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The Nature of the Lymphocytes Surrounding Reed-Sternberg Cells in Nodular Lymphocyte Predominance and in Other Types of Hodgkin's Disease

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The lymphocytes surrounding Reed-Sternberg cells may play an important role in the pathogenesis of Hodgkin's disease. In this study, T cells in different subtypes of Hodgkin's disease were analyzed in situ ***by an immunoperoxidase method employing a panel of antibodies, including several paraffin tissue-reactive monoclonal antibodies. The T cells in Hodgkin's disease-involved tissues were found to be activated CD4-positive T cells that are UCHL1+ and CD45R−. This immunophenotype is compatible with an activated helper–inducer memory T cell population. The T cells in the nodular lymphocyte predominance subtype were found to have additional positivity for Leu 7, indicating a subpopulation of CD4+ T cells, normally confined to the light zone of germinal centers of secondary follicles. (Am J Pathol 1989, 135:351–357)***

Hodgkin's disease is characterized by the presence of Reed-Sternberg cells (R-S cells) in the right setting, consisting mainly of small lymphocytes, and a variable admixture of histiocytes, eosinophils, and plasma cells.[1] Originally, the presence of lymphocytes was interpreted as a reaction of the host immune system against the neoplastic R-S cells, providing an explanation for the more favorable prognosis of the lymphocyte predominance type of Hodgkin's disease.[2] The availability of immunologic markers for lymphocyte subsets has enabled the identification of the immunophenotype of lymphocytes surrounding R-S cells *in situ*. Several studies have shown that most of these lymphocytes are CD4-positive T lymphocytes with some features of activation, such as CD38 (T10) positivity[3,4] and formation of active spontaneous E rosettes.[5] Although these findings originally were interpreted as an indication of a T helper cell nature of these lymphocytes, subsequent studies have demonstrated that CD4 expression is related to HLA class II-restricted interactions and may be found on T cells with different functional activities.[6] Several distinctions have been made; helper–inducer cells versus suppressor–inducer cells,[7] unprimed T cells versus memory T cells,[8] and helper T cells versus inflammatory T cells.[9] In addition, in germinal centers of secondary follicles, a CD4-positive T cell population coexpressing the HNK1 (Leu 7) antigen is found.[10] Here we describe a further *in situ* analysis of T lymphocytes in different subtypes of Hodgkin's disease that was performed on frozen tissue sections and on B5-fixed-paraffin sections to enable the optimal correlation of immunophenotype and morphology.

Materials and Methods

Lymph nodes of patients with different subtypes of Hodgkin's disease were partially snap frozen and partially fixed in B5 fixative and embedded in paraffin. Five cases of lymphocyte predominance nodular, five of nodular sclerosis, and five of mixed cellularity subtypes were included in this study. The clinical data on these patients are summarized in Table 1. Four-μm frozen tissue sections were prepared, air dried overnight, and fixed in acetone. Three-μm paraffin sections were prepared, deparaffinized, and treated with methanol–hydrogen peroxide to block endogenous peroxidase activity. A two-step indirect immunoperoxidase technique, employing peroxidase-conjugated rabbit anti-mouse Ig antibodies, was used.[11] Antibodies LFA1, LFA2 (CD2), LFA3, CD1, CD3, CD4, CD5, CD6, CD7, CD8, CD25, CD38, and CD20 were used only on frozen

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Table 1. *Patient Characteristics*

Patient	Age	Sex	Localization	Stage	Histology
1	29	M	Cervical	I	NLPHD
2	22	F	Cervical	I	NLPHD
3	55	F	Cervical	II	NLPHD
4	38	M	Cervical	I	NLPHD
5	56	M	Inguinal	I	NLPHD
6	46	M	Axillary	II	NS
7	77	M	Cervical	III	NS
8	21	M	Cervical	II	NS
9	23	F	Cervical	I	NS
10	34	F	Hilar	II	NS
11	54	M	Axillary	I	MC*
12	30	M	Hilar	II	MC
13	63	F	Inguinal	IV	MC
14	37	M	Cervical	III	MC
15	66	M	Paraaortic	IV	MC

* Interfollicular Hodgkin.

sections. The following paraffin tissue reactive mouse monoclonal antibodies were employed: 2H4,[7] MB1, MB2, MB3, MT1 and MT2,[11] Leu 7,[12] LN1, LN2, LN3,[13,14] and L26.[15] The characteristics of the antibodies and their sources are summarized in Table 2. Peroxidase activity was identified by staining with diaminobenzidin tetrahydrochloride (Sigma, St. Louis, MO) and H2O2. Nuclei were counterstained with Mayer's Hemalum, and the slides evaluated by light microscopy. R-S cells and their variants, as recognized in different subtypes of Hodgkin's disease, were analyzed. In lymphocyte predominance, L&H-type cells, in nodular sclerosis, mainly lacunar type, and in mixed cellularity, typical R-S cells and mononuclear variants were evaluated. Cells surrounding R-S cells were defined as a one-cell-thick layer of lymphocytes in immediate contact with the R-S cells and their mononuclear variants. A quantitative analysis was performed on paraffin tissue sections for MB1, UCHL1, and Leu7. In each

Table 2. *Characteristics of Monoclonal Antibodies*

CD	Clone	Molecular weight	Source	Reactivity in T cell areas
Frozen tissue reactive antibodies				
CD1a	T6	49	Own lab	Some IDC, no T cell reactivity
CD2	LFA2	46, 50	Springer	All T cells (E rosette receptor)
CD3	leu 4	22, 28	B&D	All T cells (T cell receptor assay)
CD4	leu 3	55	B&D	70% T cells (helper-inducer)
CD5	leu 1	67	B&D	All T cells
CD6	Tu-1	85, 130	Biotest	All T cells
CD7	WT1	40	Tax	All T cells
CD8	T8	32, 43	Own lab	30% T cells (suppr./cytotox.)
CD11a	LFA1	180	Springer	All T cells
	LFA3		Springer	No T cells; positive IDC
CD20	B1	35	Nadler	No T cells; (all B cells)
CD25	IL2 receptor	55	B&D	Few T cells
	leu 7	110	B&D	Few cells; many in follicles
CD38	OKT10	45	Ortho	Few cells
	OKI1a	28, 32	Ortho	No T cells; positive IDC
Paraffin tissue reactive antibodies				
CD45R	2H4	200, 220	Coulter	30% to 50% T cells (all B cells)
CD45R	MB1	200, 220	Own lab	30% to 50% T cells (all B cells)
CD45R	MT2	200, 220	Own lab	30% to 70% T cells (some B cells)
CD43	L26		Dako	No T cells (all B cells)
CD43	MB2	28	Own lab	No T cells (endoth., B cells)
CD43	MT1	110	Own lab	All T cells (histiocytes)
CD43	UCHL1	180	Beverley	70% to 50% of T cells
CD43	MB3	31	Own lab	IDC (invariant chain HLA II)
CD43	LN2	31	Biotest	IDC (invariant chain HLA II)
CD43	LN3	28, 32	Biotest	IDC (HLA class II)
CD43	LN1	45–85	Biotest	No T cells (germinal centers)
CD43	leu 7	110	B&D	Few cells (many in follicles)

CD, cluster designation; IDC, interdigitating cells; HLA II, human leukocyte antigen class II; IL2 receptor, interleukin 2 receptor; B&D, Becton Dickinson, Mountain View, CA; Biotest, Biotest Diagnostics, Fairfield, NJ; Dakopatts, Dako Corporation, Santa Barbara, CA; Coulter, Coulter Immunology, Hialeah, FL.

case, 100 R-S cells or variants were identified and the lymphocytes immediately surrounding them were counted. UCHL1 to MB1 ratios were also determined in T cell areas of reactive lymph nodes. We performed a double-staining technique employing a peroxidase-conjugated goat anti-mouse second-step antibody and aminoethyl carbazole as substrate for the peroxidase staining (reddish-brown), followed by rabbit anti-mouse Ig and APAAP complexes, and Naphthol AS-MX phosphate, along with Fast Blue BB for alkaline phosphatase staining.[16] This method was useful for determining UCHL1 to MB1 ratios in reactive lymph nodes and served as a control for the quantitation of these subsets in the Hodgkin cases.

Results

The immunohistologic staining results on frozen sections confirmed our and other groups previous findings and can be summarized as follows. In all types of Hodgkin's disease, a large proportion of the lymphocytes in involved areas were CD1−, CD2 (LFA2)+, CD3+, CD4+, CD5+, CD6+, CD7+, CD8−, CD11a (LFA1)+, LFA3−, CD20−, CD25−, leu 7−, and CD38+ T cells. LFA3, HLA class II, and CD25 gave strong staining of R-S cells and no staining of surrounding lymphocytes. In the nodular lymphocyte predominance type of Hodgkin's disease the results were different because most of the lymphocytes in involved areas were CD20+ B cells and the L&H-type R-S cells were also CD20+. The T cells in the nodules of lymphocyte predominance cases had a phenotype similar to that found in other types of Hodgkin's disease, except for an additional reaction with leu7 antibody.

Because morphologic details are much better preserved in the B5-fixed-paraffin tissue sections, these results could be specific to the cells directly surrounding R-S cells. The lymphocytes surrounding the R-S cells in the nodular sclerosis and mixed cellularity cases were MT1+, UCHL1+, MT2−, 2H4−, MB1−, MB2−, L26−, LN1−, LN2−, MB3−, LN3−, and leu 7−, whereas in the nodular lymphocyte predominance cases the T cells surrounding the L&H cells had additional leu 7 reactivity. Most of the cells in the nodules of the nodular lymphocyte predominance cases were L26-, MB1-, MB2-, MB3- and LN2-, and LN3-positive B lymphocytes, but these cells generally did not directly surround the R-S cells. The CD4-positive T cell population can be subdivided into CD45R+ (2H4, MB1, and MT2) and UCHL1+ subpopulations that in reactive T cell areas appear to be of approximately equal size. We have quantitated MB1-positive cells and UCHL1-positive cells surrounding R-S cells, defined as a one-cell-thick layer in immediate contact with the R-S cells. On average, approximately nine cells can be counted with no clear differences among different subtypes or among individual cases. The results for individual cases are summarized in Table 3 and represented as a UCHL1 to MB1 ratio. In addition to MB1, we also tested 2H4 and found similar results, although it stained somewhat weaker on paraffin tissue sections and also had more nonspecific background staining. The results indicated a strong predominance of UCHL1 positive cells with ratios over 50 in nodular sclerosis and mixed cellularity cases and over 15 in nodular lymphocyte predominance cases. In reactive T cell areas, however, the ratios varied between 1 and 2.5 (data not shown). We also quantitated the leu 7-positive cells and found virtually none surrounding the R-S cells in nodular sclerosis and mixed cellularity cases. However, the numbers of leu7+ cells surrounding L&H type R-S cells were similar to those of UCHL1+ cells in the nodular lymphocyte predominance type of Hodgkin's disease (Table 3). Staining for the invariant HLA class II chain with LN2 and MB3 showed cytoplasmic staining and LN3 showed membranous staining of R-S cells and absence of staining in the surrounding lymphocytes. L26, LN1, LN2, LN3, MB1, MB2, and MT2 stained R-S cells in all nodular lymphocyte predominance cases.

Discussion

The T lymphocyte population in tissues involved by nodular sclerosis or mixed cellularity type of Hodgkin's disease differs from that in normal or reactive T cell areas. There is a relative increase of CD4 positive cells that also express CD38 as a marker of activation.[3,4] To further analyze this T cell population, we used antibodies reactive with so-called "leukocyte common antigens" that are present on restricted T cell subsets. Antibodies like 2H4[7] and MB1, and MT2[11] are reactive with approximately 50% of CD4-positive T cells. These antibodies appear to recognize similar epitopes because they are reactive with antigens with molecular weights of 220 and 200 kd present on most B cells and subpopulations of T cells. MB1 can partially block binding of 2H4, whereas MT2 does not,[8] and MT2 does block MB1, whereas MB1 does not block MT2 (data not shown). Antibodies reactive with the 220 and 200 kd bands were clustered as CD45R. Antibody UCHL1 is reactive with a restricted form of the leucocyte common antigen with a molecular weight of 180 kd, and recognizes a population of approximately 50% of CD4-positive T cells.[11] The CD45R+ and the UCHL1+ cell populations have been shown to be mutually exclusive in peripheral blood,[17] and apparently also in lymphoid tissue because in double-staining immunohistochemistry, a mosaic of MB1+ and UCHL1+ cells is present with virtually no double-staining cells. In Hodgkin's disease-involved tissues there is an increase of the UCHL1+ and CD45R−

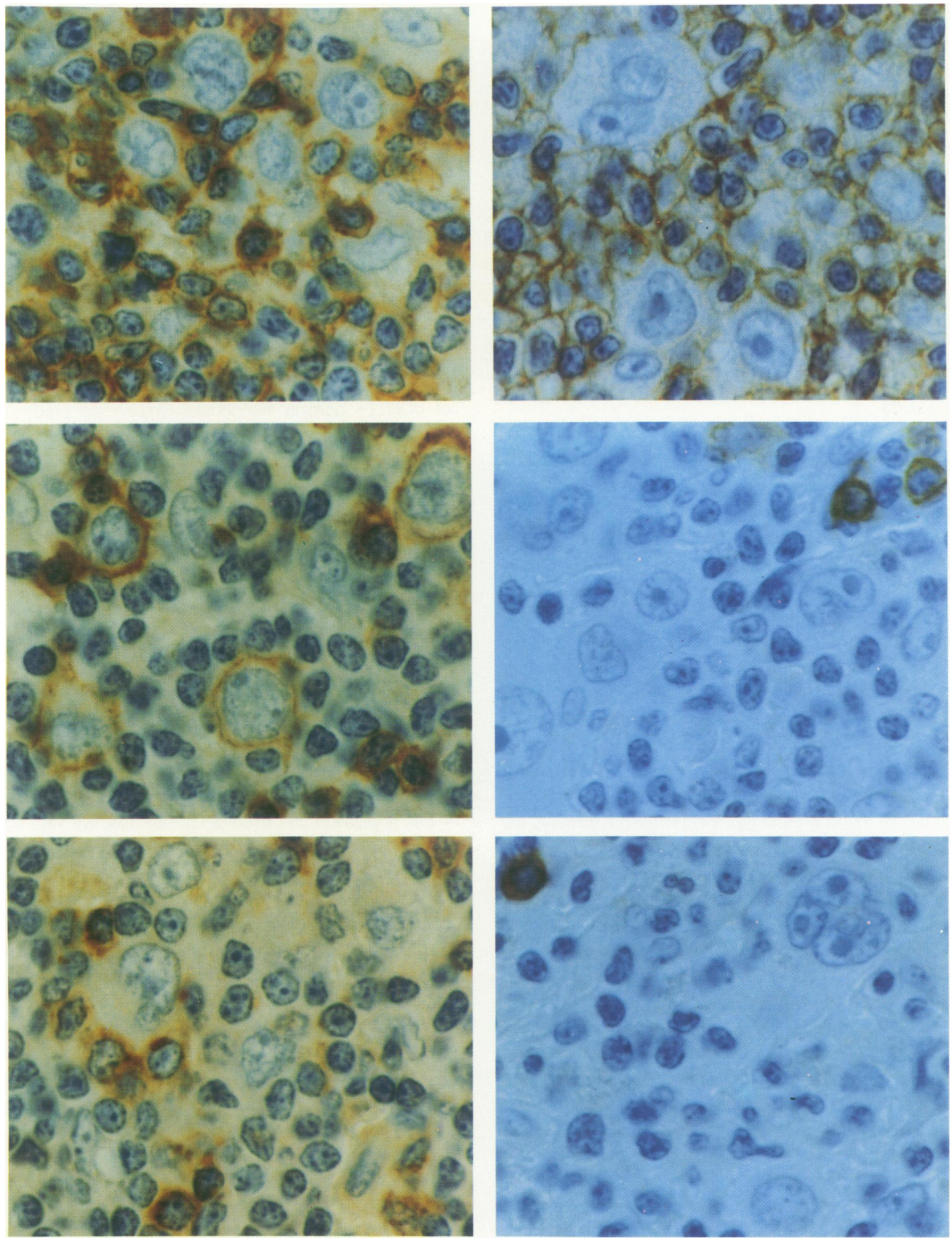

Figure 1. *Immunoperoxidase stain employing ABC method on paraffin-embedded, B-5-fixed tissue sections of a case of nodular lymphocyte predominance type of Hodgkin's disease (A, B, and C) and a case of nodular sclerosis type of Hodgkin's disease (D, E, and F).* **A** *(top left): Antibody UCHL1 gives strong staining of most small lymphocytes surrounding negative L&H-type R-S cells.* **B** *(center left): Antibody MB1 does not react with most small lymphocytes and gives strong membranous staining of L&H-type R-S cells.* **C** *(bottom left): Antibody Leu 7 reacts with most lymphocytes surrounding the R-S cells with a membranous and cytoplasmic staining pattern.* **D** *(top right): Antibody UCHL1 reacts with nearly all lymphocytes surrounding typical and lacunar R-S cells.* **E** *(center right): Antibody MB1 reacts with a few scattered lymphocytes and is not reactive with the R-S cells.* **F** *(bottom right): Antibody leu 7 reacts only with a scattered lymphocyte.*

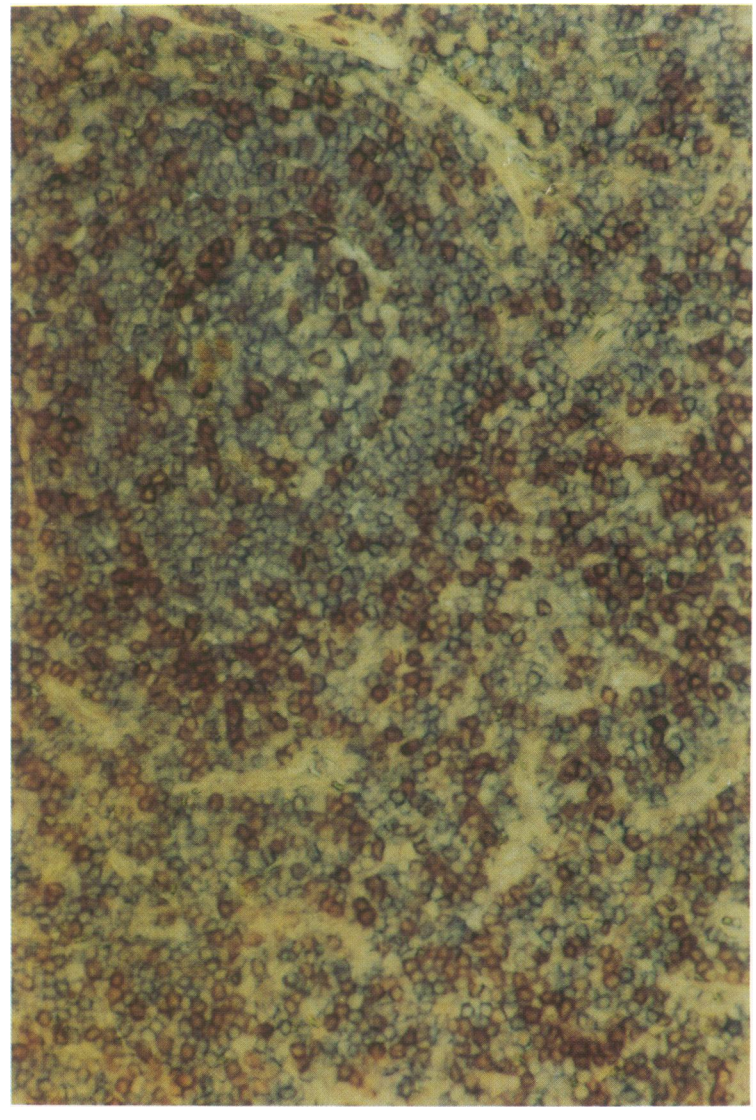

Figure 2. *Double immunostain employing ABC method and APAAP method on a paraffin-embedded tissue section of a reactive lymph node. MB1-positive cells are stained blue and UCHL1-positive cells are stained reddish-brown.*

population and a decrease of the UCHL1− and CD45R+ population. The somewhat lower ratio in the nodular lymphocyte predominance type of Hodgkin's disease results from the inclusion of B lymphocytes in the CD45R+ population. Because most lymphocytes in NLPHD nodules are B lymphocytes, some are directly bordering on the L&H-type R-S cells, despite that most lymphocytes directly surrounding these are T lymphocytes.

The significance of these findings was demonstrated by several recent studies showing that 2H4 (CD45R) is reactive with the suppressor–inducer CD4+ T cell population and not with the helper–inducer CD4+ T cell population.[6,7] This implies that the T cells in Hodgkin's disease are not suppressor–inducer T cells. In addition, it was shown that memory (ie, antigen-primed) T cells are strongly UCHL1+ and CD45R−, whereas unprimed T cells are CD45R+ and weakly UCHL1+ or UCHL1−.[8] This implies that the T lymphocytes in Hodgkin's are a memory T cell population. Several other antigens, like CDw29 (4B4), LFA2 (E rosette receptor), and LFA1 (lymphocyte function associated antigen 1), and LFA3 are also relatively strongly expressed on the memory T cell population.[8] It has been shown that CD45R+ and UCHL1− T cells lose CD45R and start to express UCHL1 after *in vitro* stimulation.[18] These data are consistent with the theory that the T cells in Hodgkin's disease are activated memory T cells that are well equipped for cell–cell interactions by the presence of high levels of lymphocyte function-associated antigens LFA1 and LFA2 (E rosette receptor). R-S cells express high levels of LFA3, which has been shown to be the ligand for the E rosette receptor,[19] and it has been demonstrated that so-called "lymphocyte rosetting" of T cells with Hodgkin cell line L428 can be inhibited by antibodies against LFA1, LFA2, and LFA3.[20]

Table 3. *Lymphocytes Surrounding Reed-Sternberg Cells*

Case	Average number of lymphocytes	Average number of leu 7 cells	UCHL1/MB1 ratio*
1	8.42	7.15	15
2	7.83	7.20	18
3	8.14	7.62	21
4	7.45	6.24	21
5	7.29	6.18	23
6	9.36	0.12	78
7	9.10	0.17	65
8	10.08	0.03	56
9	9.35	0.08	85
10	10.84	0.01	73
11	9.17	0.05	82
12	9.45	0.09	64
13	10.13	0.05	73
14	9.24	0.07	56
15	9.16	0.11	57

* The UCHL1/MB1 ratio was derived from the number of UCHI1 positive and MB1 positive cells directly surrounding a total of 100 Reed-Sternberg cells and variants. Because MB1 also stains B cells, these are included in this ratio. The effect can be seen in the ratios of the NLPHD cases that are lower than those in the other subtypes.

A further question is whether the distinction between CD4+ T cells that help B cells and produce IL4, and CD4+ T cells that mediate delayed type hypersensitivity and other inflammatory reactions producing IL2 and gamma-interferon (as has been described in mice) is also valid in humans.[9] There is no direct proof to indicate whether the lymphocytes in Hodgkin's disease produce IL2 and gamma-interferon or IL4. It appears that there must be helper cell activity according to the active germinal center formation and the plasma cell reaction that can be encountered in many cases of nodular sclerosis and mixed cellularity subtypes of Hodgkin's disease, whereas there appears to be a lack of such helper activity in the nodular lymphocyte predominance Hodgkin's disease. This is evidently because germinal centers and plasma cells are lacking and sometimes an acquired hypogammaglobulinemia can be found in this subtype of Hodgkin's disease.[21] A high percentage of CD4+, IL2-producing clones with cytolytic activity can be obtained from spleens involved by Hodgkin's disease.[22] That R-S cells strongly express IL2 receptors also suggests possible production of interleukin 2 by surrounding activated CD4+ T cells, although results on IL2, gamma interferon, and IL4 production by these subsets in humans are scarce. It was published that the CD45R+ suppressor-inducer subset produces IL2 on stimulation, whereas the CD45R− helper cells do not and actually require IL2 for their proliferation.[23] Other researchers have also found that the CD45R+ subset produces IL2 and that the CD45R− subset produces gamma-interferon and low amounts of IL2.[24] The finding that L&H type R-S cells in nodular lymphocyte predominance Hodgkin's disease are associated with Leu 7+ T cells confirms previous studies of frozen tissue sections,[25] whereas the reactivity of L&H type R-S cells with several anti-B cell antibodies and with LN1 is in agreement with the concept of a germinal center B cell origin of this cell type. The functional significance of the CD4+, Leu 7+ T cell subpopulation in germinal centers is unknown, although their almost exclusive presence in late stages of germinal center reaction suggests a regulatory role in B cell differentiation towards memory B cells. *In vitro* culture and cloning of CD4+, leu 7+ cells showed they rapidly lost the leu7 antigen, had no NK function, and were incapable of producing IL2 and B cell growth factor.[26] The exclusive presence of leu7+ lymphocytes surrounding R-S cells establishes a second immunohistologic criterion for the diagnosis of NLPHD, in addition to the finding of strong reactivity of L&H type R-S cells with several B cell specific antibodies.

References

1. Lukes RJ, Butler JJ, Hicks EB: Natural history of Hodgkin's disease as related to its pathologic picture. Cancer 1966, 19:317–344
2. Lukes RJ, Butler JJ: The pathology and nomenclature of Hodgkin's disease. Cancer Res 1966, 26:1063–1081
3. Poppema S, Bhan AK, Reinherz EL, Posner MR, Schlossman SF: In situ immunologic characterization of cellular consistuents in lymph nodes and spleen involved by Hodgkin's disease. Blood 1982, 59:226–232
4. Borowitz MJ, Croker BP, Metzgar RS: Immunohistochemical analysis of the distribution of lymphocyte subpopulations in Hodgkin's disease. Cancer Treat Rep 1982, 66:667–674
5. Galili U, Klein E, Christensson B, Biberfeld P: Lymphocytes of Hodgkin's biopsies exhibit stable E rosette formation, natural attachement and glucocorticoid sensitivity similar to immuno-activated T cells. Clin Immunol Immunopathol 1980, 16:173–179
6. Reinherz EL, Meuer SC, Schlossman SF: The human T cell receptor: analysis with cytotoxic T cell clones. Immunol Rev 1983, 74:83–128
7. Morimoto CN, Letvin JA, Distaso M, Schlossman SF: The isolation and characterization of the human suppressor inducer T cell subset. J Immunol 1985, 134:1508–1515
8. Akbar AN, Terry L, Timms A, Beverly PCL, Janossy G: Loss of CD45R and gain of UCHL1 reactivity is a feature of primed T cells. J Immunol 1988, 140:2171–2178
9. Greenbaum LA, Horowitz JB, Woods A, Pasqualini T, Reich EP, Bottomly K: Autocrine growth of CD4$^+$ cells: Differential effects of IL-1 on helper and inflammatory T cells. J Immunol 1988, 140:1555–1560
10. Poppema S, Visser L, De Ley L: Reactivity of presumed anti-natural killer cell antibody Leu 7 with intrafollicular T lymphocytes. Clin Exp Immunol 1983, 54:834–837
11. Poppema S, Hollema H, Visser L, Vos H: Monoclonal antibodies (MT1, MT2, MB1, MB2, MB3) reactive with leukocyte

subsets in paraffin-embedded tissue sections. Am J Pathol 1987, 127:418–429
12. Abo T, Balch CM: A differentiation antigen of human NK and K cells identified by a monoclonal antibody (HNK1). J Immunol 1988, 127:1024–1029
13. Epstein AL, Marder RJ, Winter JN, Fox RI: Two new monoclonal antibodies (LN-1, LN-2) reactive in B5 formalin-fixed paraffin-embedded tissues with follicular center and mantle zone human B lymphocytes and derived tumors. J Immunol 1984, 133:1028–1036
14. Marder RJ, Variakojis D, Silver J, Epstein AL: Immunohistochemical analysis of human lymphomas with monoclonal antibodies to B cell and IA antigens reactive in paraffin tissue. Lab Invest 1985, 52:497–504
15. Linder J, Ye Y, Armitage JO, Weisenburger DD: Monoclonal antibodies marking T- and B-lymphocytes in paraffin-embedded tissue, Leukocyte Typing III. Edited by AJ McMichael. Oxford, England, Oxford University Press 1987, pp 297–301
16. Cordell JL, Falini B, Erber W: Immunoenzymatic labeling of monoclonal antibodies using immune complexes of alkaline phosphatase and monoclonal anti-alkaline phosphatase (APAAP complexes). J Histochem Cytochem 1984, 32:219–229
17. Beverley PCL, Merkenschlager M, Terry L: Phenotypic diversity of the CD45 antigen and its relation to function. Immunology 1988, 1(Suppl):3–5
18. Serra HM, Krowka JF, Ledbetter JA, Pilarski LM: Loss of CD45R (LP220) represents a post-thymic T cell differentiation event. J Immunol 1988, 140:1435–1441
19. Dustin ML, Sanders ME, Shaw S, Springer TA: Purified lymphocyte function-associated antigen 3 binds to CD2 and mediates T lymphocyte adhesion. J Exp Med 1987, 165: 677–692
20. Sanders ME, Makgoba MW, Sussman EH, Luce GEG, Cossman J, Shaw S: Molecular pathways of adhesion in spontaneous rosetting of T lymphocytes to the Hodgkin's cell line L428. Cancer Res 1988, 48:37–40
21. Poppema S: Current problems in Hodgkin's disease, Advances in Pathology, Vol. 2. Pergamon Press 1982
22. Romagnani S, Maggi E, Parronchi P: Clonal analysis of T lymphocytes in spleens from patients with Hodgkin's disease: Frequent occurrence of unusual T4 positive cells which coexpress cytolytic activity and production of IL2. Int J Cancer 1986, 37:343–349
23. Salmon M, Kitas GD, Hill Gaston JS, Bacon PA: Interleukin-2 production and response by helper T-cell subsets in man. Immunol 1988, 65:81–85
24. Dohlsten M, Hedlund G, Sjogren H-O, Carlsson R: Two subsets of human CD4+ T helper cells differing in kinetics and capacities to produce interleukin 2 and interferon-γ can be defined by the leu-18 and UCHL1 monoclonal antibodies. Eur J Immunol 1988, 18:1173–1178
25. Timens W, Visser L, Poppema S: Nodular lymphocyte predominance type of Hodgkin's disease is a germinal center lymphoma. Lab Invest 1986, 54:457–461
26. Velardi A, Mingari MC, Moretta L, Grossi CE: Functional analysis of cloned germinal center $CD4^+$ cells with natural killer cell-related features: Divergence from typical T helper cells. J Immunol 1986, 137:2808–2813

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