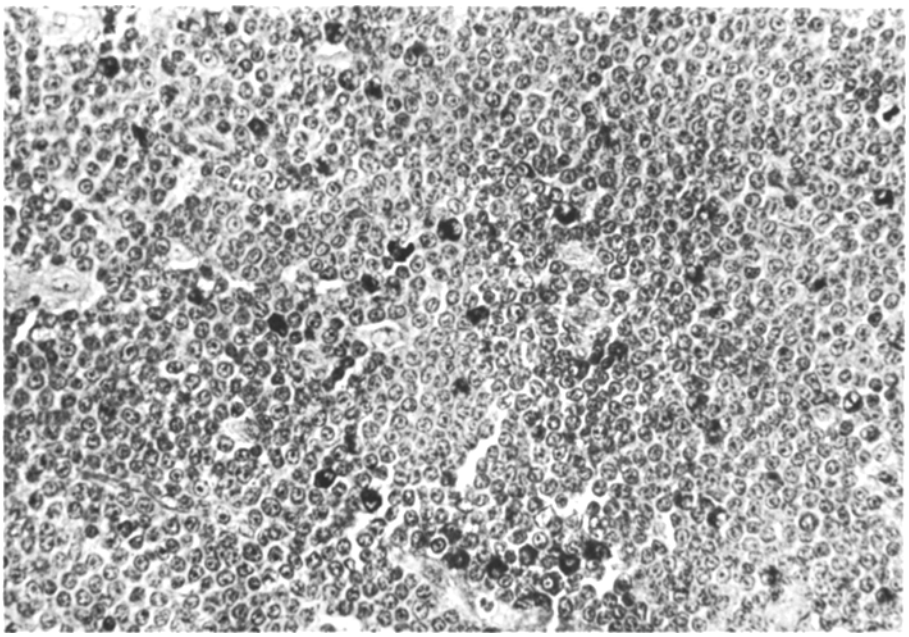

Fig. 1. Immunoglobulin-positive neoplastic cells dispersed among the other lymphoid tumor cells in LP immunocytoma. PAP staining for μ chains. ×350

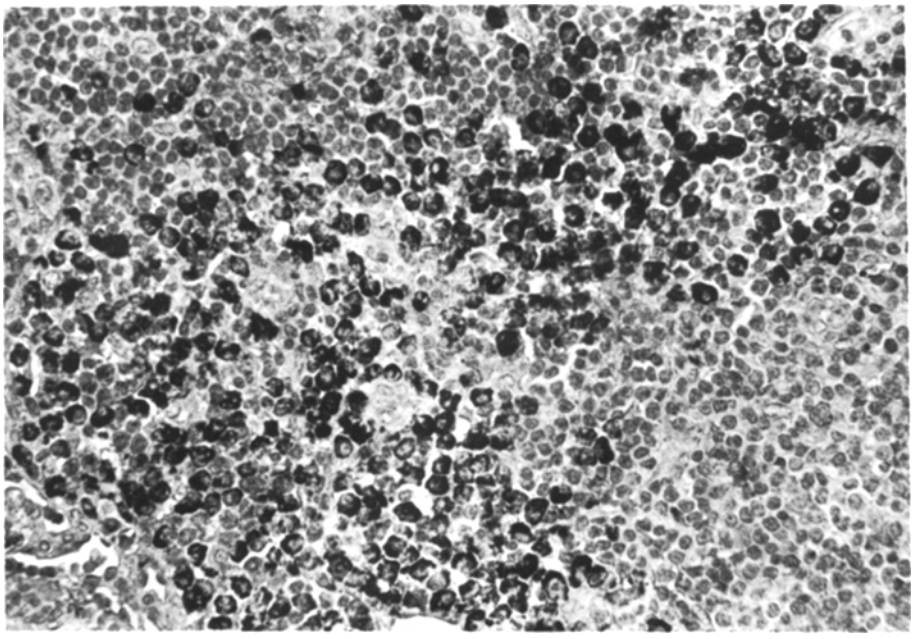

Fig. 2. Immunoglobulin-positive neoplastic cells with plasmacytic features gathered in large foci in LP immunocytoma. PAP staining for λ chains. ×400

Table 3. Immunoglobulin classes in 37 cases of LP immunocytoma (group III)

Heavy chains	Light chains			Total
	κ	λ	$\kappa+\lambda$	
μ	19	6	–	25
γ	1	1	–	2
α	–	–	1	1
None	5	1	–	6
$\mu+\gamma$	–	1	–	1
$\mu+\alpha$	–	1	–	1
$\gamma+\alpha$	–	1	–	1
Total	25	11	1	37

Table 4. Frequency of plasmacytoid cells in the four groups of cases

Group	n	Plasmacytoid cells			
		–	–/+	+	++
I	46	17	29	0	0
II	10	0	7	3	0
III	37	1	4	21	11
IV	6	0	0	3	3
Total	99	18	40	27	14

– = none, –/+ = less than 10 cells per section, + = up to one cell per high-power field, ++ = two or more cells per high-power field

The *third* group included the cases with a large number of monotypically or bitypically positive neoplastic cells (Figs. 1 and 2). The average number of CIg-positive cells was always higher than one cell per high-power field. The monotypically Ig-positive cells were found either gathered in variable-sized clusters or dispersed throughout the whole section (Figs. 1 and 2). Although in a number of cases CIg-positive neoplastic cells were seen in the vicinity of vessels, CIg-positive cells were also always found among the other neoplastic lymphoid cells that were not near vessels. The frequency of the various Ig classes is given in Table 3. The most frequent heavy chain class was μ, which was detected alone in 25 cases and together with γ or α chains in one case each. Gamma chains were demonstrated alone in only two cases and together with μ or α chains in one case each. Alpha chains were the least common heavy chains, seen once alone and twice together with μ or γ chains. In six cases, we could not demonstrate any heavy chains; only light chains were detectable. Light chains were demonstrated in all cases. The κ to λ ratio was 2.4:1. In one case, both κ and λ chains were found in tumor cells; it was not possible to clarify by the present method whether the two light chains were localized in the same or different tumor cells.

Table 5. Frequency of neoplastic and nonneoplastic Marschalkó plasma cells in the four groups of cases

Group	*n*	Neoplastic plasma cells				Reactive plasma cells			
		−	−/+	+	++	−	−/+	+	++
I	46	46	0	0	0	0	30	11	5
II	10	10	0	0	0	0	8	2	0
III	37	22	6	2	7	14	19	4	0
IV	6	4	2	0	0	0	4	2	0
Total	99	82	8	2	7	14	61	19	5

− = none, −/+ = less than 10 cells per section, + = up to one cell per high-power field, ++ = two or more cells per high-power field

In the *fourth* group, the plasma cells showed the same polyclonal pattern as in group I.

With the immunological data in mind, we restudied the morphology of the cases in the four groups and compared the following histologic features with the PAP staining results: frequency of plasmacytoid cells, plasma cells, and basophilic blast cells; presence of proliferation centers; and occurrence of PAS positivity. In addition, we determined the number of epithelioid venules and hemosiderin deposits.

Using Giemsa staining, we distinguish between plasmacytoid cells and plasma cells on the basis of the amount and basophilia of the cytoplasm. Plasma cells have more abundant and more intensely basophilic cytoplasm (like plasma cells of the Marschalkó type) than do plasmacytoid cells, which thus fall between lymphocytes and typical plasma cells in appearance. Plasmacytoid cells (Table 4) were evident in relatively large numbers in group II and especially in groups III and IV, whereas more than one third of the cases in group I did not show any. Only occasional plasmacytoid cells were found in many cases in groups I and II. A large number of these cells were demonstrated only in cases of LP immunocytoma (11 out of 37 cases in group III) and in three of the six cases that were diagnosed as LP immunocytoma but did not show evidence of monoclonal Ig in the plasmacytoid cells (group IV). In one case of LP immunocytoma, there were no plasmacytoid cells and also no plasma cells; but there were some immunoblasts (see below), with a frequency of more than one blast cell per high-power field. A moderate or large number of neoplastic plasma cells of the Marschalkó type (Table 5) were demonstrated in only nine cases of immunocytoma of the lymphoplasmacytic subtype. In contrast, a variable, but usually small number of reactive plasma cells were found in most cases in all groups. Although it was not always easy to distinguish the reactive plasma cells from neoplastic plasma cells, the reactive plasma cells could be identified with a sufficient degree of confidence because of their more mature appearance (smaller nuclei with typical chromatin) and their localization in perivascular, subcapsular, or fibrosed areas. The true nature of such plasma

Table 6. Frequency of basophilic blast cells (immunoblasts) in the four groups of cases

Group	*n*	Basophilic blast cells			
		−	−/+	+	++
I	46	21	25	0	0
II	10	0	8	2	0
III	37	0	15	13	9
IV	6	0	3	2	1
Total	99	21	51	17	10

− = none, −/+ = less than 10 cells per section, + = up to one cell per high-power field, ++ = two or more cells per high-power field

Table 7. Occurrence of proliferation centers (pseudofollicular pattern) in the four groups of cases

Group	*n*		Proliferation centers			
			Present		Absent	
I	46		38		8	
II	10		7		3	
III (LP immunocytoma)	37		19		18	
Lymphoplasmacytic subtype		9		0		9
Lymphoplasmacytoid subtype		28		19		9
IV	6		4		2	
Total	99		68		31	

cells was revealed by PAP immunostaining, however, when they showed a somewhat stronger reaction and a polyclonal Ig content.

A variable number of large basophilic blast cells (immunoblasts) were found in all groups of cases (Table 6), the largest number in the LP immunocytoma group (Group III). The cases of B-CLL (group I) contained only occasional immunoblasts (25 cases) or none (21 cases). Rather than these cells, cases of B-CLL always showed some so-called paraimmunoblasts (Lennert and Mohri, 1978), i.e., large cells with a morphology resembling that of immunoblasts, but with only weak or moderate basophilia (gray instead of blue with Giemsa staining). Such paraimmunoblasts also occurred in small numbers in cases in groups II, III, and IV.

Proliferation centers, or a so-called pseudofollicular pattern (Table 7), were seen in all groups of cases, but more frequently (82.6%) in definite cases of B-CLL (group I) than in LP immunocytoma (group III, 51.4%). If the immunocytoma group is divided into the lymphoplasmacytoid and lymphoplasmacytic subtypes, it becomes evident that the lymphoplasmacytic subtype never shows a pseudofollicular pattern, whereas the lymphoplasmacytoid subtype is more like B-CLL, i.e., it showed a pseudofollicular pattern in 67.9% of the cases.

In cases showing PAS positivity (Table 8), the reaction was either globular (in nucleus and/or cytoplasm) or diffuse. In cases with globular inclusions,

Table 8. Occurrence of PAS positivity in the four groups of cases

Group	PAS positivity			Total/*n*
	Globular inclusions		Diffuse	
	Intra-nuclear	Intra-cyto-plasmic		
I	0	0	0	0/46
II	0	0	1	1/10
III	7	4	7	18/37
IV	0	0	1	1/6

however, diffuse PAS positivity could also be seen in the cytoplasm of some cells. Lymphoplasmacytoid cells or plasma cells with globular PAS-positive inclusions were found only in LP immunocytoma (group III), namely, in 11 out of 37 cases. PAS-positive globules were also detected outside plasmacytoid cells, especially in the cytoplasm of histiocytes; in two cases (one of B-CLL and one of LP immunocytoma), such globules were abundant. With PAP immunostaining, however, they proved to be negative for CIg. The composition of these inclusions was not clear.

An increase in epithelioid venules was observed mainly in cases of LP immunocytoma (20 out of 37 cases), especially in cases with a diffuse growth pattern. Ten of the cases in group I and three of the cases in group II, however, also showed moderate to pronounced proliferation of venules.

Considerable amounts of hemosiderin were found in two cases of the lymphoplasmacytic subtype and in one case of the lymphoplasmacytoid subtype of LP immunocytoma. Hemosiderosis was never seen in B-CLL.

Discussion

The cases that were negative with PAP immunostaining were excluded, because the negative reaction usually had a technical basis. The morphologically similar lymphomas with lymphocytic predominance, namely, B-CLL and LP immunocytoma, could then be categorized into four groups according to the results of the PAP staining. The cases in the first group were definitely CLL of the B-cell type. The third group contained the definite cases of LP immunocytoma. The cases in the second group fell between the first and third groups and were therefore called "borderline" cases. In B-CLL, we found only polyclonal plasmacytoid cells. In LP immunocytoma, there were moderate or large numbers of monoclonal plasmacytoid cells or plasma cells. Borderline cases contained only occasional but *monoclonal* plasmacytoid cells or plasma cells. The fourth group has not been clarified. Cases in this group showed a moderate or large number of lymphoplasmacytoid cells in which the PAP technique did not demonstrate monoclonal Ig. There are two possible answers to the question of the reason for the Ig negativity: (1) The Ig content of the lymphoplasmacytoid

cells was too low to be detected by the PAP technique. (2) The lymphoplasmacytoid cells did not produce Ig, although they contained a prerequisite for Ig production, namely, an increased amount of RNA as indicated by the basophilia of the cytoplasm. We do not know, however, whether the rough endoplasmic reticulum of the lymphoplasmacytoid cells was well enough developed for detectable Ig production. This will have to be investigated by electron microscopy. Thus, we cannot yet classify the six cases in group IV as B-CLL or LP immunocytoma.

Most of the cases presented here are part of a series of the Kiel Lymphoma Study Group and are thus unselected. They therefore provide a rough idea of the frequency of B-CLL and LP immunocytoma in West Germany. The following percentages are estimates of the relative proportion of cases of B-CLL and LP immunocytoma: 49.5% B-CLL, 39.8% LP immunocytoma, and 10.6% borderline cases (the actual number of cases of B-CLL is probably somewhat higher).

We also looked for morphological criteria that would enable a distinction to be made between B-CLL and LP immunocytoma. It became evident that there are strong and weak criteria. Strong criteria for LP immunocytoma are PAS positivity, especially globular inclusions in the nuclei or cytoplasm of tumor cells, and a moderate or large number of plasmacytoid cells or plasma cells and precursors (immunoblasts). Weak criteria for LP immunocytoma include a diffuse growth pattern, an increase in epithelioid venules, and hemosiderosis. We did not find a large number of plasmacytoid cells or plasma cells and immunoblasts in B-CLL. Most cases showed a pseudofollicular pattern, no increase in epithelioid venules, and no hemosiderosis. Nevertheless, the borderline between B-CLL and LP immunocytoma is not sharp. In addition to the so-called borderline cases, we observed cases of B-CLL with a few plasmacytoid cells or plasma cells and immunoblasts. We also found one case that was proved by PAP immunostaining to be LP immunocytoma, but did not contain recognizable cells of the plasma cell series except immunoblasts.

At present, it appears to be advisable to apply the PAP technique to all cases that are histologically suspected of being B-CLL or LP immunocytoma, this being the only way to prove that plasma cells with a monoclonal Ig content are part of the lymphoma and to thus permit the definite diagnosis of LP immunocytoma. When the plasma cells show a polyclonal pattern, we can be sure that the neoplasm is B-CLL in most cases. This conclusion is not absolute, however, not only because of the unclarified cases in group IV, but also because it is not always possible to identify monoclonal neoplastic plasma cells among polyclonal reactive plasma cells – both types of cells may be present together in the same lymph node, in which case the nature of the monoclonal Ig-producing cells is not obvious. Thus, the PAP technique does not provide a straightforward answer; the results have to be analyzed very critically. Finally, there will still be cases in which a distinction between B-CLL and LP immunocytoma cannot be made by histologic methods and the PAP technique alone.

Our findings in LP immunocytoma agree with the previously reported data on the type of Ig in CLL: IgM was predominant in both neoplasms, and κ chains were more frequent than λ chains. In three cases of LP immunocytoma,

two heavy chain classes were found simultaneously; this pattern may be called "biclonal", or, better, "bitypic". In one case, we demonstrated α chains and both κ and λ chains. With the method used in this study it was not possible to determine whether or not the two light chain types were present simultaneously in single tumor cells. Other investigators (Seligmann, 1979) maintain, however, that the simultaneous occurrence of both light chain types in a single cell is impossible.

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Received April 4, 1979