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Immunoglobulin Specificity of Low Grade B Cell Gastrointestinal Lymphoma of Mucosa-Associated Lymphoid Tissue (MALT) Type

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The specificity of the tumor cell immunoglobulin in three cases of low grade B cell gastrointestinal mucosa-associated lymphoid tissue (MALT) lymphoma has been studied. Using anti-idiotypic antibodies to detect the reactivity of tumor immunoglobulin in tissue sections from the patients and other individuals, we observed specificity for normal tissue components in all three cases studied. Reactivity in one case was with follicular dendritic cells, in the second case with a novel antigen on mucosal post capillary venules, and, in the third case, a broad pattern of reactivity was observed. This study suggests that autoimmunity may play a role in the pathogenesis of gastric lymphoma. (Am J Pathol 1993, 142:285–292)

Low grade B cell lymphomas of mucosa-associated lymphoid tissue (MALT) occur most commonly in sites that are normally devoid of lymphoid tissue.[1] However, these sites may acquire reactive lymphoid tissue, which resembles normal MALT, exemplified by Peyer's patches of the terminal ileum.[1,2] Thus, in the salivary gland and thyroid, MALT acquired in the course of autoimmune responses in Sjögren's syndrome and Hashimoto's thyroiditis provides the necessary background for the development of the characteristic MALT lymphomas that occur at these sites.[3,4] The immune response preceding the onset of the most common MALT-type lymphoma, that arising in the stomach, is less clearly understood, but it has recently been proposed that it is associated with infection with *Helicobacter pylori*.[5] Follicular dendritic cells (FDC), sites of antigen accretion, frequently persist within MALT-type lymphomas and are often associated with blast transformation of tumor cells and increased rate of tumor cell proliferation.[1,6] This suggests that the growth of these low grade tumors might be dependent on the FDC network, possibly as a source of continued antigenic stimulation. Study of the specificity of the tumor immunoglobulin (Ig) could therefore give significant clues to the possible stimulus for B cell proliferation and the nature of the preceding immune response. In this report, we have studied the specificity of human monoclonal antibodies derived from three low grade B cell gastrointestinal lymphomas of MALT type to determine whether they react with autoantigens.

Materials and Methods

Tissues

Three cases of low grade primary B cell gastric lymphoma of MALT type (patients 1 to 3), were received fresh in the laboratory. The case of patient 1, which has been reported previously,[7] also involved the small intestine, and it was the intestinal tumor mass from which the cells to be used in the experiments described were taken. Suspensions of the tumor cells were frozen down in aliquots for subsequent immunizations and for heterohybridoma fusions. Tumor and uninvolved gastric mucosa were studied in all cases and apparently normal spleen removed during gastrectomy was studied in patients 1 and 3. Blocks of all tissue to be studied using immunohistochemistry were snap-frozen in liquid nitrogen and stored at −70 C.

A panel of snap-frozen normal tissues from other individuals (tonsil, $n = 3$; peripheral lymph node, $n = 3$; Peyer's patches, $n = 3$; appendix, $n = 6$; kidney, n

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= 2; lung, *n* = 2; breast, *n* = 2; thyroid, *n* = 2; pancreas, *n* = 2) and lymphomas of a range of histogenetic types (*n* = 19) were included in this study.

Preparation of Anti-Idiotypic Antibodies

Murine anti-idiotypic antibodies were produced[8] to detect the tumor Ig from each patient. Mice were immunized with tumor cells from each patient and clones were screened using indirect immunoperoxidase immunohistochemistry with a rabbit anti-mouse Ig secondary reagent (Dako Ltd, High Wycombe, Bucks, UK) and diaminobenzidine substrate.[9] Antibodies were selected for their reaction with frozen sections of the appropriate tumors but not frozen sections of tonsil tissue. Because the anti-idiotypes were ultimately to be used to detect the binding of tumor Ig in tissues from unrelated individuals, it was important that the anti-idiotypes should recognize private idiotopes, and that there should be little or no reactivity of anti-idiotypes themselves in tissues from other individuals. Anti-idiotypes were therefore screened and selected for lack of reactivity with a panel of normal and malignant lymphoid tissues using immunohistochemistry as described below. Enzyme-linked immunosorbent assays (ELISA) were subsequently used to confirm that the anti-idiotypic antibodies were in fact recognizing tumor Ig, but not serum Ig.

Preparation of Tumor Ig

A source of tumor Ig was required for this study, first, to use in ELISA to confirm that the anti-idiotypes were in fact anti-idiotypes, and second, to study the reactivity of tumor Ig in tissues from other individuals.

Tumor Ig was obtained from each of the three lymphomas studied by preparing a detergent extract of the tumor cell membranes. A total of 5×10^7 tumor cells were incubated for 2 hours in 1 ml of 1% Brij 58 (Sigma Ltd, Poole, Dorset) in physiological saline at 4 C. To provide an alternative, more abundant source of the tumor Ig in patients 1 and 2, where the amount of detergent extract was limited, tumor cells were directly fused with the mouse myeloma line NSO to give heterohybrid cell line secreting human Ig detected by ELISA with anti-idiotypes as described below.[7] Secreting lines were unstable and were maintained by repeatedly cloning out and selecting secreting subclones.

Enzyme-Linked Immunosorbent Assay

The reactivity of the anti-idiotypes with Ig derived from the tumors was confirmed using ELISA. ELISA plates were coated with rabbit polyclonal antibodies to either human IgM, IgG, IgA, κ or λ chain in phosphate-buffered saline, pH 9.0, overnight at 4 C. All subsequent dilutions and washes were in phosphate-buffered saline (pH 7.6) and 0.05% Tween. Coated plates were incubated successively with either tumor Ig or pooled normal human serum, doubling dilutions of murine anti-idiotypic antibodies, and rabbit anti-mouse Ig peroxidase conjugate (Dako). Plates were washed extensively between all incubations. Binding was visualized using the *O*-phenylenediamine reagent (Sigma) and the optical densities read in a Uniscan II ELISA reader at 492 nm.

Immunohistochemistry

Immunohistochemical studies were carried out on 8-μ acetone-fixed frozen sections.

Reactivity of Anti-Idiotypes

The reactivity of the murine anti-idiotypic antibodies in tissue sections was studied using indirect immunoperoxidase with peroxidase conjugated rabbit anti-mouse secondary antiserum (Dako) and diaminobenzidine substrate.[9]

Reactivity of Tumor Ig on Tissues from Other Individuals

The reactivity of the tumor Ig from each patient on tissue sections from other individuals was detected by incubating sections in the tumor Ig and using the anti-idiotypic antibody to localize the tumor Ig binding, followed by rabbit anti-mouse peroxidase conjugate and the diaminobenzidine reagent. To determine whether epithelial positivity observed in sections of small bowel and appendix was due to the nonspecific binding of polymeric Ig to secretory component (SC), fixed sections were preincubated in rabbit polyclonal antibodies to SC (Dako Ltd) before staining using human tumor Ig.

Immunofluorescence

Acetone-fixed sections were incubated in murine anti-idiotypic antibody to the lymphoma in patient 2 and rabbit polyclonal antibody to CD3 (Dako) simultaneously and then washed. Binding of these antibodies was detected using goat anti-mouse Ig conjugated to rhodamine isothiocyanate (Southern Biotechnology Ltd) and swine anti-rabbit Ig conjugated to fluorescein isothiocyanate (Dako). Sections

were mounted and observed using fluorescence microscopy at the appropriate wavelengths.

Results

Enzyme-Linked Immunosorbent Assay Characterization of Anti-Idiotypic Antibodies

ELISA assays confirmed that the murine monoclonal antibodies recognizing each of the three tumors were specific for the Ig of the individual tumors. They only reacted with the Ig of the appropriate tumor and did not react with pooled human serum. All anti-idiotypes detected the tumor Ig bound to anti-IgM-coated plates. The reactivity was light chain-restricted; patients 1 and 3 recognized appropriate tumor Ig on the κ-coated plate only and patient 2 recognized appropriate tumor Ig on the λ-coated plate only (Figure 1).

Immunohistochemical Reactivity of Anti-Idiotypic Antibodies

The reactivity of the anti-idiotypic antibodies is summarized in Table 1.

Reactivity of Anti-Idiotypic Antibodies in Patients' Tumors

Anti-idiotypic antibodies produced against all three of the tumors reacted with the entire tumor masses against which they were raised. Immunohistochemical reactivity of the anti-idiotypic antibodies within the corresponding tumors was not restricted to the tumor cells. This was thought to be due to tumor Ig bound to normal tissue components within the tumor masses, although at this stage, the possibility of cytophilic binding of complexes could not be excluded. In patient 1, binding to FDCs was observed; in patient 2, binding to venules was observed; and in patient 3, generalized positivity of the reactive cell infiltrate was observed (Table 1, Figure 2). Binding to epithelial cells was also observed in each case.

Reactivity of Anti-Idiotypic Antibodies in Tissues from Other Individuals

Anti-idiotypic antibodies in patients 1 and 3 did not react with any cells in any tissues from other individuals including 3 tonsils, 3 peripheral lymph nodes, 3 Peyer's patches, 6 appendices and 19 lymphomas of different histogenetic types. In patient 2, double

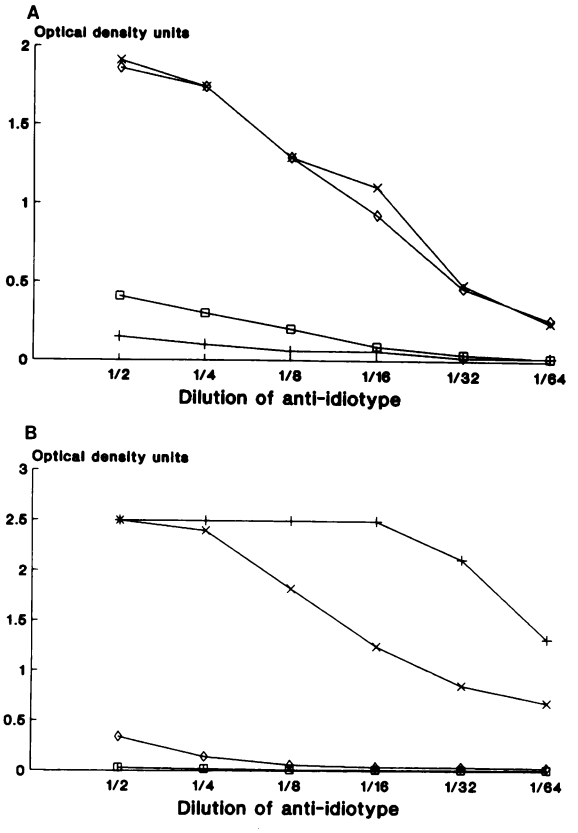

Figure 1. *Results of ELISA using plates coated with rabbit polyclonal antibodies to Ig κ (+), λ (◇), and μ (×) chains, followed by human tumor Ig from patient 2* (A) *and patient 3* (B). *Pooled normal human serum was included on μ coated plates in place of the tumor Ig in* (A) *and* (B) *as controls* (□). *All wells were then incubated in doubling dilutions of anti-idiotypic antibodies to the tumor Ig in patients 2* (A) *and 3* (B). *The binding of the anti-idiotypic antibodies was detected using rabbit anti-mouse Ig conjugated to horse radish peroxidase. O-phenylenediamine substrate was used. Optical density units are shown on the vertical axes and reciprocal dilutions of the anti-idiotypic antibodies on the horizontal axes.*

immunofluorescence in tissue sections showed that the anti-idiotypic antibody reacted with a small scattered population of T cells (<1% of the total lymphoid cells) in all other lymphoid tissues studied, but with no other lymphomas (Table 1).

Reactivity of Anti-Idiotypic Antibodies in Patients' Uninvolved Tissues

The pattern of reactivity of anti-idiotypic antibodies in each of the patients' uninvolved tissues varied from patient to patient. In patient 1, reactivity with FDCs and isolated cells in the marginal zone was observed in apparently uninvolved spleen.[7] In patient 2, venules were recognized by the anti-idiotypic antibody in uninvolved gastric mucosa. In patient 3, all cells at the tumor site and in the apparently uninvolved spleen were positive (Figure 3). In the uninvolved gastric mucosa in patient 3, the gastric epi-

Table 1. *Reactivity of Anti-Idiotypic Antibodies on Tonsil and the Patients' own Tissues*

Patient no.	Percent reactivity		Reactivity in patient	
	Tumor cells	Unrelated tonsil	Additional reactivity at tumor site	Reactivity at distant sites
1	100 (small intestinal tumor cells)	0	FDCs; epithelium	Spleen: FDCs; some marginal zone cells
2	100	<1	Venules	Stomach: venules
3	100	0	All cells at tumor site	Spleen: entire spleen positive; stomach: lamina propria positive

thelium was negative but the entire lamina propria was strikingly positive.

Immunohistochemical Reactivity of the Tumor Ig in Tissues from Other Individuals

The reactivity of tumor Ig from each patient in tissues from other individuals was studied immunohistochemically using the anti-idiotypic antibodies to detect the bound tumor Ig. As expected in patients 1 and 2, the detergent extract and the heterohybridoma supernatant gave an identical result. Because the detergent extract was abundantly available in patient 3, it was not necessary to produce a heterohybridoma.

Reactivity of Tumor Ig from Patient 1

The Ig from patient 1 recognized follicular dendritic cells in all normal lymphoid tissues studied (Figure 4a). The Ig in patient 1 also bound strongly to crypt epithelium in the intestine and appendix, but this binding could be inhibited by preincubating sections in polyclonal antibody to SC. Weak staining was also observed in the pancreatic ducts and acini, bronchiolar respiratory epithelium, and renal tubules and Bowman's capsules.

Reactivity of Tumor Ig from Patient 2

The Ig from patient 2 recognized postcapillary venules in Peyer's patches and appendix but not tonsil or peripheral lymph node (Figure 4, b and c). Staining of smooth muscle surrounding some medium sized veins and arteries in various organs and of the luminal surface of pancreatic ducts and renal tubules was observed.

Reactivity of Tumor Ig from Patient 3

Ig from patient 3 recognized a cytoplasmic antigen in all cells in sections of tonsil, spleen, and appendix (Figure 4d). Reactivity with epithelium, smooth muscle, vessels, and connective tissue was observed in the various organs studied. There was no inhibition of binding of Ig to intestinal epithelium in patient 3 by preincubation of sections in antibody to SC.

Discussion

We have found that the tumor Ig expressed by three cases of low grade primary B cell MALT lymphoma showed specificity for normal tissue constituents. In each case, this was evident both in the patients' own tissues and in tissues from other individuals. The antigens in two (patients 1 and 2) had very restricted tissue distribution, being expressed by follicular dendritic cells in one patient and mucosal postcapillary venules and some vascular smooth muscle in another. Broader reactivity in and on cells of many lineages was observed in patient 3. Intense reactivity of tumor Ig with crypt epithelial cells seen in patient 1 was considered to be due to the binding of tumor IgM to SC, since it was blocked by preincubating sections with rabbit polyclonal antiserum to SC. Binding of human polymeric antibody to SC in tissue sections has been reported previously.[10] Little or no binding to epithelium was observed in patient 2. In patient 3, the low level of binding to epithelium was not inhibited by preincubation in antiserum to SC, and this was probably specific.

Because low grade B cell lymphomas of MALT are relatively rare tumors, we were only able to study three cases in detail. The pattern of autoreactivity in these cases of lymphoma of MALT type, which are characteristically $CD5^-$,[11] is different from that reported in other types of low grade B cell lymphoma. The malignant cells in most cases of chronic lymphocytic leukemia, which are $CD5^+$ tumors, express polyspecific antibodies that bind to multiple antigens with low affinity.[12,13] This reflects the nature of the Ig secreted by $CD5^+$ B cells which, in healthy individuals, commonly binds with low affinity to many antigens, including autoantigens.[14] In a recent study of the Ig specificity of the malignant

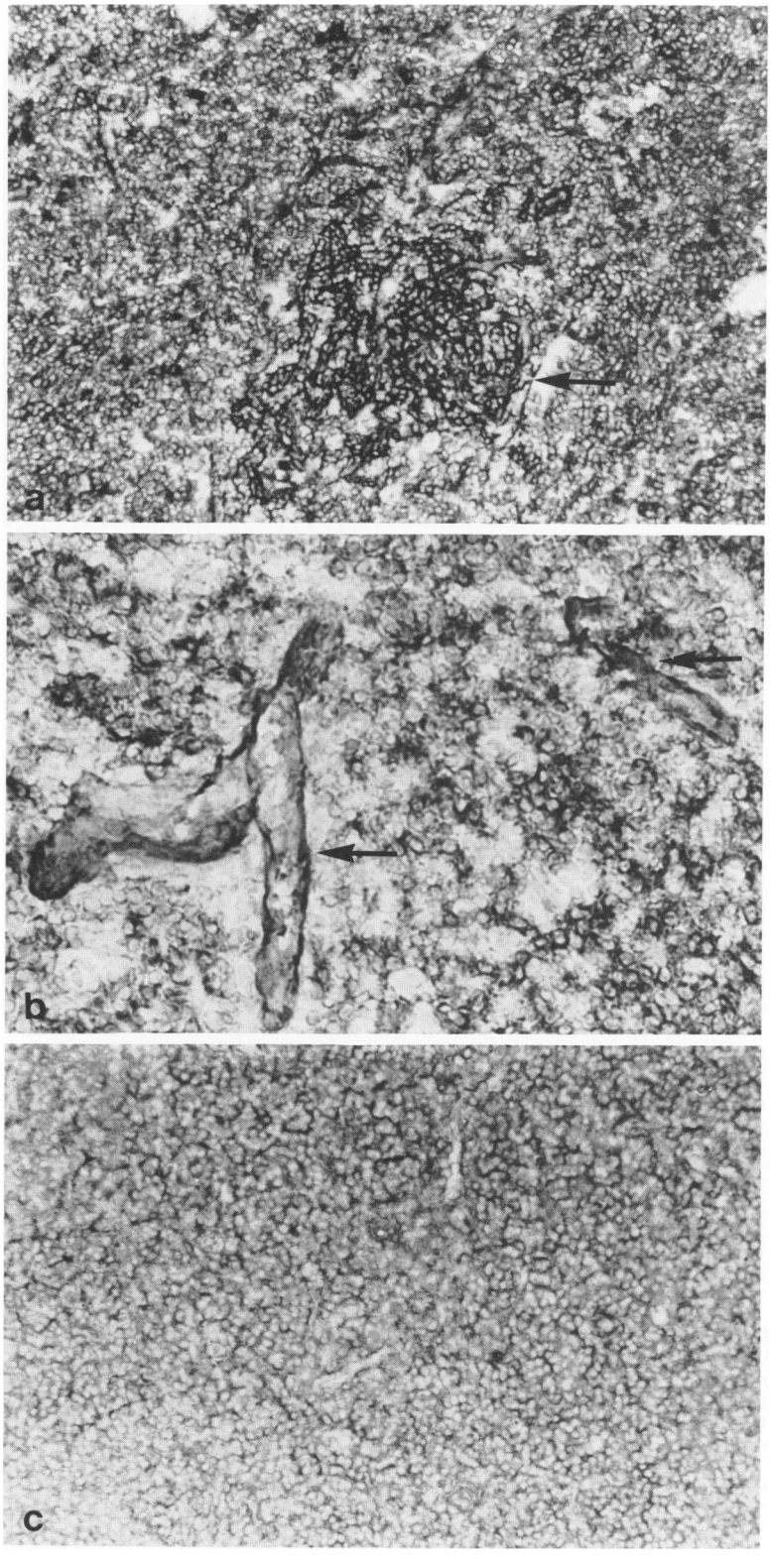

Figure 2. *Cryostat sections of lymphomas in patients 1 to 3 stained with the anti-idiotypic antibodies raised against them. All tumor cells in each case are recognized by the anti-idiotypes. In addition, the anti-idiotype in patient 1 recognizes FDCs (arrows) and in patient 2 recognizes venules (arrows). All cells in the tumor mass, which commonly includes an infiltrate of reactive cells, are recognized by the anti-idiotype in patient 3 (immunoperoxidase; original magnification, ×50).*

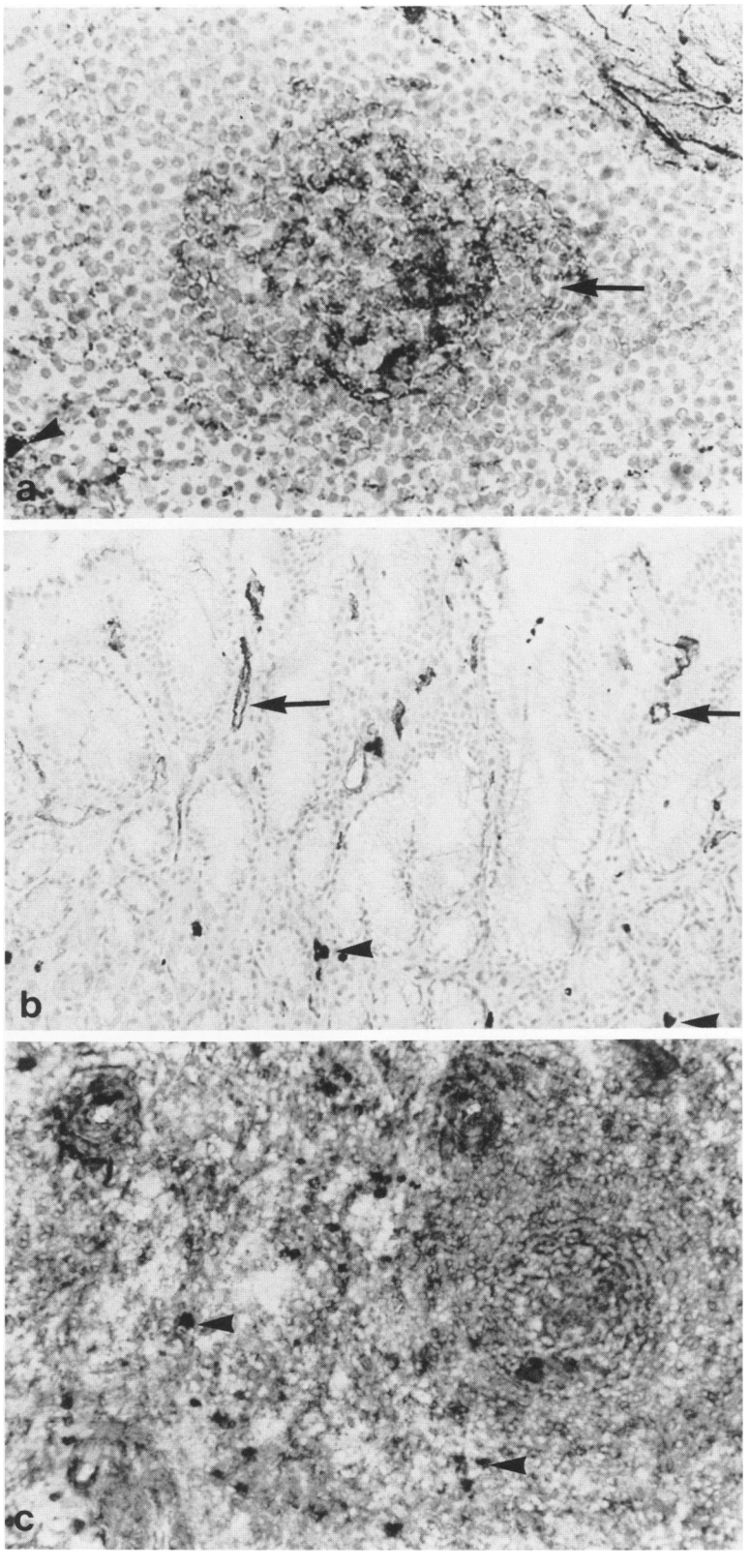

Figure 3. *Frozen sections of apparently uninvolved spleen* (**a** *and* **c**) *and gastric mucosa* (**b**) *from patients 1* (**a**), *2* (**b**), *and 3* (**c**) *stained with the anti-idiotypic antibodies raised against the tumors in these patients. Staining from the specific binding of tumor Ig at distant sites is detected on the FDCs in patient 1 (arrows), venules in patient 2 (arrows), and generalized positivity is observed in patient 3. Nonspecific staining from endogenous peroxidase in granulocytes is indicated with arrowheads (immunoperoxidase; original magnification:* **a**, ×*100;* **b** *and* **c**, ×*50).*

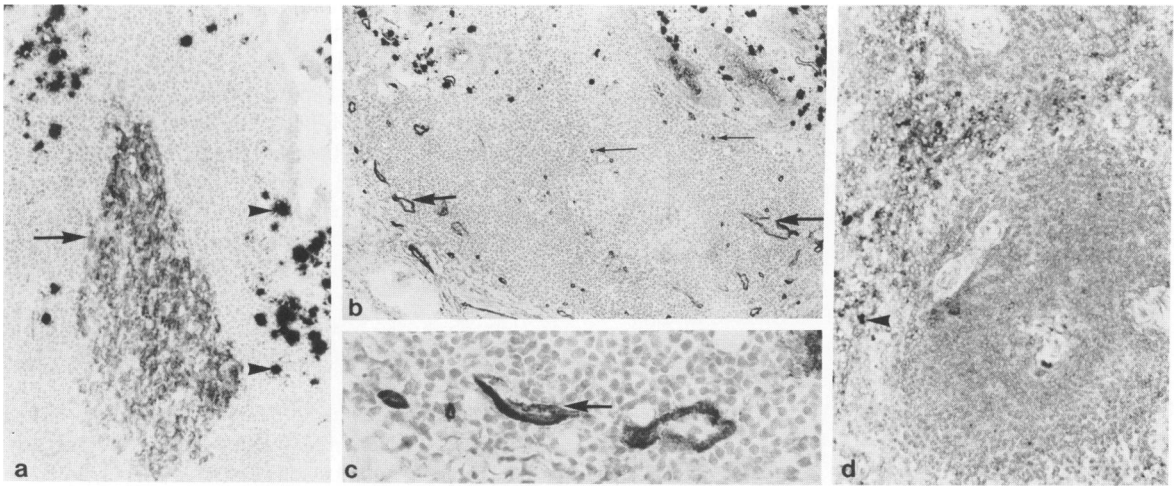

Figure 4. *Frozen sections of appendix* (**a** *to* **c**) *and spleen* (**d**) *from unrelated individuals stained with tumor Ig from patient 1* (**a**), *patient 2* (**b** *and* **c**), *and patient 3* (**d**). *Sections of appendix were preincubated in antibodies to SC to minimize nonspecific binding of tumor Ig. Tumor Ig from patient 1* (**a**) *recognizes FDCs (arrows). Tumor Ig from patient 2 recognizes postcapillary venules (large arrows). Note the scattered population of lymphocytes recognized by the anti-idiotype (small arrows). The tumor Ig in patient 3 binds to most cells in spleen. Nonspecific staining from endogenous peroxidase in granulocytes is indicated by arrowheads (immunoperoxidase; original magnification:* **a** *and* **d**, ×50; **b**, ×25; **c**, ×200).

cells in 31 cases of follicular lymphoma, only 8 showed reactivity with autoantigens. Of these 8, 2 were polyspecific, 4 were reactive with only with the Fc fragment of IgG, and 2 recognized the Fc fragment of IgG and nuclear antigens.[15] The tumor Ig from patients 1 and 2 in this study clearly recognized distinct and different populations of cells. This is not the pattern that would be expected of polyspecific reactivity, in which multiple antigens are recognized. It is possible, however, that the tumor Ig in patient 3 in our study is polyspecific because it shows weak reactivity with cells of many lineages, suggesting that it may be binding weakly to multiple antigens.

Focal networks of FDCs are characteristically present in low grade MALT lymphomas, often associated with blast transformation of the tumor cells.[6] This suggests that the lymphomas may be dependent on FDC for their growth. The most comprehensively investigated aspects of FDC function have been the retention and processing of antigen.[16,17] These cells clearly have other functions, however; some are defined, such as cellular adhesion, and others are still to be described.[18,19] At the onset of our studies, we considered it possible that MALT type lymphomas are dependent on the FDC network as a source of stimulation due to bound complexes of antibody with antigen. The results of this study have shown that the tumor Ig derived from MALT type lymphomas binds autoantigen. It is possible that these lymphomas are responsive to autoantigens and are driven by antigen in their early stages. Further experimentation is clearly required before the relationship, if any, between the lymphoma cells and the antigens that they recognize is understood.

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