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Phenotyping of Proliferating Lymphocytes in Angioimmunoblastic Lymphadenopathy and Related Lesions by the Double Immunoenzymatic Staining Technique

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Biopsy specimens of lymph nodes with the histologic characteristics of angioimmunoblastic lymphadenopathy with dysproteinemia (AILD) were obtained from 9 cases (4 cases of AILD and 5 cases of AILD-like T lymphoma [AILD-T]) and histologically analyzed by the use of a double immunoenzymatic staining technique with the combination of a monoclonal antibody against lymphocyte membrane antigen and that against human DNA polymerase α (pol α), which is detectable in the nucleus of the cells in G1, S, and G2 phases. In all 9 cases, the pol α$^+$ proliferating cells had a peripheral T-cell phenotype with T11 and Leu-4 antigens, whereas proliferating B cells with B1 antigen were rarely observed. As for T-cell subset antigens, the proliferating T cells had T4$^+$ helper/inducer phenotype in 7 cases, while T8$^+$ suppressor/killer T cells proliferated in 2 cases, although a significant number of T4$^+$ proliferating cells were also recognized. The study on malignant lymphomas that evolved in the 2 cases showed that the T-subset antigens on major proliferating tumor cells were the same as those found in the preceding AILD lesions, suggesting that lymphoma T cells originate from the AILD lesion. The results suggested that AILD without histologic manifestations of malignancy and AILD-T may be a neoplastic disease derived from either subset of peripheral T cells. (Am J Pathol 1987, 127:279–287)

ANGIOIMMUNOBLASTIC lymphadenopathy with dysproteinemia (AILD)[1,2] also referred to as immunoblastic lymphadenopathy (IBL),[3] is a systemic disease characterized by generalized lymphadenopathy, hepatosplenomegaly, and immunologic abnormalities. The morphologic features in lymph nodes are diffuse effacement of nodal architecture, polymorphic cellular infiltration, including small lymphocytes, plasma cells and immunoblasts, and proliferation of arborizing small vessels. In spite of its fatal outcome, the disease was originally considered to be a nonneoplastic, reactive process of the B-cell system. Suchi, in this group, also reported the disease with quite similar histologic and clinical features and called it immunodysplastic disease because he regarded this type of lesion as a proliferative disease of immunocompetent cells with the suppression of humoral and cellular immunity.[4] After the proposal of these new disease entities, malignant lymphomas arising in AILD were reported by Nathwani et al.[5] In addition, IBL-like T-cell lymphoma, or adult T-cell lymphoma with hyperimmunoglobulinemia, both of which had atypical lymphoid elements in addition to the features originally described as AILD, was reported by Shimoyama et al[6] and Watanabe et al,[7] and

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they later reported that tumor cells in this lesion had T suppressor/killer (s/k) phenotype.[8,9] Thus, the differential diagnosis between AILD without any histologic manifestations of malignancy and T-cell lymphoma with the features of AILD became highly important.

Because the hybridoma technique has provided monoclonal antibodies against various antigens on lymphocytes specific to stages of differentiation and subpopulation, immunohistologic analysis on frozen sections and cell suspension analysis have been conducted with these antibodies.[10,11] Immunohistologic study offers more precise information about the topographic arrangement and localization of cells with different phenotypes, but it is often difficult to provide an accurate diagnosis for the lesions with polymorphic cellular infiltration, such as AILD.

Recently, we developed a double immunoenzymatic staining technique to determine the surface phenotype of proliferating lymphocytes using a monoclonal antibody against DNA polymerase α (pol α)[12,13] present in the nucleus of cells in G1, S, and G2 phases,[14] together with those detecting lymphocyte membrane antigens.[15] Examination of lymph nodes with the characteristics of AILD using this technique revealed that the proliferating cell population in most cases had a single phenotype, either T helper/inducer (h/i) or T s/k subset antigen, suggesting that the AILD lesion as such may be regarded as a malignant proliferation of peripheral T cells.

Materials and Methods

Patients

This study is based on 9 patients, selected by histologic features in lymph node biopsy specimens, from which frozen tissues were available. The materials consisted of 11 lymph nodes, as Case 3 and 4 provided two biopsies at different times. The fresh materials were divided into three parts, one for routine histologic examination, another for suspension cell analysis, and the rest for immunohistologic analysis.

The histologic criteria for AILD are based on the report of Frizzera et al[2]: 1) extensive alteration of nodal architecture; 2) abundance of small arborizing vessels; 3) pronounced proliferation of immune reactive cells, such as lymphocytes, plasma cells, immunoblasts, and eosinophils. AILD-like T lymphoma (AILD-T) is diagnosed for the lesions with the zonal proliferation of various transformed cells, including clear cells, atypical lymphocytes, and/or immunoblasts, in addition to the morphologic features of AILD. The term "AILD lesions" is used in this study to refer to both AILD and AILD-T. Histologic diagnosis was performed by two of us (T.S. and R.N.).

Antibodies

Mouse monoclonal antibody against human DNA pol α was kindly provided by Dr. F. Hanaoka, of Tokyo University School of Pharmaceutical Science.[12,13] Other monoclonal antibodies, including B1 (cluster of differentiation [CD] 20, molecular weight [Mr] 35 kd [K], Coulter Immunology, Hialeah, Fla) for B cells, T11, T4, T8 (CD2, 4 and 8, Mr 55 K, 55K, and 32K, respectively, Coulter Immunology), and Leu-4 (CD3, Mr 19/29K, Becton Dickinson Monoclonal Center, Inc., Mountain View, Calif) for T cells, were obtained commercially.

Immunofluorescence Assay of Suspension Cells

Lymphocyte suspensions were prepared from fresh samples of the diagnostic materials by teasing through stainless steel wire mesh in RPMI-1640 medium containing 10% fetal calf serum. Cells were separated on Ficoll-Paque (Pharmacia, Uppsala, Sweden) by density gradient centrifugation, when necessary, for removal of contaminating erythrocytes. The surface phenotype of lymphocytes was determined by indirect membrane immunofluorescence with fluorescence isothiocyanate (FITC)-conjugated F(ab')$_2$ fragment of goat antimouse IgG and IgM (Coulter Immunology) as a second antibody. The percentage of cells showing surface fluorescence was determined by counting approximately 200 mononuclear cells.

Double Immunoenzymatic Staining

The double immunoenzymatic staining procedure using periodate-lysine-paraformaldehyde (PLP)-fixed tissues was described previously.[15] In brief, the sequential immunostaining procedure was carried out as follows: First, immunoperoxidase staining against pol α in the nucleus using the avidin-biotin-peroxidase complex (ABC) method[16] was performed with a Vectastain ABC Kit (Vector, Burlingame, Calif); then the immunoalkaline phosphatase monoclonal anti-alkaline phosphatase (APAAP)[17] method was conducted with an APAAP Kit (Zymed Laboratories, San Francisco, Calif). The sections were stained for peroxidase with 3,3'-diaminobenzidine-tetrahydrochloride as described previously[18] and then stained with second primary antibodies. As substrate solution for an alkaline phosphatase reaction, naphthol-AS-phosphate (Sigma Chemical Co., St. Louis, Mo) and fast blue BB (Sigma) were dissolved in dimethylformamide and diluted with 0.05 M propandiole buffer, pH 9.75. To inhibit endogenous tissue enzyme activity, 0.25 mM levamisole (Sigma) supplemented the incubation medium. Consequently, the nucleus of proliferating cells was stained in brown, the lymphocyte membrane antigen in blue.

Results

Clinical Findings

The clinical and laboratory findings at presentation and following biopsy, as well as the therapy and follow-up data, are summarized in Table 1. The patients were 3 men and 6 women with ages ranging from 34 to 90 years. Seven of 9 had generalized lymphadenopathy, and 2 had localized lymphadenopathy without systemic symptom. Five of 8 patients examined had a prominent, polyclonal elevation in immunoglobulins (Cases 4, 6, 7, 8, and 9), and 1 patient had a tendency toward elevation in IgA (Case 5). Malignant lymphomas evolved in 2 cases (Cases 3 and 4).

Morphologic Findings

Histologic findings are also summarized in Table 1, and the representative area of AILD (Figure 1, Case 1) and AILD-T (Figure 2, Case 8) are illustrated. In lymph node biopsies of all patients, the normal architecture was effaced, and there were no residual germinal centers. The normal lymphoid population was replaced by polymorphic infiltration, composed of small lymphocytes, immunoblasts, plasma cells, histiocytes, and eosinophils, and associated with an increase of arborizing vessels in all cases (Figure 1). A considerable number of mitotic figures were observed, except in Cases 3a and 3b. Foci of atypical immunoblasts (Figure 2A) or zonal proliferation of medium-sized cells possessing a moderate amount of pale cytoplasm and small round nuclei (pale cell) (Figure 2B) was found in Cases 5, 6, 7, and 8. In Cases 1 and 3a and 3b, abundant epithelioid histiocytes were observed.

The first biopsied lymph node (3a) of Case 3, which was not available for the study with double immunoenzymatic staining, and the second (3b) had the

Table 1—Clinical Characteristics of 9 Patients With AILD Lesions

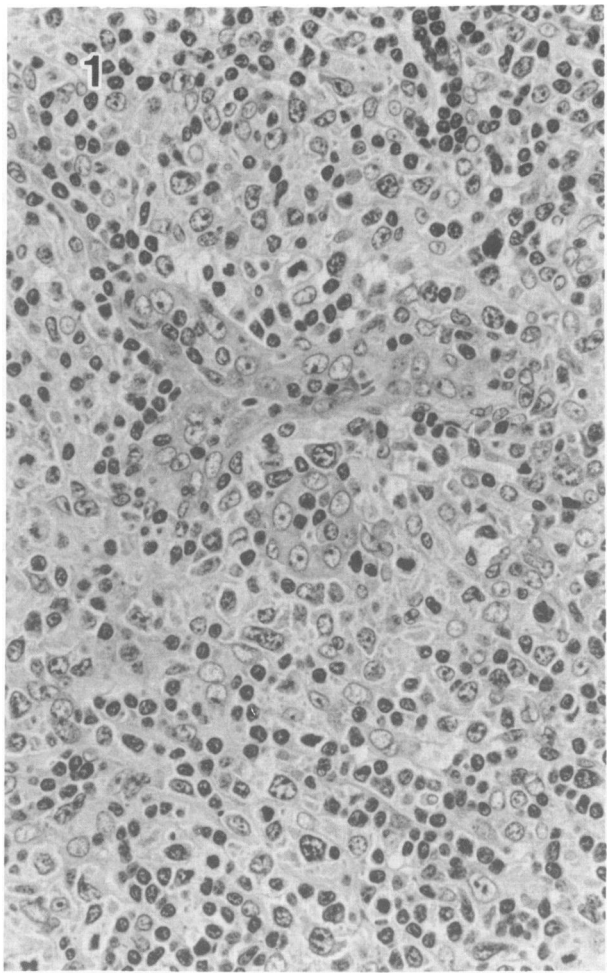
Case	Age/Sex	Histologic diagnosis*	Clinical presentation	Ig level (mg/dl)†	Treatment‡	Follow-up
1	45/F	AILD	Localized lymphadenopathy	G: 1295 A: 173 M: 178	THP-ADM	Alive without disease, 31 months
2	76/F	AILD	Localized lymphadenopathy	ND	None	Alive without disease, 26 months
3a§	39/M	AILD	Generalized lymphadenopathy	G: 920 A: 168 M: 270	COPP	Alive with disease, 72 months; remission 3 years; then relapse with AILD lesion; remission 2 years; then relapse with lymphoma (3c)
b	42	AILD				
c	44	ML, diffuse large				
4a§	34/F	AILD	Generalized lymphadenopathy, fever, skin rash, autoimmune hemolytic anemia	G: 2310 A: 485 M: 1266	PDN	Dead of disease, 23 months; without complete remission; lymphoma 13 months after initial symptom (4c)
b	34	AILD				
c	35	ML, diffuse medium				
5	47/M	AILD-T	Generalized lymphadenopathy	G: 1320 A: 456 M: 206	CHOP	Alive without disease, 21 months
6	90/F	AILD-T	Generalized lymphadenopathy	G: 2469 A: 689 M: 272	VEMP	Alive without disease, 13 months
7	60/M	AILD-T	Generalized lymphadenopathy	G: 1920 A: 420 M: 200	CHOP	Alive without disease, 25 months; remission 1 year; acute monocytic leukemia
8	53/F	AILD-T	Generalized lymphadenopathy, fever, erythema	G: 2240 A: 633 M: 188	CHOP	Alive without disease, 8 months
9	51/F	AILD-T	Generalized lymphadenopathy, fever, skin rash	G: 3117 A: 679 M: 118	CHOP	Alive without disease, 7 months

*AILD, angioimmunoblastic lymphadenopathy with dysproteinemia, without histologic manifestations of malignancy; AILD-T, AILD lesion with histologic manifestations of malignancy; ML, malignant lymphoma.

†Ig, immunoglobulin; G, IgG; A, IgA; M, IgM; ND, not done.

‡THP-ADM, 4′-O-tetrahydropyranyl adriamycin; COPP, cyclophosphamide + vincristine + procarbazine + prednisolone; PDN, prednisolone; CHOP, cyclophosphamide + hydroxydaunorubicin + vincristine + prednisolone; VEMP, vincristine + cyclophosphamide + 6-mercaptopurine + prednisolone.

§The material not examined by double immunoenzymatic staining.



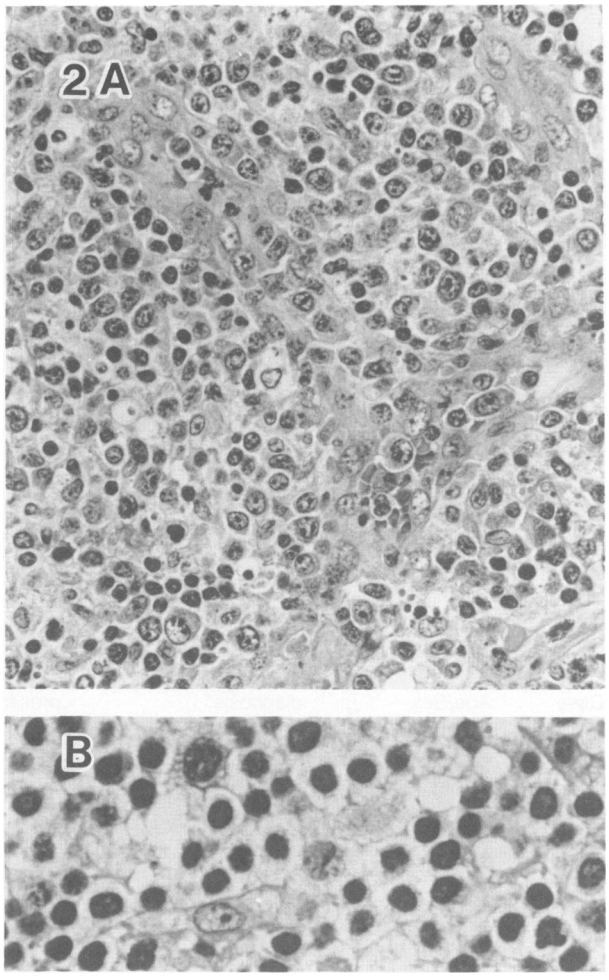


Figure 1—Case 1 lymph node diagnosed as AILD. Arborizing vessels with hypertrophic endothelium and polymorphic cellular composition, including small lymphocytes, plasma cells, and immunoblasts, are evident. (H&E, ×370) **Figure 2**—Case 8 lymph node diagnosed as AILD-T. The focus of atypical immunoblasts (**A**) and that of "clear cells" (**B**) are observed, in addition to marked proliferation of small vessels as illustrated in **A**. (H&E, **A**, ×370; **B**, ×740)

same lesion diagnosed as AILD. Diffuse lymphoma without the characteristics of AILD was found in the third biopsied lymph node (3c), 5 years after initial symptoms. In the first biopsied lymph node (4a) of Case 4, which was not available for the immunologic study, there were pronounced polymorphism of infiltrate and proliferation of arborizing vessels; and in the second (4b), after 3 months' steroid therapy, a moderate thickening of the walls of the proliferating vessels was seen, as described by Frizzera et al.[2] Although immunoblasts, plasma cells, and eosinophils decreased in number, resulting in rather monotonous proliferation of medium-sized lymphocytes, clusters of immunoblasts were found in sinuses. In the third biopsied lymph node (4c), the histologic characteristics of AILD disappeared, and diffuse lymphoma developed. The number of vessels diminished, and large atypical immunoblasts were scattered within the monotonous proliferation of medium-sized lymphocytes with mild atypia.

Membrane Immunofluorescence Analysis of Suspension Cells

Cell suspensions were prepared from biopsied lymph node specimens and stained with monoclonal antibodies to study cell surface antigens. The results were summarized in Table 2 (Case 4 was not available for this analysis). The predominant cell population isolated from AILD lesion was $T11^+$ and Leu-4^+ peripheral T cells in all cases examined. Although 10–35% of $B1^+$ B cells were also found, the monoclonality of immunoglobulin was not observed. As for T-cell subset antigens, the number of T cells with $T4^+$ h/i phenotype was greater than that with $T8^+$ s/k phenotype in all but Case 8, where the ratio of $T4^+$ and

Table 2—Cell Composition of Lymph Node with AILD Lesion Studied by Membrane Immunofluorescence

		Surface phenotype (% positive†)										
		T cell				B cell	Heavy chain (Ig)				Light chain (Ig)	
Case	Histologic diagnosis*	CD2‡ T11	CD3 Leu4	CD4 T4	CD8 T8	CD20 B1	Υ	α	μ	δ	κ	λ
1	AILD	60	64	48	17	35	21	0	35	29	16	19
2	AILD	33	44	45	4	9	12	3	8	2	7	2
3b	AILD	69	56	37	12	28	0	0	16	33	14	10
c	ML	85	38	20	28	0	0	0	0	0	0	0
4	AILD	ND										
5	AILD-T	64	50	39	18	18	2	11	4	4	10	3
6	AILD-T	81	61	49	24	20	6	7	7	14	14	11
7	AILD-T	44	64	53	6	15	1	1	16	6	9	16
8	AILD-T	67	43	10	35	17	2	1	5	0	3	2
9	AILD-T	77	66	44	25	16	3	3	5	2	6	2

*See footnote to Table 1.
†The percentage of positive cells was estimated by counting at least 200 cells by indirect immunofluorescence assay.
‡CD, cluster of differentiation.

$T8^+$ cells was reversed. In the malignant lymphoma (3c) that evolved in Case 3, the expression of Leu-4, T4, and T8 antigens on the tumor cells was suppressed.

Immunohistologic Analysis by Double Immunoenzymatic Staining

PLP-fixed sections from each specimen were stained by the ABC method with various monoclonal antibodies (data not shown, except those with anti-pol α in Table 3). The predominant cell population in the lymph nodes with the AILD lesion was Leu-4^+ peripheral T cells in all cases, but a certain number of B cells were also found in both clustered and scattered patterns in most cases. Both subsets of T cells intermingled with various proportions, concordant with the results obtained by the analysis of suspension cells as mentioned above. We found more than 30% pol α^+ cells in all but Case 3b, in which the mitotic figures were rarely observed on routine histologic preparations as described above.

To determine the phenotype of the proliferating cell population, double immunoenzymatic staining was employed. The percentage of membrane antigen-positive cells among the pol α^+ population was studied in the representative area on the sections and summarized in Table 3. The pol α^+, proliferating cells were clearly recognized among the heterogenous population constituting AILD lesions; ie, most of them were $T11^+$ and Leu-4^+ peripheral T cells, whereas those with B1 antigen were rarely observed. As for the

Table 3—Surface Phenotype of Proliferating Cells in Lymph Nodes With AILD Lesion Studied by Double Immunoenzymatic Staining

			Pol α and				
			T cell				B cell
Case	Histologic diagnosis*	Pol α	CD2‡ T11	CD3 Leu4	CD4 T4	CD8 T8	CD20 B1
1	AILD	49%†	ND	74%§	80	6	4
2	AILD	63	ND	27	37	0	7
3b	AILD	12	ND	50	49	8	8
c	ML	54	72	9	25	1	1
4b	AILD	31	83	66	42	52	6
c	ML	27	91	64	10	61	14
5	AILD-T	66	ND	51	64	6	4
6	AILD-T	53	59	56	51	5	5
7	AILD-T	53	ND	49	64	1	3
8	AILD-T	89	88	74	23	61	0
9	AILD-T	49	45	25	24	10	9

*See footnote to Table 1.
†The percentage of pol α^+ cells was estimated in representative parts of the lesion by counting at least 300 cells.
‡See footnote to Table 2.
§The percentage of membrane antigen-positive cells was estimated by counting at least 300 pol α^+ cells.

T-cell subset antigens, most of them were T4$^+$ h/i T cells in 6 cases (Case 1, 2, 3b, 5, 6, and 7). The results for Case 1 are illustrated in Figure 3A, B, and C, and those for Case 6 are illustrated in Figure 3D, E, and F. Case 9 was also T4-dominant, but there were observed pol α^+ cells without any lymphocyte membrane antigens examined. In Cases 4b and 8, the number of pol α^+ cells with T8 antigen was larger than that with T4 antigen, although the latter cell population was also present in a considerable number, ie, 42% and 23%, respectively. The results in specimen 4b are shown in Figure 4A, B, C, and D.

In the malignant lymphoma that evolved in Case 3c, T4$^+$ cells proliferated predominantly and were the same phenotype as the main proliferating population in specimen 3b, although a significant number of the tumor cells had only T11 antigen, without expressing Leu-4, T4, or T8 antigens. In Case 4, T8$^+$ cells proliferated predominantly, with a considerable number of T4$^+$ cells in specimen 4b diagnosed as AILD, as described above. The immunoblasts clustered in sinuses in specimen 4b, however, showed T8 phenotype (Figure 4C and D), and the medium-sized pol α^+ cells in specimen 4c were also T8$^+$ (Figure 4E and F). The large proliferating cells in specimen 4c were, however, negative for T8, and were positive only for T11 among T cell antigens tested (Figure 4G).

Discussion

Double immunoenzymatic staining of AILD lesions from 9 cases (consisting of 4 AILD and 5 AILD-T cases) demonstrated that 1) the proportion of pol α^+ cells was generally high, 2) the proliferating cell population in most cases had a single phenotype of peripheral T cells, and 3) pol α^+ cells in 7 cases were of the T h/i phenotype, while those in 2 cases were of the T s/k phenotype. Moreover, no clear difference was observed between AILD and AILD-T regarding the surface phenotype of proliferating cells. As described in the introduction, AILD or IBL was originally reported as a benign proliferative process of the B-cell system without a precise immunohistologic study having been conducted. Suchi, however, suspected that these lesions might be a malignant proliferation of lymphocytes and called it an immunodysplastic disease. The present study by double immunoenzymatic staining showed that the pol α^+ proliferating population had a single surface phenotype; ie, there was monoclonal proliferation of T cells in 8 of 9 cases, including 3 cases of AILD without histologic features indicating malignancy, suggesting that the lesions are a malignant proliferation of mature T cells. Even in the rest of one case (Case 4b), relative increase in T8$^+$ cells was clearly recognized and, moreover, the clustered immunoblasts had only T8 antigen, as illustrated in Figure 4C and D. It is noted that the analysis by double immunoenzymatic staining could show clearly the predominantly proliferating cell population, when compared with conventional single immunoenzymatic staining or suspension cell analysis. A cytogenetic study by Kaneko et al[19] also revealed that clonal chromosome abnormalities were generally recognized in AILD even before the evolution of malignant lymphomas. In this connection, the study on serial samples from Case 3 and 4 provided us important information. Pol α^+ cells were relatively small in number in the specimens of each case diagnosed as AILD, but specimen 3b clearly showed that pol α^+ cells were h/i T cells, and specimen 4b had predominantly s/k proliferating T cells, as discussed above; ie, the proliferating cell population in both specimens already showed the same phenotype as that observed in the malignant lymphomas that developed later, suggesting that the transformed T cells were probably hidden in AILD without overt histologic changes showing malignancy. Recently, Weiss et al[20] carried out Southern blot analysis of AILD and AILD-T and found the rearrangements of T-cell receptor β chain (TCR β) genes in 8 of 11 cases, indicating that these lesions contain a clonal proliferation of T lymphocytes. These results and ours indicate that AILD and AILD-T may be a neoplastic disease derived from mature T cells.

AILD was originally considered to be a nonneoplastic, reactive process of the B-cell system as described above.[3] There were also reports of cases with monoclonal hypergammaglobulinemia, indicating neoplastic proliferation of the B-cell system.[21,22] Moreover, just recently Griesser et al[23] observed that 4 of 11 cases of AILD showed rearrangements of immunoglobulin heavy chain (Ig H) genes, and more complex rearrangement patterns of both Ig H and TCR genes were reported by Lipford et al.[24] These studies, altogether, suggest that a part of AILD lesions is benign or malignant B cell disease, which may or may not be related to a regulatory T-cell defect.[22,25] However, the cases in this study did not show a monoclonal proliferation of B cells, although a polyclonal elevation of serum Ig was observed in 5 of 8 cases examined. Epidemiologic and/or genetic bias between the Japanese cases in this study and the Caucasian cases studied by other investigators may explain the difference in the results. In this regard, it is noted that T-cell lymphoma, including adult T-cell lymphoma/leukemia, is more common in Japan than in Western countries. None of the cases in this study, however, showed evidence of infection of human T-

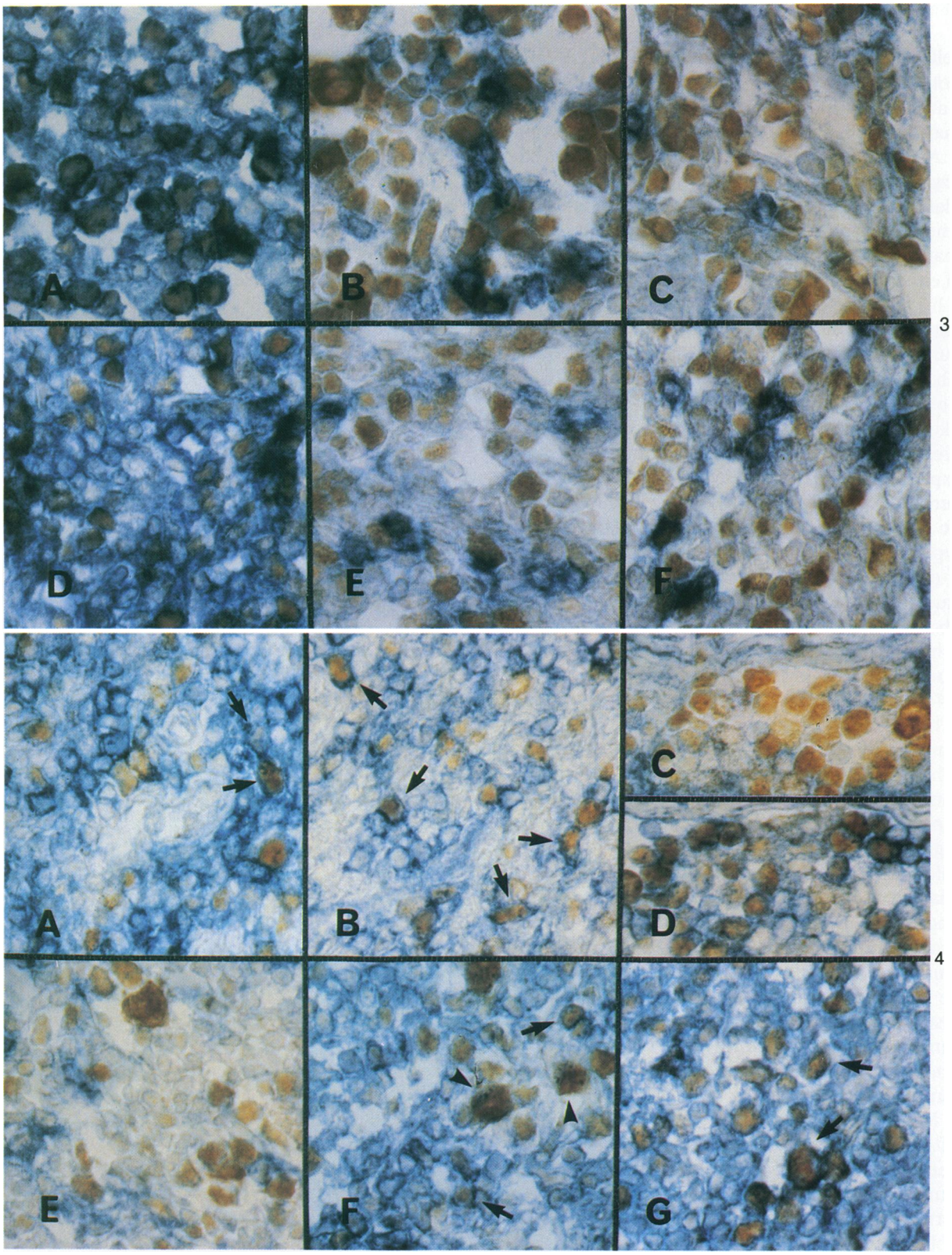


Figure 3—Double immunoenzymatic staining of Case 1 and Case 6 lymph nodes with anti-pol α and anti-lymphocyte membrane antigens. Lymph nodes of Case 1 diagnosed as AILD (**A, B,** and **C**) and Case 6 diagnosed as AILD-T (**D, E,** and **F**) are stained with anti-pol α and T4 (**A** and **D**), T8 (**B** and **E**), or B1 antibody (**C** and **F**). The nucleus of pol α^+ proliferating cells stained by the ABC method is visualized in brown, and the membrane antigens stained by the APAAP method are in blue. The percentage of pol α^+ cells in Case 1 and Case 6 lymph nodes is 49% and 53%, respectively, and most of them are positive for T4 (**A** and **D**), but negative for T8 (**B** and **E**) or B1 (**C** and **F**). (×550) See Table 3. **Figure 4**—Double immunoenzymatic staining of two lymph node specimens sequentially obtained from Case 4. Specimen 4b, diagnosed as AILD (**A, B, C,** and **D**), and specimen 4c, diagnosed as malignant lymphoma (**E, F,** and **G**), are stained by double immunoenzymatic technique with anti-pol α and T4 (**A, C,** and **E**), T8 (**B, D,** and **F**), or T11 antibody (**G**). In specimen 4b, 31% of lymphocytes are positive for pol α, and the main proliferating cell population is positive for T8 (**B,** *arrow* indicates both pol α^+ and T8$^+$ cells), although a considerable number of proliferating T4$^+$ cells are also found (**A,** *arrow* indicates both pol α^+ and T4$^+$ cells). Most immunoblasts in the sinus have pol α in the nucleus, and they are positive for T8 (**D**) but negative for T4 (**C**). In specimen 4c, 27% of lymphocytes are positive for pol α, and most of the medium-sized lymphocytes are positive for T8 (**F,** *arrow* indicates both pol α^+ and T8$^+$ cells) but not for T4 (**E**). The large, pol α^+ cells, however, lack T8 antigen (**F,** *arrowhead* indicates pol α^+ but T8$^-$ cells), although they are positive for T11 (**G,** *arrow* indicates both pol α^+ and T11$^+$ cells). (**A, B, E, F,** and **G,** ×550; **C** and **D,** ×1100) See Table 3.

cell leukemia/lymphoma virus Type I (HTLV I) (data not shown). Double immunoenzymatic staining of Caucasian AILD lesions may provide us with information on whether AILD of B-cell origin exists or not.

Another important finding obtained in this study regarding the phenotype of the proliferating cell population is that 7 of 9 cases were of the T h/i phenotype, whereas 2 cases were of the T s/k phenotype, showing that lesions with features of AILD may be composed of either type of T-cell subset, although those with the T h/i phenotype seem to be dominant. The immunophenotyping of AILD by Weiss et al[20] showed results quite similar to ours regarding T-cell subset antigens. These results, however, seem to contradict those of Tobinai et al[8] and Shimoyama et al,[9] that most IBL-like (AILD-like) T-cell lymphoma cases in Japan express the T s/k phenotype. This discrepancy may be explained by the immunologic methods used, ie, suspension cell analysis versus double immunoenzymatic staining of proliferating cell population. Phenotyping of many more cases will be able to determine T-cell subsets of AILD lesions. It is also noted in this study that, in the T8-predominant cases, such as Case 4b and 8, a considerable number of $T4^+$ proliferating T h/i cells were also recognized, although only a small number of $T8^+$ proliferating cells were observed in T4-dominant lesions. This finding suggests that these T h/i cells may proliferate reactively by the lymphokines produced by $T8^+$ neoplastic cells or various other immunologic interactions. The present study showed clearly that there were two different T phenotypes in the AILD lesions studied; but no major histologic differences, including the appearance of clear cells, were seen. It has been speculated that histologic characteristics of certain peripheral T-cell lymphomas, such as polymorphic cellular infiltration and proliferation of small vessels in AILD lesions and clusters of histiocytes in Lennert's lymphomas, may be related to the function of neoplastic T cells. Our results may indicate that lymphokines with a similar function were produced by either type of T-cell lymphoma, and they may cause similar histologic characteristics.

In conclusion, lymph node lesions with the features of AILD may be considered as a neoplastic disease of peripheral T cells of both subtypes, and double immunoenzymatic staining will be highly useful in determining the phenotype of tumor cells in lesions with polymorphic infiltration.

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