Immunologic Surface Markers in Non-Hodgkin's Lymphomas

Jeffrey Cossman, MD, Bertram Schnitzer, MD, and Michael J. Deegan, MD

Tissues from 21 patients with non-Hodgkin's lymphomas were examined for immunologic cell surface markers. Patterns of distribution of complement receptor (CR) B lymphocytes and Fc receptor (FcR)-bearing histiocytes in tumor tissue were evaluated and compared to routine histologic preparations of the tumors and to normal tissue. The lymphomatous infiltrates from all 6 cases of nodular, poorly differentiated lymphocytic lymphoma (NPDLL) consisted of dense populations of CR B lymphocytes. Involved tissue from 7 of 8 patients with diffuse, poorly differentiated lymphocytic lymphoma (DPDLL) was predominately comprised of CR B lymphocytes. Discrete nodules of CR B cells were present in a lymph node replaced by DPDLL. FcR were identified on the cells from 1 of 3 cases of histiocytic lymphoma. None of the 4 cases of undifferentiated lymphoma possessed demonstrable surface markers in tissue section; however, the cell suspension from 1 case contained a high percentage of CR B cells. Both CR and T cell markers were present on the cells of DPDLL of childhood. (Am J Pathol 87:19–32, 1977)

THE CURRENT CLASSIFICATION of the malignant lymphomas is based on both cell morphology and the pattern of growth (nodular or diffuse). Recent developments in the identification of immunologic cell surface markers have been applied to neoplasms arising from lymphoreticular cells.[1] Among the non-Hodgkin's lymphomas, a B lymphocytic origin has been attributed to well-differentiated lymphocytic lymphoma;[2] poorly differentiated lymphocytic lymphoma (PDLL), both nodular (N) and diffuse (D);[3–6] Burkitt's lymphoma;[7] and histiocytic lymphoma (HL).[8,9] Mycosis fungoides,[10] DPDLL of childhood with mediastinal mass,[1,11] and some cases of adult DPDLL are derived from T lymphocytes.[12]

In the present study, we examined the histologic distribution of cells bearing specific receptors in the tumor-bearing tissue from 21 patients with non-Hodgkin's lymphomas using the frozen section rosette assay. Tissues from 13 nonlymphoma patients served as controls. In tissue section and in cell suspension, complement receptor (CR) B lymphocytes are identified by their attachment to sheep erythrocytes (E) coated with IgM (A) and complement (C) (IgMEAC).[13] Receptors for the Fc fragment of

From the Department of Pathology, The University of Michigan Medical School, Ann Arbor, Michigan.

Accepted for publication November 8, 1976.

Address reprint requests to Dr. Jeffrey Cossman, Department of Pathology, University of Michigan Medical Center, Ann Arbor, MI 48109.

the immunoglobulin molecule (FcR) will bind E sensitized with IgG in both tissue sections and cell suspensions.[14,15] FcR histiocytes can be distinguished from FcR-positive B lymphocytes by using subagglutinating concentrations of IgG.[15] T cells form rosettes with unsensitized E only in cell suspension (Table 1).[16]

Materials and Methods

Patient Population

Twenty-one patients with non-Hodgkin's lymphomas undergoing either laparotomy or biopsy served as tissue sources. Diagnoses were based on the classification of Rappaport.[17] Evaluation of the pattern of growth (nodular or diffuse) was assessed on the original diagnostic biopsy. The source of tissue and diagnosis for each case are presented in Table 2. None of the patients were undergoing therapy at the time the tissue was obtained for this study (see Table 2 for previous therapeutic regimen).

Preparation of Tissue

Portions of spleens and lymph nodes containing lymphoma were frozen in O.C.T. embedding medium (Ames Co., Div. Miles Laboratories, Elkhardt, Ind.) and stored at −70 C until use. Adjacent tumor areas were fixed in formalin and routinely prepared for histologic diagnosis. Seven spleens and six lymph nodes from nonlymphoma patients were similarly prepared and served as controls.

For cell suspensions, normal lymph nodes or areas of involvement from tissue containing lymphoma were minced, pressed through a stainless steel mesh, and suspended in Medium 199 (Microbiological Associates, Bethesda, Md.). Cells were then washed three times in Medium 199 and centrifuged on a Ficoll-Hypaque (LSM, Litton/Bionetics, Kensington, Md.) gradient to isolate mononuclear cells. Viability was assessed by trypan blue exclusion and was always greater than 90%. The final concentration of the cell suspension was brought to 2×10^6 cells/ml.

Preparation of Reagent Sheep Red Blood Cells

The techniques are identical to those previously described.[18] Sheep erythrocytes (E) (10^9 cells/ml) in Veronal-buffered saline (VBS) were sensitized with either rabbit anti-sheep whole erythocyte IgM fraction (Cordis Laboratories, Miami, Fla.) (1:600) or IgG fraction (Cappel Laboratories, Downington, Pa.) (1:120). The concentration of IgG used was less than half the minimum hemagglutinating concentration in order to avoid binding to the B lymphocytic FcR.[15] IgM and IgG were incubated with E for 10 minutes at room temper-

Table 1—Rosette Formation by Human Cells in Tissue Section

Cell	Reagent			Unsensitized sheep erythrocytes
	IgGEA	IgMEAC	IgMEA	
B Lymphocyte	−	+	−	−
T Lymphocyte	−	−	−	+*
Histiocyte (macrophage)	+	−	−	−

IgGEA = sheep erythrocytes coated with IgG, IgMEAC = sheep erythrocytes coated with IgM and complement, IgMEA = sheep erythrocytes coated with IgM.

* Single cell suspensions only.

ature and finally diluted to 10^6 cells/ml (IgMEA and IgGEA, respectively). Complement (fresh whole mouse serum diluted 1 : 10 in VBS) was added to stock IgMEA, incubated for 30 minutes at 37 C, washed three times, and brought to a final concentration of 10^6 cells/ml. IgMEA, IgMEAC, and IgGEA thus prepared were used for rosette assays on both whole tissue sections and cell suspensions. Washed unsensitized E (10^6 cells/ml) were used for T-cell identification in cell suspensions.

Rosette Assay

Air-dried 8-μ-thick frozen sections were layered with IgMEA, IgMEAC, or IgGEA and incubated for 30 minutes at room temperature. Nonadherent reagent red cells were washed off with cold buffer, and the sections were fixed in Perfix (Applied Bioscience, Paterson, N.J.). Sections were stained with hematoxylin and eosin and examined by both brightfield and darkfield microscopy. Multiple serial sections from each case were examined, and the assay was performed in duplicate on two different occasions. Adherence of reagent red blood cells was assessed according to a scale of 0 to 4+.

Cell suspensions (2×10^6 cells/ml) were incubated with IgMEA, IgMEAC, or IgGEA for 30 minutes at 37 C with rotation. For T-cell determination, cell suspensions were incubated with E for 5 minutes at 37 C, centrifuged at 200 *g* for 5 minutes, and incubated 1 hour at 4 C.[16] Following incubation the cell suspensions were placed in a hemocytometer, and mononuclear cells were classified according to the number of adherent reagent red blood cells (0, 1–2, and 3 or more). Two hundred cells were counted in each instance.

Results

Normal

Frozen sections of all normal tissues exhibited attachment of IgMEAC to lymphoid follicles (Figure 1A). IgGEA adhered to splenic red pulp and the medullary areas of lymph nodes and spared the follicular areas (Figure 1B). Neither IgMEAC or IgGEA adhered to thymic-dependent (T cell) areas, i.e., the paracortex of lymph nodes and the splenic periarteriolar lymphatic sheath. IgMEA did not adhere to tissues and thus served as a control for IgMEAC.

Nodular, Poorly Differentiated Lymphocytic Lymphoma

In all 6 cases of NPDLL, IgMEAC was consistently adherent to the nodular areas of lymphoma and spared the internodular zones (Figure 2A and Table 2). IgGEA, when present, was attached to normal appearing histiocytes in the internodular areas and did not adhere to the nodules (Figure 2B and Table 2). IgGEA clearly circumscribed and outlined the nodules of NPDLL.

Diffuse, Poorly Differentiated Lymphocytic Lymphoma

Tissues from 7 of the 8 patients with DPDLL demonstrated intense attachment of IgMEAC to the areas involved by lymphoma (Table 2). Two lymph nodes (Cases 10 and 12) and a lung infiltrated by DPDLL (Case 14) contained CR B lymphocytes in a diffuse pattern. In Case 7 the

Table 2—Immunologic Markers in Tissue Section

Case	Diagnosis*	Therapy†	Tissue	IgMEAC‡	IgGEA‡
1	NPDLL	0	Spleen	4+N	1+S
2	NPDLL	0	Lymph node	4+N	0
3	NPDLL	R(3)	Lymph node	4+N	0
4	NPDLL	0	Lymph node	2+N	0
5§	NPDLL	0	Lymph node	4+N	2+S
6	NPDLL	R(5)	Spleen	4+N	1+S
7	DPDLL	R(7) & C(3)	Lymph node	3+N	1+D
8	DPDLL	0	Spleen	4+N	1+S
9	DPDLL	0	Spleen	4+N	4+S
10	DPDLL	0	Spleen	4+N,D	1+S
10	DPDLL	0	Lymph node	4+D	0
11	DPDLL	0	Lymph node	1+D	0
12	DPDLL	R(7/12)	Lymph node	3+D	0
13	DPDLL	0	Spleen	4+N,D	3+S
14	DPDLL	0	Lung	4+D	0
15	HL	0	Spleen	1+D	3+D
16	HL	0	Maxillary sinus	0	0
17§	HL	0	Stomach	0	0
18	UL-NB	C(2/12)	Lymph node	0	0
19	UL-NB	0	Lymph node	0	0
20	UL-NB	0	Tonsil	0	0
21	UL-B	0	Lymph node	0	0
22	PDLL-C	0	Lymph node	3+D	0

* PDLL = poorly differentiated lymphocytic lymphoma, N = nodular, D = diffuse, C = childhood, HL = histiocytic lymphomas, UL = undifferentiated lymphoma, NB = non-Burkitt's, B = Burkitt's.

† C = chemotherapy, R = radiation therapy; numbers refer to last therapy prior to biopsy (in years).

‡ 0-4+: N = nodular, D = diffuse, S = surrounds nodules.

§ Cases 5 and 17 are from the same patient.

lymph node architecture was obliterated by a diffuse infiltrate of poorly differentiated lymphocytes (Figure 3A and B) and was diagnosed as DPDLL. However, when the sections were incubated with IgMEAC, a nodular pattern of adherent reagent red blood cells was obtained (Figure 3C). The four spleens involved by DPDLL (Cases 8, 9, 10, and 13) were from patients diagnosed as having DPDLL on the basis of initial lymph node biopsy. In each of these spleens the lymphoma preferentially involved the splenic Malpighian corpuscles but had also infiltrated the red pulp in small aggregates (Figure 4A). IgMEAC selectively adhered to the areas of lymphomatous involvement (Figure 4B). IgGEA outlined the IgMEAC-positive areas in a pattern similar to that observed in NPDLL (Figure 4C).

Histiocytic Lymphoma

Of the 3 cases examined, surface markers were identified in only one (Case 15). In this case, IgGEA was present in a diffuse pattern (Table 2 and Figure 5). Rare IgMEAC-positive cells were also seen.

Undifferentiated Lymphoma

One of the 4 cases was Burkitt's lymphoma, while the other 3 cases were of non-Burkitt's type. None of the 4 cases exhibited immunologic markers in tissue section. The cell supension of a tonsil from 1 of the non-Burkitt cases (Case 20) contained 23% CR (IgMEAC)-positive cells (Table 3).

Poorly Differentiated Lymphocytic Lymphoma of Childhood

A 10-year-old boy initially presented with an enlarged supraclavicular lymph node, mediastinal mass, and a malignant pleural effusion; he was not leukemic. The cells from the supraclavicular node and pleural effusion did not have the characteristic cytologic features of Sternberg sarcoma. IgMEAC adhered diffusely to the frozen tissue sections of the supraclavicular lymph node mass (Table 2). Cell suspensions of both the involved lymph node and a malignant pleural effusion from this patient were also examined for surface markers. Greater than 90% of the cells obtained from both sources were morphologically poorly differentiated lymphocytes. A high percentage of cells from both lymph node and pleural effusion bore complement receptors (IgMEAC positive). Interestingly, many of the lymphoma cells also formed E (T cell) rosettes (Table 3).

Discussion

The follicular center cell origin of NPDLL, first suggested by Lennert [19] on the basis of morphologic studies and expanded by Lukes and Collins,[20] has now been well supported by immunologic studies which demonstrate the derivation of the cells of this disorder from B lymphocytes. These cells

Table 3—Immunologic Surface Markers in Cell Suspensions

		Reagent red cells/lymphoid cell (% of 200 cells)		
	Reagent	0	1-2	≥3
Case 20—tonsil	IgMEA	97.0	3.0	0.0
	IgMEAC	51.5	25.5	23.0
	IgGEA	93.0	7.0	0.0
	E	90.0	4.5	5.5
Case 22—lymph node	IgMEA	93.5	3.5	3.0
	IgMEAC	17.0	27.0	56.0
	IgGEA	87.0	10.0	3.0
	E	31.5	5.5	63.0
Case 22—pleural fluid	IgMEA	94.0	3.0	3.0
	IgMEAC	49.0	29.0	22.0
	IgGEA	91.0	3.5	5.5
	E	58.0	24.0	18.0
Normal lymph nodes (mean of 3)	IgMEA	96.0	4.0	0.0
	IgMEAC	66.0	8.0	26.0
	IgMEA	92.0	5.0	3.0
	E	52.0	12.0	36.0

See Table 1 for abbreviations.

bear surface immunoglobulin [4-6] and contain intracytoplasmic immunoglobulin.[21] The present study confirms the findings of Jaffe *et al.*[3] and Gajl-Peczalska *et al.*[5] in that NPDLL is comprised of complement receptor B lymphocytes. The normal counterpart of the NPDLL cell is the CR-bearing follicular center cell. The involvement of follicular (B) cell areas of lymphoid tissue by NDPLL lends further support to the concept that cells of lymphoreticular neoplasms preferentially migrate to those areas in which their normal counterparts reside.[22]

The present investigation includes the largest series of DPDLL studied to date by the frozen section rosette assay and provides evidence for a B-cell origin in a majority (7 of 8) cases. This finding is consistent with surface immunoglobulin (sIg) studies of DPDLL.[4,5] Lack of surface markers in 1 of our cases of DPDLL (Case 11) may reflect a difference in degree of differentiation in which CR and FcR are not expressed on a cell of B-lymphocytic lineage. Alternatively, these CR-negative, FcR-negative cells may be derived from T lymphocytes. Cases of adult DPDLL with T-cell markers have been reported.[12] In the other 7 cases of DPDLL that we studied the areas of lymphoma were predominately comprised of CR B lymphocytes, and in 4 of these the follicular (B) cell areas of spleen were preferentially involved. Therefore, these 7 cases are presumably of B (follicular center) cell origin. A B-cell origin of some cases of DPDLL may be indicative of a previous NPDLL which has evolved into a diffuse form. In NPDLL and in many cases of DPDLL there is a similar propensity for the initial involvement of B-cell areas of lymphoid tissue. The small clusters of CR lymphoma cells in the splenic red pulp suggest an early transition to diffuse involvement. Of interest in this regard is Case 7, in which there was a diffuse, monomorphous infiltrate of poorly differentiated lymphocytes with obliteration of normal nodal architecture. However, a distinct nodular pattern of CR B cells was seen following incubation with IgMEAC. These positive areas were not residual follicles since normal lymph node elements were absent. Perhaps this case represents a late stage of transition from nodular to diffuse lymphoma in which complement receptors are lost by those cells which no longer remain in the nodular aggregates. Such a loss of surface receptors may reflect changes in the degree of cellular differentiation as the lymphoma evolves into a diffuse form.

Fc receptors have been previously identified on the surface of histiocytic lymphoma cells,[8,9] and some cells bore monoclonal sIg, suggesting a B-cell origin.[8] In the present study, FcR were identified on the cells of 1 of 3 cases of histiocytic lymphoma, perhaps reflecting a derivation from a "true" histiocyte; however, such a conclusion cannot be drawn with

certainty on the basis of this one surface marker. Two cases of histiocytic lymphoma lacked FcR and CR, a finding which was not unexpected since many cases of histiocytic lymphoma have been devoid of surface markers.[1] The majority of those cases that do possess detectable markers are B lymphocytic by virtue of sIg[8] and intracytoplasmic immunoglobulin.[21] The histiocytic lymphoma of stomach (Case 17) that we examined is from the same patient as in Case 5, a lymph node with the diagnosis of NPDLL. These two specimens are morphologically distinct and presumably represent two separate neoplasms. Development of histiocytic lymphoma from NPDLL and the simultaneous existence of these two lymphomas with identical surface markers have been described.[1] However, the differences in both morphology and immunologic surface markers between the two neoplasms from the present case would tend to favor the simultaneous presence of two distinct lymphomas.

Surface markers were not detected on the cells of 2 of the 3 cases of non-Burkitt's undifferentiated lymphoma (UL-NB). One case (Case 20) did contain a group of CR cells when examined in cell suspension. A relatively high proportion (25.5%) had only one or two reagent red cells (IgMEAC) attached to their surfaces, and IgMEAC did not adhere to the cells in tissue section. This would suggest that the CR possessed by these cells is either sparsely distributed on the cell membrane or has a low avidity for complement. A B-lymphocytic origin of UL-NB was found in all 4 cases examined by Brouet *et al.*, who demonstrated monoclonal sIg in each case.[23] Fc receptors were sought in 2 cases and were present in both.[23] The case of Burkitt's lymphoma in the present study lacked FcR and CR. All previous studies of Burkitt's lymphoma have been carried out on cell suspensions, and the majority of these investigations have been shown to be monoclonal proliferations of sIg-bearing B cells.[1,7]

Poorly differentiated lymphocytic lymphoma of childhood with mediastinal mass in generally of T-cell origin.[1,11] Lymphoma cells from the present case (Case 22) had CR and formed E (T cell) rosettes in suspension. These cells were positive with IgMEAC in tissue section as well. The cell suspension of the lymph node was particularly intriguing since the percentages of IgMEAC and E rosettes are both greater than 50%. Morphologically, nearly all cells in the suspension were malignant; thus some of these lymphoma cells may have possessed both receptors. Other investigators have reported finding both B- and T-cell markers on individual cells from lymphoproliferative disorders.[1,9,23-27] Cells bearing both B and T markers may represent a malignant proliferation of those lymphocytes found in small numbers in normal human peripheral blood that bear CR as well as form E rosettes.[24,28]

Summary

In the present study, NPDLL and most cases of DPDLL were shown to be derived from B lymphocytes of the follicular center cell type. In at least 1 case of DPDLL an apparent transition from a nodular to a diffuse pattern was evident immunologically but not by routine morphology. One case of histiocytic lymphoma was comprised of FcR-bearing cells, a finding consistent with a histiocytic origin. One case of undifferentiated lymphoma was shown to be of B lymphocytic lineage; however, the CR of these cells were less readily detectable than those of normal B cells. Both T- and B-cell markers were found on the surface of the cells from a case of DPDLL of childhood.

These results are in accord with the work reported by other investigators. The majority of the previous studies have relied on data obtained from individual cell suspensions prepared by mincing tumor-containing tissue. The advantage of this method is that a large array of surface markers can be identified on the living cells. However, the precise histologic location and pattern of distribution of cells possessing specific markers cannot be evaluated. By examining intact tissue we have demonstrated the distribution of complement receptor and Fc-receptor–bearing cells in a number of cases of malignant lymphoma. The importance of studying tissue sections is particularly clear in DPDLL, where the assay for immunologic markers has demonstrated the close relationship of some of these cases to NPDLL which would have otherwise been undetected.

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All sections are stained with hematoxylin and eosin.

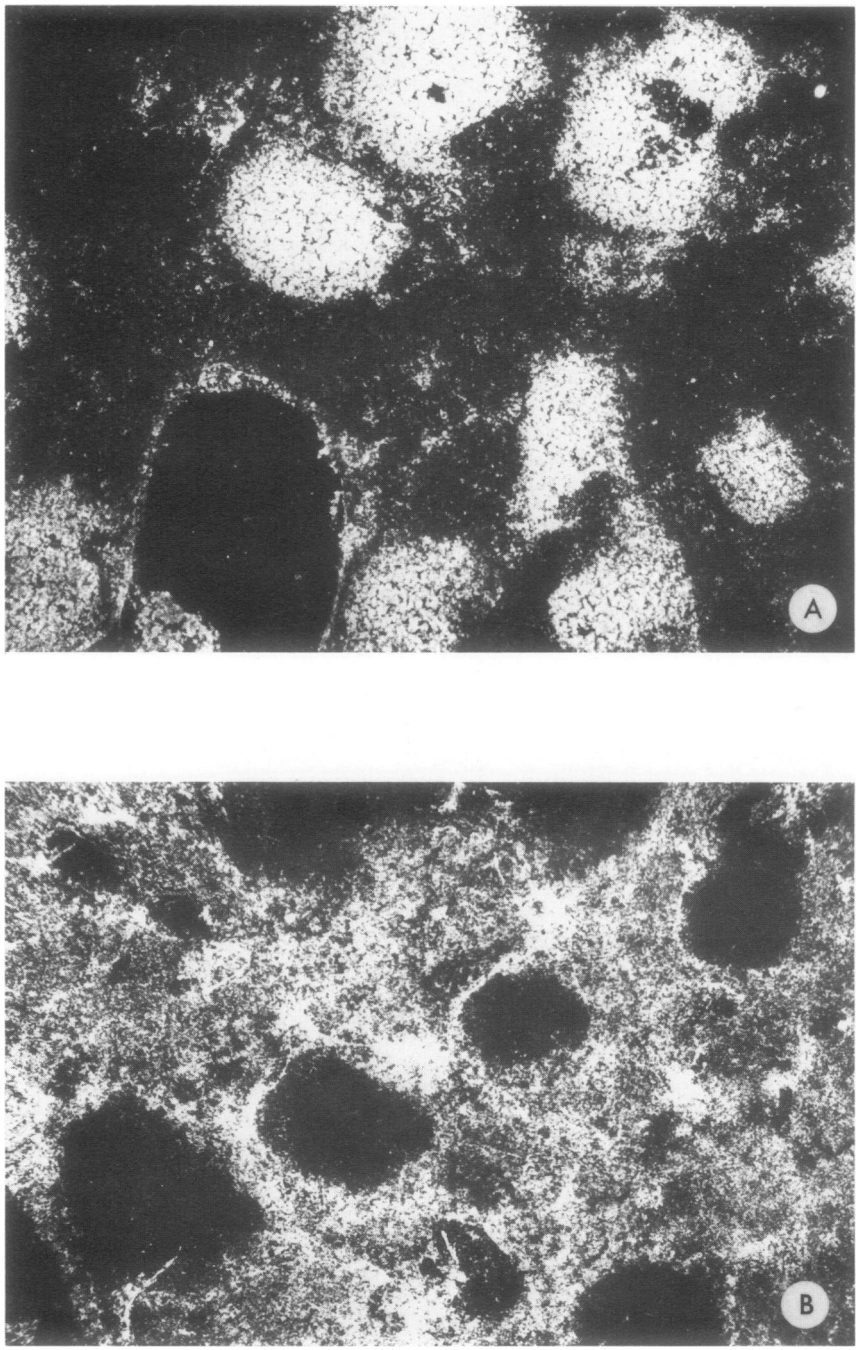


Figure 1—Normal spleen. **A**—IgMEAC; reagent red blood cells adhere to splenic follicles (*white areas*) (Darkfield, × 25). **B**—IgGEA; IgGEA adheres to red pulp histiocytes (*white areas*) with sparing of follicles (*black areas*) (Darkfield, × 25).

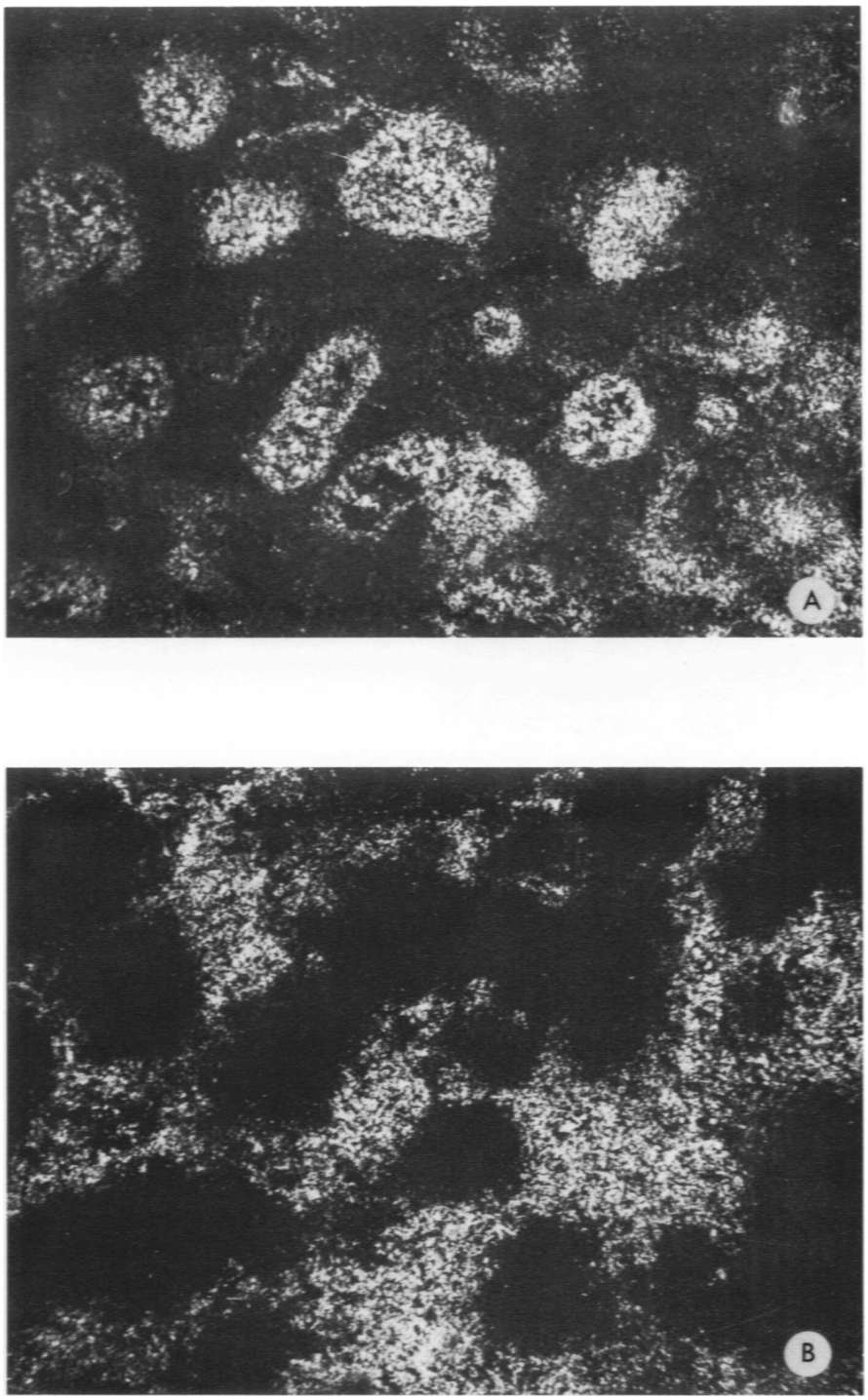
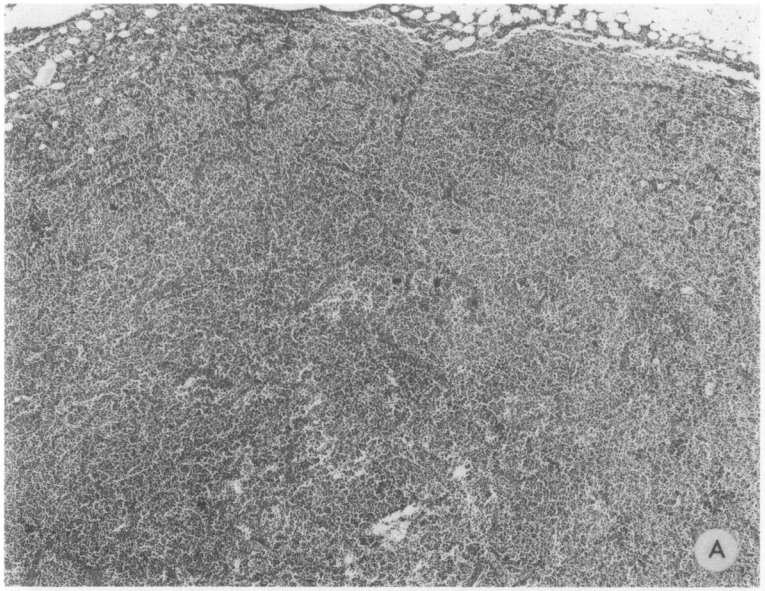
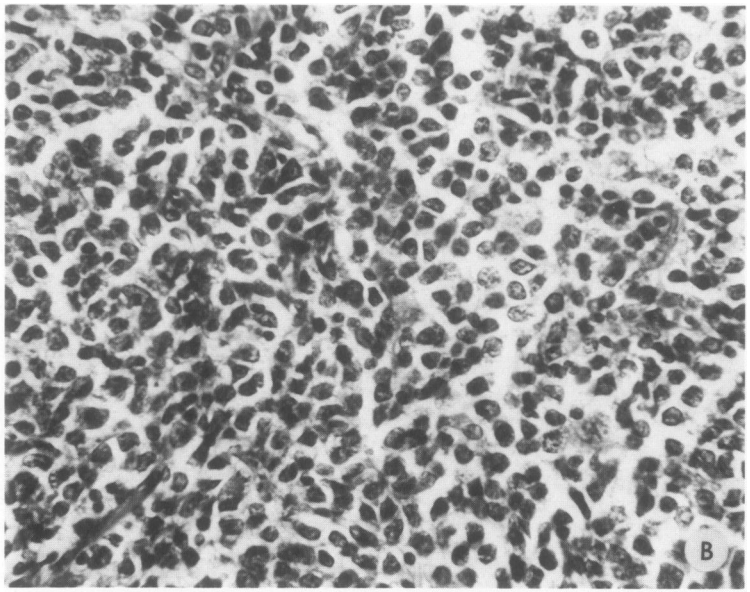


Figure 2—NPDLL in lymph node (Case 5). **A**—IgMEAC; reagent red cells adhere to the nodular areas of lymphoma (Darkfield, × 25). **B**—IgGEA (same field as **A**); IgGEA is present in the internodular areas and spares the nodules (Darkfield, × 25).





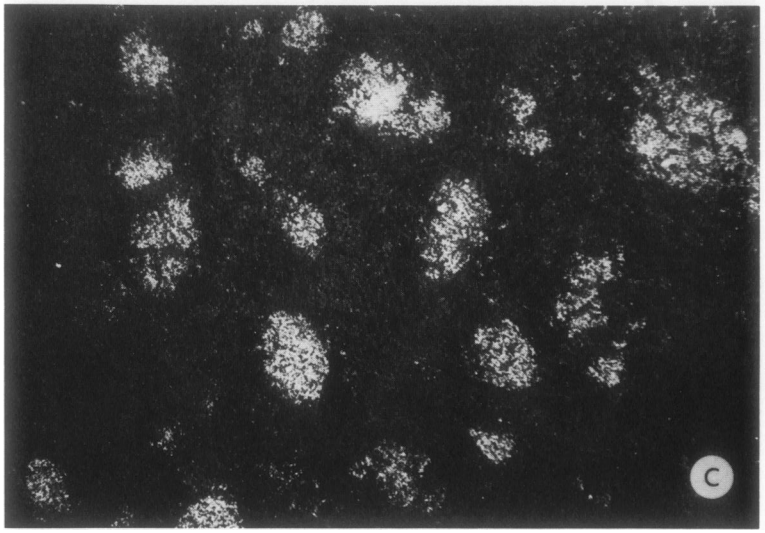


Figure 3—DPDLL in lymph node (Case 7). **A**—This formalin-fixed, paraffin-embedded section demonstrates replacement of the lymph node by lymphoma in a diffuse pattern (Brightfield, × 30). **B**—Detail of **A**; the lymphoma is comprised of poorly differentiated lymphocytes with sharply angulated nuclei (Brightfield, × 400). **C**—IgMEAC; discrete nodules of complement receptor cells are present within this histologically diffuse lymphoma (Darkfield, × 25).

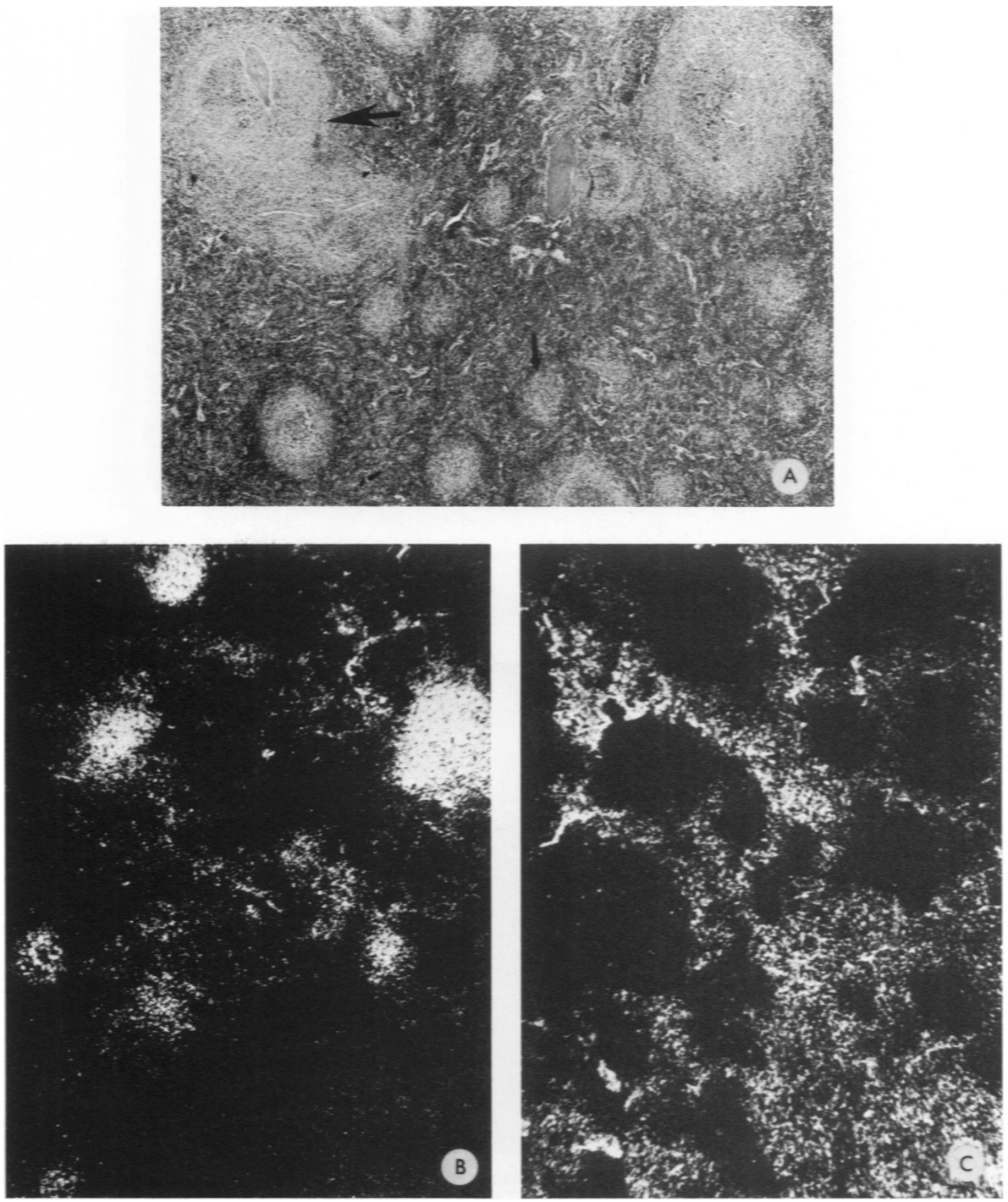


Figure 4—Spleen involved by DPDLL (Case 10). **A**—Multiple aggregates of lymphoma are seen in this paraffin-embedded section. Some of these are follicles expanded by lymphoma (*large arrow*) while several smaller clusters of lymphoma cells are present in the red pulp (*small arrow*). (Brightfield, ×30) **B**—IgMEAC; both large and small areas of lymphoma contain complement receptor cells (Darkfield, × 25). **C**—IgGEA (same field as **B**); the areas of lymphomatous involement are spared while the intervening red pulp contains Fc-receptor cells (Darkfield, × 25).

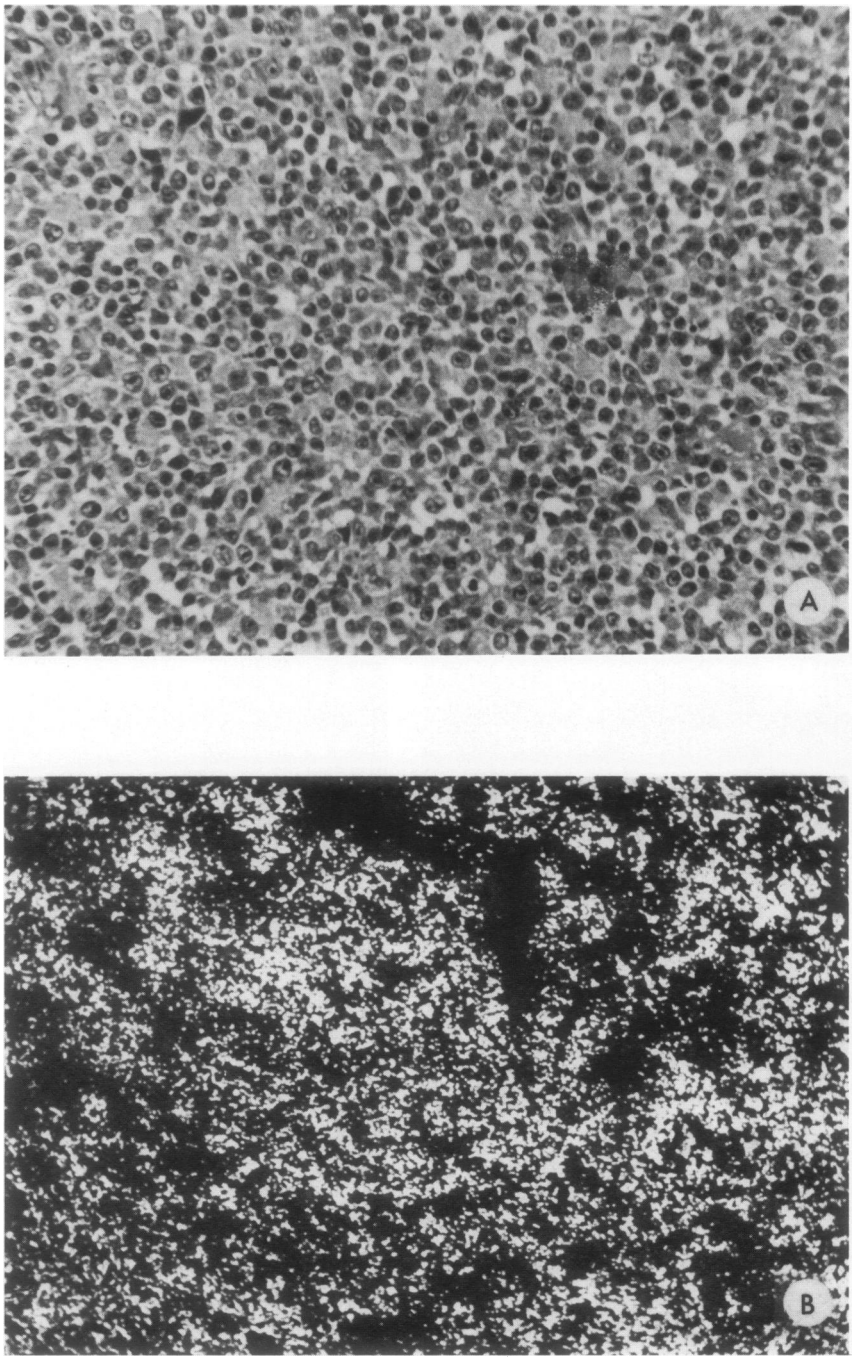


Figure 5—Diffuse histiocytic lymphoma (Case 15). **A**—Cells with large, vesicular nuclei and prominent nucleoli characteristic of histiocytic lymphoma are present in a diffuse pattern (Brightfield, × 300). **B**—IgGEA; the lymphoma is comprised of a diffuse collection of Fc-receptor-bearing cells (Darkfield, × 25).