Low-Grade Lymphomas

Expression of Developmentally Regulated B-Cell Antigens

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A series of low-grade B-cell lymphomas was analyzed for a battery of immunologic determinants by flow cytometry and immunohistochemistry. Histologically distinctive subclasses of these lymphomas, well-differentiated lymphocytic (WDL), intermediately differentiated lymphocytic (IDL), and follicular center cell (FCC) lymphoma, were found to be readily distinguishable by their expression of immunologic determinants that are known to be developmentally regulated in normal B cells. Although all cases expressed monoclonal surface immunoglobulin (sIg), HLA-DR, and the surface membrane proteins recognized by antibodies B1 (p32) and BA1, staining with other monoclonal antibodies revealed unique immunologic phenotypes for each subclass: WDL p65 (Leu 1)+, p24 (BA2)−; IDL p65+, p24+; FCC p65−, p24−. Additionally, the fluorescence intensities (number of determinants per cell) obtained for sIg, BA-1, and B1, but not HLA-DR, were significantly different among the three lymphoma subclasses. The relative fluorescence intensities of each of these three markers followed the same pattern: FCC>IDL>WDL. Taken together, these distinguishing features suggest that low-grade B-cell lymphomas represent arrested, and possibly sequential, stages of B-cell differentiation. (Am J Pathol 1984, 115: 117–124)

LYMPHOCYTIC MALIGNANCIES associated with a low-grade natural history include well-differentiated ("small") lymphocytic lymphoma (WDL), its leukemic counterpart, chronic lymphocytic leukemia (CLL), nodular (follicular center cell, FCC) lymphomas, and intermediately differentiated lymphocytic ("mantle-zone") lymphoma (IDL).[1–6] Despite their indolent clinical behavior, these lymphomas are not usually totally eradicated by conventional therapeutic modalities.[2,5] Each of the entities can be distinguished on morphologic grounds and are, with rare exception, B-cell neoplasms, as evidenced by monoclonal immunoglobulin production and expression of complement receptors by neoplastic cells.[6,7]

Because malignant lymphomas may well represent monoclonal expansions arrested at a particular stage of differentiation,[8] we questioned whether the low-grade lymphomas expressed distinguishing immunologic phenotypes that might relate them to stages of normal B-cell differentiation. Several monoclonal (hybridoma) antibodies such as B1, BA1, and BA2 have been found to react with certain B-cell surface antigens preferentially expressed during discrete stages of B-cell differentiation.[9–11] We investigated the expression of these antigens as well as other developmentally regulated B-cell markers, surface immunoglobulin (sIg)[9,11–13] and HLA-DR (Ia),[9,11,13] in a series of low-grade B-cell lymphomas using flow cytometry and immunohistology. The profile that emerged demonstrated a unique phenotype for each subclass of lymphoma, such that the various low-grade B-cell lymphomas appear to represent sequential, arrested stages in the terminal differentiation of B cells.

Materials and Methods

Lymph node biopsies were obtained from 34 patients with low-grade B-cell lymphomas. Each bore monoclonal surface immunoglobulin with a single light chain type. Twelve cases were classified as malignant lymphoma, intermediately differentiated lym-

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Table 1—Immunologic Determinants of Low-Grade B-Cell Lymphomas*

Lymphoma	Determinant		
	Leu1(p65)	BA2(p24)	J5(gp95)
WDL	+ +	–	+ (5/10)†
IDL	+ +	+ +	+ + (11/12)
FCC	–	–	+ (4/10)

* Surface membrane markers expressed by low grade B cell lymphomas are indicated as follows: +, all cases positive, mean of percentage of stained cells between 15-50%; + +, all cases positive, mean of percentage of stained cells >50%; –, all cases negative.

† Proportion of positive/total cases where only a fraction of cases were positive.

phocytic (IDL), 12 patients had follicular lymphomas (nodular pattern) (FCC), and 10 had well-differentiated lymphocytic lymphoma (WDL). All follicular lymphomas were either of the poorly differentiated lymphocytic type (6 cases) or mixed-cell type (4 cases). Follicular large cell "histiocytic" lymphomas were excluded because these are associated with a more aggressive clinical course.[14]

Lymph node biopsies were prepared and stained with monoclonal antibodies for analysis by flow cytometry and immunohistochemistry as described.[7,15,16] Prior to staining of cell suspensions, endogenous cytophilic immunoglobulin was shed by placing cells in a 37 C water bath for 30 minutes. To remove shed immunoglobulin, cells were carefully layered over 10 ml of heat-inactivated fetal calf serum in a 15-ml conical tube, centrifuged at 300*g* for 20 minutes, and then washed three times in RPMI-1640. Monoclonal antibodies to human antigens used in this study included anti-γ, anti-α_1, anti-α_2, anti-δ, anti-κ, and anti-λ immunoglobulins, anti-HLA-DR (Ia) and Leu 1 (p65)[17] (Becton-Dickinson, Sunnyvale, Calif); anti-immunoglobulin μ (Bethesda Research Laboratories, Gaithersburg, Md); Lyt-3 (New England Nuclear, Boston, Mass)[18]; J5, common acute lymphoblastic leukemia antigen (CALLA) (from Dr. Jerome Ritz, Harvard University)[19]; BA-1 and BA-2(p24) (from Dr. Tucker Le Bien, University of Minnesota)[9,10]; and B1 (p32) (Coulter Corp., Hialeah, Fla).[20] Secondary antibody was fluorescein-conjugated, affinity-purified goat anti-mouse IgG, heavy and light chains (Kirkegaard and Perry, Gaithersburg, MD). Surface membrane μ chain was also identified by direct immunofluorescence with affinity-purified, fluorescein-conjugated $F(ab')_2$ fragment of goat anti-human immunoglobulin μ heavy chain (Tago, Co., Burlingame, Calif). Fluorescent antibody analyses were performed by flow cytometric analysis of 10,000 cells with a FACS-II (Becton-Dickinson FACS Systems, Sunnyvale, Calif), coupled to a PDP 11-34 computer (Digital Equipment Corporation, Maynard, Mass). Linear scale histograms (1023 channels) were analyzed by integrating the number of cells in positive channels and subtracting the similarly calculated value for the nonreactive mouse ascites control. When this percentage exceeded 15% a sample was scored as positive. The FACS-II was calibrated daily with uniform 1.515-μ diameter fluorescent microspheres (Polysciences, Inc., Warrington, Pa) and used at identical settings for each run so that fluorescence intensity could be compared using the same monoclonal antibody on different specimens. Florescence intensity was determined by computer calculation of the median channel number of stained cells. The mean fluorescence intensities of each determinant were compared between subclasses by the Student *t* test.

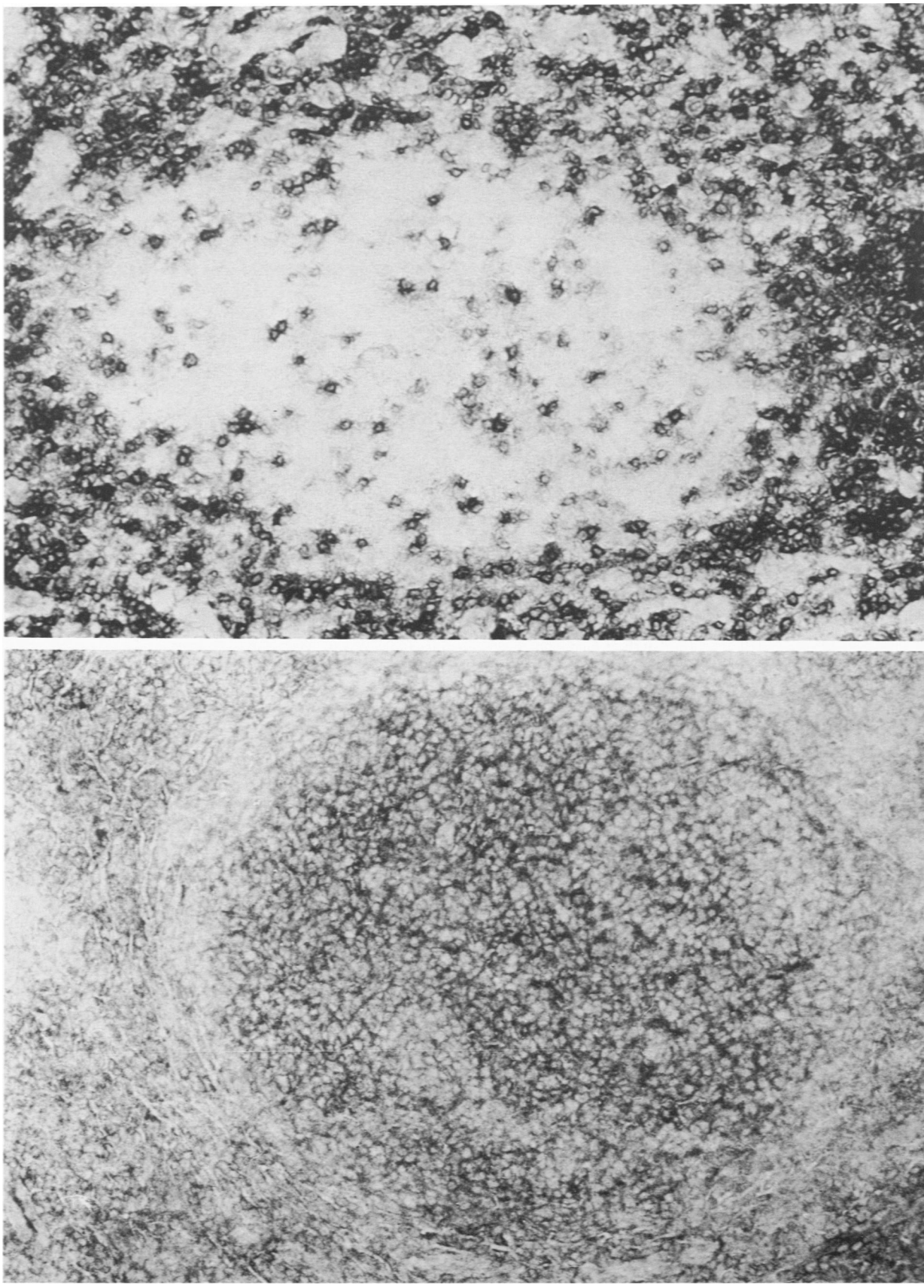
Air-dried, acetone-fixed frozen sections of each case were stained with the same mouse monoclonal antibodies as above by the avidin-biotin complex (ABC) method.[16]

Results

Cells from all cases bore sIg with a single light chain type, which was most often κ in WDL (7κ, 3λ) and FCC (8κ, 4λ) and λ in IDL (4κ, 8λ). Most cases expressed surface μ heavy chain. One case of IDL and 3 of FCC expressed only γ chains, whereas 3 FCC cases and 1 WDL case stained for both μ and γ. Surface δ chains were detected in some cases from all three subclasses.

Neoplastic cells from all cases of WDL and IDL expressed p65 in cell suspension as well as tissue section. In FCC the means for total T cells (Lyt3) and p65 were identical, 35%. Thus, in FCC p65 appeared to be expressed only by admixed normal T cells, because normal T cells, but not B cells, in peripheral blood and lymphoid tissues bear the p65 antigen.[17] Furthermore, in frozen tissue sections of FCC the p65+ cells were predominantly outside neoplastic follicles (Figure 1A). The majority of cases of IDL stained for p24 (BA2), whereas WDL and FCC did not react with BA2 either in suspension or frozen

Figure 1A—A frozen section of a follicular center cell lymphoma (FCC) stained with Leu1(p65) by the avidin-biotin complex (ABC) method shows staining of the small cells outside of the neoplastic follicle. Another T-cell antibody, Lyt-3, also showed the same pattern of staining in FCC. Thus, the p65+ cells in FCC were admixed T cells rather than the malignant B cells. FCCs were composed of an average of 35% T cells as assessed by flow cytometry. (×650) **B**—Frozen section of follicular center cell lymphoma (FCC) stained with anti-CALLA (J5) shows selective reactivity with the cells of the neoplastic follicle. (×650)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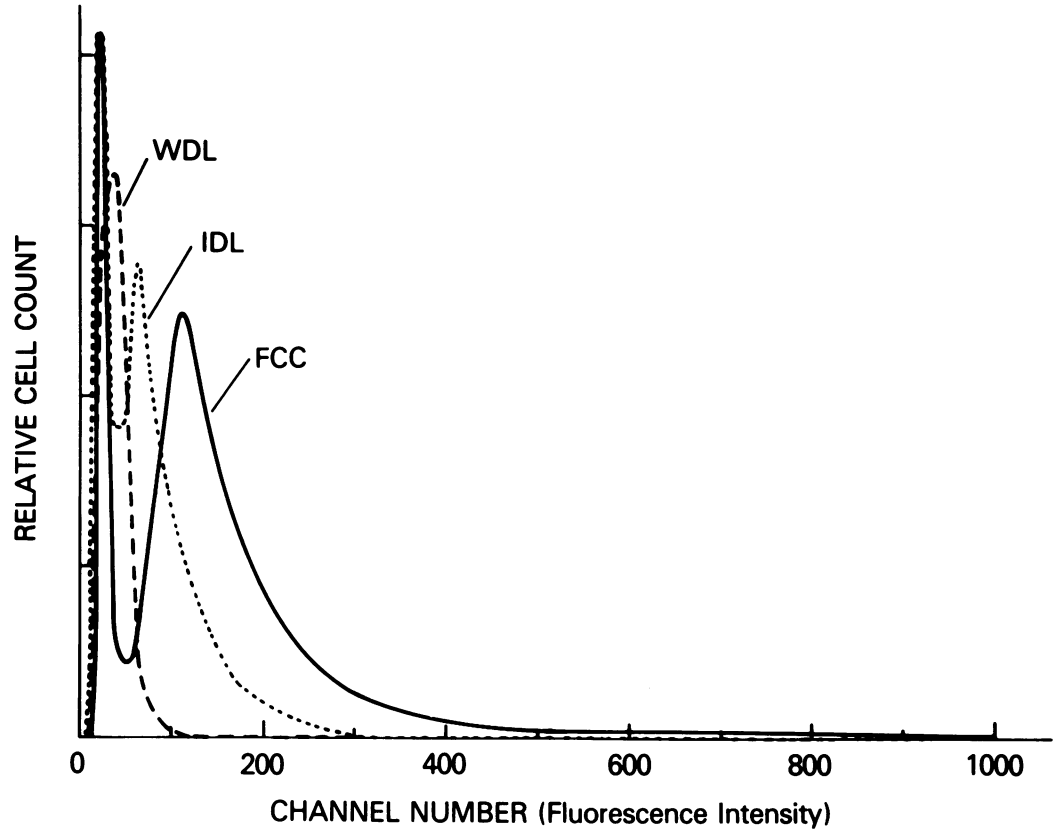

Figure 2—Histogram of FACS II analysis of surface immunoglobulin in representative cases of WDL, IDL, and FCC. Staining with BA1 and B1 gave similar homogeneous profiles and the same sequential pattern of fluorescence intensity, FCC>IDL>WDL.

section (Table 1). J5 (CALLA) stained 4 of 10 FCC, 11 of 12 IDL, and 5 of 10 WDL (Table 1, Figure 1B).

Four markers were expressed in all cases: sIg, B1, BA1, and HLA-DR. Cells stained for sIg, B1, and BA1 and analyzed by the FACS had a homogeneous profile on a linear fluorescence histogram (Figure 2). Also, the intensity of the fluorescence for these markers was greatest in FCC and least in WDL (Figure 2). The fluorescence intensity derived from the FACS analysis of each sample was calculated as the median fluorescence channel number. This value provides an approximate determination of the number of antigen sites per cell, and comparisons of the mean of these relative fluorescence values were made between subclasses. Statistically significant differences ($P < 0.05$) were obtained for sIg, B1, and BA1, but not HLA-DR when each subclass was compared with the other (WDL versus IDL, WDL versus FCC, and IDL versus FCC) (Figure 3).

Discussion

Our studies indicate that low-grade B-cell non-Hodgkin's lymphomas are immunologically distinguishable as three subsets corresponding to the histologic subclasses WDL, IDL, and FCC. The unique array of immunologic markers within each group is evidence that these are not only morphologic variants of B-cell lymphomas but may represent distinct biologic entities as well. Indeed, they may well represent consecutively arrested stages of differentiation along the continuum of B-cell development. The notion that B-cell neoplasms have reached a blocked step in B-cell maturation is supported by studies demonstrating their inherent capacity to differentiate further when exposed *in vitro* to exogenous signals such as allogeneic T lymphocytes,[21,22] phorbol diester (TPA),[15,23] and/or mitogens.[21,22,24] It is likely that the surface membrane determinants of B cells recognized by monoclonal antibodies are developmentally regulated, thus allowing for their consecutive acquisition and loss during differentiation. This results in distinctive phenotypes for each stage, as is the case for T cells.[25]

Certain changes in membrane phenotype associated with B-cell differentiation have already been established: as a B cell differentiates toward a plasma cell, there is progressive loss of surface immunoglob-

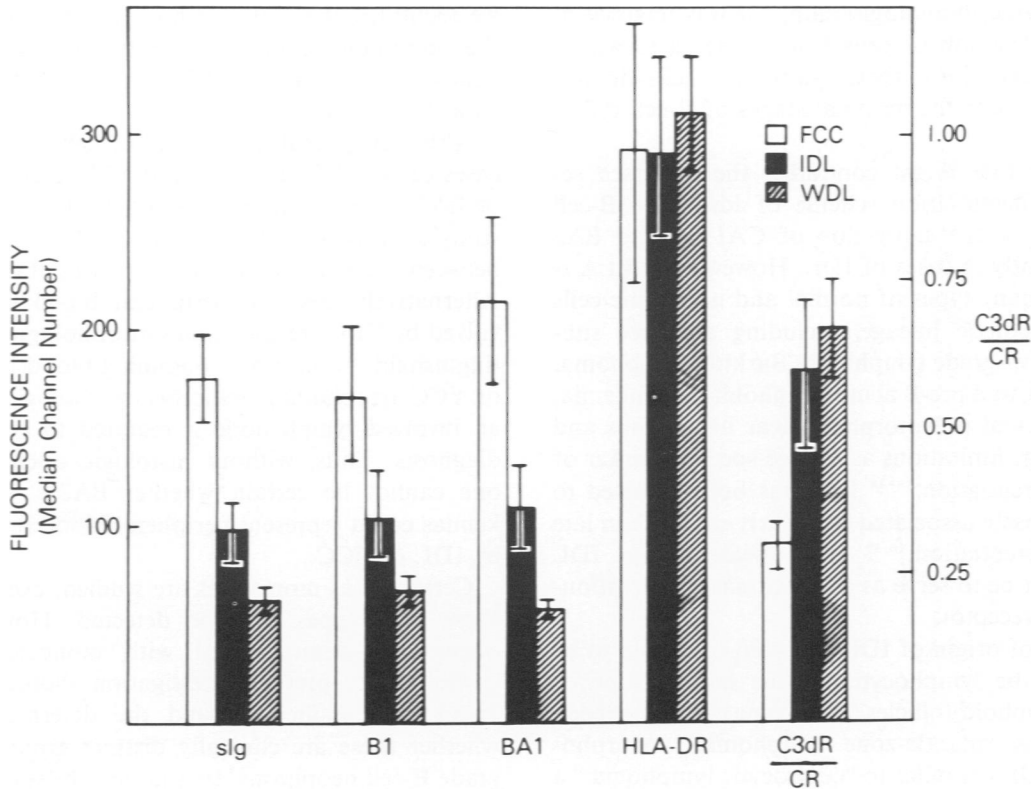

Figure 3 – Relative fluorescence intensities of HLA-DR, B1, BA1, and sIg in the low-grade B-cell lymphomas. The median channel number (fluorescence intensity) of stained cells was determined by flow cytometry (FACS-II), and the mean of these values was calculated for each subclass of lymphoma. Statistically significant differences ($P < 0.05$) were obtained between each subclass for the mean fluorescence intensity of BA1, B1, and sIg, but not HLA-DR. The ratio of cells bearing complement receptors specific for C3d only (C3dR) to total complement receptors bearing cells (CR), as previously determined,[7] is also displayed.

ulin,[12,13] BA1,[9] and B1,[11] but prolonged retention of HLA-DR.[9,11,13] Most remarkably, we observed a sequential decline in the expression of sIg, BA1, and B1 when proceeding from FCC to IDL to WDL. We have previously encountered the identical sequence, but in reverse, when investigating complement receptor subsets[7] (Figure 3). Interestingly, the expression of the C3d receptor transiently increases on mitogen-activated B cells as they approach the stage of immunoglobulin secretion.[26] Thus, the proposed sequence encountered in B-cell neoplasms appears to mirror the sequence of normal B-cell differentiation.

If the three subclasses of low-grade B-cell lymphoma are serially linked according to differentiation status, the present evidence would support WDL as the lymphoma closest to the plasma cell stage, ie the most differentiated. WDL is much more likely to have plasmacytoid morphologic characteristics and an associated serum immunoglobulin spike than is IDL or FCC.[1,4] Cells of WDL and its leukemic counterpart, chronic lymphocytic leukemia, have faint sIg[27] and readily secrete immunoglobulin *in vitro* when stimulated by TPA.[28] This differentiation step is associated with rapid accumulation of immunoglobulin messenger RNA, predominantly of the form coding for the secretory rather than membrane-bound μ chain,[28] a pattern similar to that seen in murine plasma cells.[29-31] Thus, these cells are at the threshold of the terminal differentiation stage of abundant immunoglobulin secretion. By contrast, FCC and IDL can be induced to secrete immunoglobulin; but to accomplish this, they require the addition of allogeneic T cells along with TPA (unpublished results).

Exclusive expression of γ but not μ heavy chains by 3 of 12 FCC and simultaneous expression of both μ and γ in 3 additional cases could involve either of two possible mechanisms. In one the Cμ gene could have been deleted with subsequent joining of complementary switch sequences 5′ to the Cγ gene.[32] Alternatively, a single large transcript encoded from an intact C region gene could be spliced to produce mature Cγ-mRNA in IgG-bearing cells or even Cμ-mRNA in the cells expressing both μ and γ.[33] The configurations of the C genes in these lymphomas is being investigated. Although sIgD is usually absent on plasma cells ac-

tively secreting immunoglobulin,[34] it was retained at least on a fraction of cells from some cases within each subclass. Thus, these lymphoma cells do not fully recapitulate the terminal stages of B-cell differentiation.

Evidence that might contradict the proposed sequential differentiation scheme of low-grade B-cell lymphomas is the expression of CALLA and BA2 predominantly in cases of IDL. However, CALLA is found on many types of normal and neoplastic cells of hematopoietic lineage, including all three subclasses of low-grade lymphoma, Burkitt's lymphoma, precursor B, and pre-B acute lymphoblastic leukemia, and on normal polymorphonuclear neutrophils and therefore has limitations as a stage-specific marker of B-cell differentiation.[35-38] BA2 has been reported to be more closely associated with early rather than late B-cell differentiation.[10,39] Its appearance on IDL alone might be to serve as a functional or activation-associated receptor.

The cell of origin of IDL has been suggested to be related to the lymphocytes of the mantle zone of normal lymphoid follicles,[6] and some cases have been classified as "mantle-zone lymphoma."[4] Morphologically, IDL is similar to "centrocytic lymphoma," a B-cell lymphoma that is also p65 (Leu 1)+ and, like the cases of IDL presented here, has a striking predominance of λ light chain expression.[40] The regulation of this preference for λ production in IDL is not known, but presumably nonproductive attempts at rearrangement of both κ chain genes occurred in these cells, because λ gene expression is preceded by κ gene deletion or rearrangements.[41] We have previously demonstrated that the majority of IDLs contain alkaline phosphatase activity in their cell surface membranes.[3] Cells of the normal mantle zone are B cells with cell membrane alkaline phosphatase,[3] but in frozen section they lack p65 and CALLA.[42] Although the normal counterpart of the IDL cell remains elusive, the neoplastic cells of FCC clearly relate to the normal germinal center[43]; and, therefore, IDL and WDL may well be counterparts of postfollicular cells.

Expression of the T-cell-associated antigen p65 by WDL is not unexpected, because the majority of cases of CLL are p65+.[44-46] That IDL was also p65+ lends additional support to the serial linkage of IDL and WDL and might relate them to the rare normal B cell of lymph node and tonsil found to be p65+.[47] Significant here is the lack of Leu 1 staining by the neoplastic cells of FCC in contrast to IDL. Because some IDLs have a nodular pattern and a cytologic similarity to FCC, these two histologic types could be confused. This could lead one to conclude that some FCC lymphoma cells are Leu 1+. In the present study we identified the IDLs without prior knowledge of the immunologic marker results and have demonstrated a phenotype for IDL (Leu 1+) that is distinct from FCC (Leu 1−).

Although a small percentage of cells from 5 of 10 cases of B-cell CLL were reported by Kersey et al to be BA2+,[10] none of the cases of WDL in the present study expressed BA2. This could reflect a difference between nodal and blood-borne phases of the disease. Alternatively, because peripheral blood smears involved by CLL are sometimes morphologically indistinguishable from those containing blood-borne IDL or FCC lymphoma, tissue histopathologic study of an involved lymph node is required to confirm the diagnosis. Thus, without histologic documentation one cannot be certain whether BA2+ B-cell leukemias could represent peripheral blood involvement by IDL or FCC.

Certainly, as more cases are studied, exceptions to these phenotypes may be detected. However, the phenotypes demonstrated with monoclonal antibodies in the present investigation should assist in histologic classification and the determination of whether these are clinically distinct groups of low-grade B-cell neoplasms. In addition, because conventional therapy does not prevent relapse in low-grade lymphomas, the therapeutic use of monoclonal antibodies directed at neoplastic cells may offer an alternative means of control. The efficacy of highly specific monoclonal anti-idiotype antibody has already been demonstrated in one patient with follicular lymphoma.[48] The present study has identified distinct surface antigen combinations in low-grade B-cell lymphomas. These antigens could provide specific points of attack by generally available monoclonal antibodies not only as an alternative strategy for passive immunotherapy but also for *in vitro* treatment of autologous bone marrow prior to reinfusion. Clinical trials employing these techniques are under way.

Note Added in Proof

Recently, several studies have appeared which confirm and extend the findings we report here. In three separate investigations it was discovered that expression of p65 (Leu 1, OKT1) by low-grade FCC could be detected in only 0 of 25,[49] 2 of 42,[50] and 4 of 13 cases.[51] Perhaps these few reported p65+ cases share histologic features with the nodular form of IDL. Further evidence supporting the similarity of "centrocytic lymphoma" and IDL was demonstrated in a recent study in which "centrocytic lymphoma," like IDL, was found to have a surface Igλ predomi-

nance (11 of 17 cases) and expression of Leu 1 (p65), BA2 (p24), and alkaline phosphatase in most cases.[52]

References

1. Pangalis GA, Nathwani BN, Rappaport H: Malignant lymphoma, well-differentiated lymphocytic: Its relationship with chronic lymphocytic leukemia and macroglobulinemia of Waldenstrom. Cancer 1977, 39:99–1010
2. Stein RS, Cousar J, Flexner JM, Graber SE, McKee LC, Krantz S, Collins RD: Malignant lymphomas of follicular center cell origin in man: III. Prognostic features. Cancer 1979, 44:2236–2243
3. Nanba K, Jaffe ES, Braylan RC, Soban EJ, Berard CW: Alkaline phosphatase-positive malignant lymphoma: A subtype of B-cell lymphomas. Am J Clin Pathol 1977, 68:535–542
4. Weisenburger DD, Nathwani BN, Diamond LW, Winberg CD, Rappaport H: Malignant lymphoma, intermediate lymphocytic type: A clinico-pathologic study of 42 cases. Cancer 1981, 48:1415–1425
5. Garvin AJ, Simon R, Young RC, DeVita VT, Berard CW: The Rappaport classification of non-Hodgkin's lymphomas: A closer look using other proposed classifications. Semin Oncol 1980, 7:234–243
6. Mann RB, Jaffe ES, Berard CW: Malignant lymphomas—a conceptual understanding of morphologic diversity. Am J Pathol 1979, 94:105–175
7. Cossman J, Jaffe ES: Distribution of complement receptor subtypes in non-Hodgkin's lymphomas of B cell origin. Blood 1981, 58:20–26
8. Salmon SE, Seligmann M: B-cell neoplasia in man. Lancet 1974, 2:1230–1233
9. Abramson C, Kersey J, Le Bien T: A monoclonal antibody (BA-1) reactive with cells of human B lymphocyte lineage. J Immunol 1981, 126:83–88
10. Kersey JH, Le Bien TW, Abramson CS, Newman R, Sutherland R, Greaves M: A human leukemia associated and lymphohemopoietic progenitor cell surface structure identified with monoclonal antibody. J Exp Med 1981, 153:726–731
11. Stashenko P, Nadler LM, Hardy R, Schlossman SF: Expression of cell surface markers after human B lymphocyte activation. Proc Natl Acad Sci USA 1981, 78:3848–3852
12. Ferroni M, Viale G, Risso A, Pernis B: A study of the immunoglobulin classes present on the membrane and in the cytoplasm of human tonsil plasma cells. Eur J Immunol 1976, 6:562–565
13. Halper J, Fu SM, Wang CY, Winchester R, Kunkel HG: Patterns of expression of human "Ia-like" antigens during the terminal stages of B cell development. J Immunol 1978, 120:1480–1484
14. Osborne CK, Norton L, Young RC, Garvin AJ, Simon RM, Berard CW, Hubbard S, DeVita VT Jr: Nodular histiocytic lymphoma: An aggressive nodular lymphoma with potential for prolonged disease-free survival. Blood 1980, 56:98–103
15. Cossman J, Neckers LM, Arnold A, Korsmeyer SJ: Induction of differentiation in a case of common acute lymphoblastic leukemia. N Engl J Med 1982, 307: 1251–1254
16. Hsu S, Raime L, Fanger H: A comparative study of the peroxidase—anti-peroxidase method and an avidin-biotin complex method for studying polypeptide hormones with radioimmunoassay antibodies. Am J Clin Pathol 1981, 75:734–738
17. Engelman EG, Warnke R, Fox RI, Dilley J, Benike CJ, Levy R: Studies of a human T lymphocyte antigen recognized by a monoclonal antibody. Proc Natl Acad Sci USA 1981, 78:1791–1795
18. Kamoun M, Martin PJ, Hansen JA, Brown MA, Siadek AW, Nowinski RC: Identification of a human T lymphocyte surface protein associated with the E-rosette receptor. J Exp Med 1981, 153:207–212
19. Ritz J, Pesando JM, Notis-McConarty J, Lazarus H, Schlossman SF: A monoclonal antibody to human acute lymphoblastic leukemia antigen. Nature 1980, 283:583–585
20. Stashenko P, Nadler LM, Hardy R, Schlossman SF: Characterization of a human B lymphocyte-specific antigen. J Immunol 1980, 125:1678–1685
21. Fu SM, Chiorazzi N, Kunkel HG, Halper JP, Harris SR: Induction of in vitro differentiation and immunoglobulin synthesis of human leukemic B lymphocytes. J Exp Med 1978, 148:1570
22. Saiki O, Kishimoto T, Kuritani T, Muraguchi A, Yamamura Y: *In vitro* induction of IgM secretion and switching to IgG production in human B leukemic cells with the help of T cells. J Immunol 1980, 124:2609–2614
23. Totterman TH, Nilsson K, Claesson L, Simonsson B, Armon P: Differentiation of chronic lymphocytic leukemia cells *in vitro*: I. Phorbolester-induced changes in the synthesis of immunoglobulin and HLA-DR. Human Lymph Differ 1981, 1:13–16
24. Robert KH, Moller E, Gahrtos G, Eriksson H, Nilsson K: B-cell activation of peripheral blood lymphocytes from patients with chronic lymphatic leukemia. Clin Exp Immunol 1978, 33:302–308
25. Reinherz EL, Kung PC, Goldstein G, Levey RH, Schlossman SF: Discrete stages of human intrathymic differentiation: Analysis of normal thymocytes and leukemic lymphoblasts of T-cell lineage. Proc Natl Acad Sci USA 1980, 77:1588–1590
26. Gormus BJ, Crandall RB, Shands JW: Endotoxin-stimulated spleen cells: Mitogenesis, the occurrence of the C3 receptor, and the production of immunoglobulin. J Immunol 1974, 112:770–775
27. Slease RB, Wistor R, Sher I: Surface immunoglobulin density on human peripheral blood mononuclear cells. Blood 1979, 54:72–87
28. Cossman J, Neckers LM, Braziel RM, Trepel J, Korsmeyer SJ, Bakhshi A: In vitro enhancement of immunoglobulin gene expression in chronic lymphocytic leukemia. J Clin Invest (In press)
29. Alt FW, Bothwell ALM, Knapp M, Siden E, Mather E, Koshland M, Baltimore D: Synthesis of secreted and membrane-bound immunoglobulin μ chains is directed by mRNA that differ at their 3′ end. Cell 1980, 20: 293–301
30. Rogers J, Early P, Carter C, Calame K, Bond M, Hood L, Wall R: Two mRNA's with different 3′ ends encode membrane-bound and secreted forms of immunoglobulin μ chain. Cell 1980, 20:303–312
31. Yuan D, Tucker PW: Effect of lipopolysaccharide stimulation on the translation of messenger RNA for cell surface immunoglobulin. J Exp Med 1982, 156:962–974
32. Davis MM, Calame K, Early PW, Livant DL, Joho R, Weissman IL, Hood L: An immunoglobulin heavy-chain gene is formed by at least two recombinational events. Nature 1980, 283:733–739
33. Rabbitts TH: Evidence for splicing of interrupted immunoglobulin variable and constant region sequences in nuclear RNA. Nature 1978, 275:291–296
34. Preud'homme JL: Loss of surface IgD by human B lymphocytes during polyclonal activation. Eur J Immunol 1977, 7:191–193
35. Ritz J, Nadler LM, Bhan AK, Notis-McConarty J, Pesando JM, Schlossman SF: Expression of common acute lymphoblastic leukemia antigen (cALLA) by

lymphomas of B cell and T cell lineage. Blood 1981, 58:648–652
36. Korsmeyer SJ, Arnold A, Bakhshi A, Ravetch JV, Siebenlish U, Hieter PA, Sharrow SO, LeBien TW, Kersey JH, Poplack DG, Leder P, Waldmann TA: Immunoglobulin gene rearrangement and cell surface antigen expression in acute lymphocytic leukemia of T cell and B cell precursor origins. J Clin Invest 1983, 71:301–313
37. Cossman J, Neckers LM, Leonard WJ, Greene WC: Polymorphonuclear neutrophils express the common acute lymphoblastic leukemia antigen. J Exp Med 1983, 157:1064–1069
38. Braun MP, Martin PJ, Ledbetter JA, Hansen JA: Granulocytes and cultured human fibroblasts express common acute lymphoblastic leukemia associated antigens. Blood 1983, 61:718–725
39. Le Bien TW, Bollum FJ, Yasmineh WG, Kersey JH: Phorbol ester-induced differentiation of a non-T, non-B leukemic cell line: Model for human lymphoid progenitor cell development. J Immunol 1982, 128:1316–1320
40. Stein H, Gerdes J, Mason DY: The normal and malignant germinal centre. Clin Haematol 1982, 11:531–559
41. Hieter PA, Korsmeyer SJ, Waldmann TA, Leder P: Human immunoglobulin κ light chain genes are deleted or rearranged in λ-producing B cells, Nature 1981, 290: 368–372
42. Poppema S, Bhan AK, Reinherz EL, McCluskey RT, Schlossman SF: Distribution of T cell subsets in human lymph nodes. J Exp Med 1981, 153:30–41
43. Jaffe ES, Shevach EM, Frank MM, Berard CW, Green I: Nodular lymphoma—Evidence for origin from follicular B lymphocytes. N Engl J Med 1974, 290:813–819
44. Royston I, Majda JA, Baird SM, Meserve BL, Griffiths JC: Human T cell antigens defined by monoclonal antibodies: The 65,000-dalton antigen on T cells (T65) is also found on chronic lymphocytic leukemia cells bearing surface immunoglobulin. J Immunol 1980, 125: 725–731
45. Boumsell L, Coppin H, Pham D, Raynall B, Lemerle J, Dausett J, Bernard A: An antigen shared by a human T cell subset and B cell chronic lymphocytic leukemic cells: Distribution on normal and malignant lymphoid cells. J Exp Med 1980, 152:229–234
46. Martin PJ, Hansen JA, Nowinski RC, Brown MA: A new human T cell differentiation antigen: Unexpected expression on chronic lymphocytic leukemia cells. Immunogenetics 1980, 121:429–439
47. Caligaris-Cappio F, Gobbi M, Bofill M, Janossy G: Infrequent normal B lymphocytes express features of B-chronic lymphocytic leukemia. J Exp Med 1982, 155: 623–628
48. Miller RA, Maloney DG, Warnke R, Levy R: Treatment of B cell lymphoma with monoclonal anti-idiotype antibody. N Engl J Med 1982, 306:517–522
49. Harris NL, Bhan AK: Distribution of T-cell subsets in follicular and diffuse lymphomas of B-cell type. Am J Pathol 1983, 113:172–180
50. Burns BF, Warnke RA, Doggett RS, Rouse RV: Expression of a T-cell antigen (Leu-1) by B cell lymphomas. Am J Pathol 1983, 113:165–171
51. Knowles DM, Halper JP, Azzo W, Wang CY: Reactivity of monoclonal antibodies Leu1 and OKT1 with malignant human lymphoid cells. Cancer 1983, 52:1369–1377
52. Swerdlow SH, Habeshaw JA, Murray LJ, Dhaliwal HS, Lister TA, Stansfield AG: Centrocytic lymphoma: A distinct clinicopathologic and immunologic entity. Am J Pathol 1983, 113:181–197

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