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Expression of Sialyl-Lewis X, an E-selectin Ligand, in Inflammation, Immune Processes, and Lymphoid Tissues

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The carbohydrate structure sialyl-Lewis X (SLe^x) can function as a ligand for E-selectin, formerly known as endothelial leukocyte adhesion molecule-1 (ELAM-1). This study was performed to analyze the expression of SLe^x by leukocytes and other cell types in the context of inflammatory and immune processes. Human peripheral blood cells were examined by flow cytometry using monoclonal antibody CSLEX1 directed against SLe^x. Cell surface SLe^x was found in abundance on nearly all isolated polymorphonuclear leukocytes (PMN) and monocytes, and at low levels on a substantial portion (up to 40%) of natural killer cells. This moiety was expressed also on approximately 10% of peripheral blood T cells. Immunohistochemistry was performed on various human tissues involved in inflammatory or immune processes and on secondary lymphoid tissues. In acute appendicitis, endothelial cells of postcapillary venules expressed E-selectin, and most PMN, both within vessels and extravasated, expressed SLe^x. A substantial number of monocytes/macrophages in inflamed appendiceal, synovial, and dermal tissues also reacted with antibody CSLEX1; however, only rare tissue macrophages in uninflamed nonlymphoid sites showed expression of SLe^x. These observations are consistent with the concept that SLe^x on circulating PMN and monocytes functions as a ligand for endothelial E-selectin in the development of inflammatory reactions. SLe^x-positive lymphocytes also were seen, notably, T lymphocytes in inflamed skin. An unexpected finding was that the CSLEX1 antibody also reacted with venular endothelium in certain lymphoid tissues and in inflamed appendix, but not with endothelium in normal appendix. Whether the SLe^x antigen identified on endothelium represents* de novo *expression or passive adsorption remains to be determined. (Am J Pathol 1992, 141:1397–1408).

E-selectin, formerly known as endothelial leukocyte adhesion molecule-1 (ELAM-1), is a glycoprotein of M_r 115,000 that is expressed on microvascular endothelial cells in response to inflammatory mediators such as tumor necrosis factor and interleukin 1.[1,2] Expression of E-selectin on cultured endothelial cells peaks within 4 to 6 hours of cytokine stimulation,[1] and a similar time course was found in an experimental model of inflammation in baboon after cutaneous injection of single cytokines or endotoxin *in vivo*.[3,4] Experimental evidence supports the conclusion that there is an adhesive role for E-selectin in the recruitment of polymorphonuclear leukocytes (PMN) to sites of inflammation.[1–6] E-selectin also has been found to contribute to the adhesion of monocytes[7–9] and a subpopulation of T lymphocytes[10–12] *in vitro*, and to be expressed on endothelial cells at sites of mononuclear leukocyte infiltration.[3,11,13–15] Two other cytokine-inducible endothelial adhesion molecules appear to support leukocyte adhesion and extravasation during inflammation, namely, intercellular adhesion molecule (ICAM)-1[16] and inducible cell adhesion molecule-110/vascular cell adhesion molecule-1 (INCAM-110/VCAM-1),[17–19] both members of the immunoglobulin gene superfamily. Intercellular adhesion molecule interacts with the β2 integrins CD11a/CD18 and CD11b/CD18,[20–25] and may contribute to the adhesion of all leukocyte types. Induc-

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ible cell adhesion molecule-110/vascular cell adhesion molecule-1 supports the adhesion of lymphocytes and monocytes but not PMN through an interaction with its counter-receptor the α4β1 integrin, VLA-4.[18,26,27]

Based on predictions from the sequence of cloned cDNAs, E-selectin has a mosaic structure composed of an N-terminal lectinlike domain followed by an epidermal growth factor (EGF) domain and six nonidentical repeats of a motif found in a family of complement regulatory proteins.[2,28] Two other molecules have been found to have similar domain structures P-selectin (CD62), known also as PADGEM,[29] or GMP-140,[30] is found in storage granules of platelets and endothelial cells and can be rapidly redistributed to the cell surface after stimulation by several mediators, including thrombin and histamine.[31–33] Structural information predicts a transmembrane protein with an N-terminal lectin domain, an EGF repeat, and nine complement regulatorylike repeats.[34] L-selectin is expressed on most lymphocytes, monocytes, and PMN; it is also known by several other names, including LAM-1, Leu8, TQ1, LECCAM 1, LECAM-1 P-selectin.[35–39] Like E- and L-selectin contains an N-terminal lectin domain, an EGF repeat, and complement regulatory repeats. Together, these three molecules constitute a new gene family that has been referred to as the Selectins[40] or LECCAMS.[41]

The structural similarity between the N-terminal domain of E-selectin and the carbohydrate binding regions of C-type lectins[2,42] has prompted the suggestion that carbohydrate moieties on the surface of PMN or other leukocytes might serve as ligands for E-selectin. Recently, data derived from several experimental approaches have supported the conclusion that one ligand for E-selectin is sialyl-Lewis X (SLe^x; NeuAcα2,3Galβ1,4 [Fucα1,3]GlcNAc).[7,43–46] Before the recognition that SLe^x may be a ligand of E-selectin, its cellular and tissue distribution had been investigated in several studies.[47–55] Various normal cell types, including peripheral blood PMN and monocytes, and esophageal glandular and renal tubular epithelium, were shown to express SLe^x.[47,49,51,52,54] Multiple human tumors, particularly adenocarcinomas of gastrointestinal tract and lung, have been found to elaborate more SLe^x than corresponding benign cells,[48–50,53,55] an observation of substantial interest in cancer biology. SLe^x has also been reported to be involved in P-selectin-mediated adhesion.[33,56,57]

The current study describes the expression of SLe^x on leukocytes and other cell types at sites of inflammatory and immunologic processes and within secondary lymphoid tissues. Sialyl-Lewis X was found on PMN and monocytes/macrophages in areas of inflammation, consistent with the concept that E-selectin and its ligands are involved in leukocyte adhesion and extravasation. New points are highlighted. First, a smaller proportion of PMN and monocytes/macrophages in the tissues bear SLe^x than their counterparts in the blood, suggesting that this structure may be lost during extravasation or residence in tissue. Second, a significant portion (5% to 15%) of T lymphocytes in peripheral blood were found to express SLe^x, possibly contributing to the adhesion of a subset of lymphocytes to E-selectin.[10–12] Finally, venular endothelium in lymphoid tissues and inflamed appendix stained with the antibody CSLEX1 used for detection of SLe^x; several potential mechanisms that may account for this endothelial staining are discussed.

Materials and Methods

Analysis of Peripheral Blood Leukocytes

Expression of SLe^x and other antigens on human peripheral blood leukocytes was determined by flow cytometric analysis of cells after single or double staining with monoclonal antibodies. Human peripheral blood was obtained from healthy volunteers; PMN or mononuclear cell populations were isolated by a standard method using a Ficoll-Hypaque gradient.[58] To assess SLe^x expression, 5×10^5 cells were incubated with a saturating concentration of antibody CSLEX1[48] (murine monoclonal anti-SLe^x antibody, IgM, donated by Cytel Corp., La Jolla, CA, and also obtained from American Type Culture Collection, Rockville, MD, 10 μg/ml) for 45 minutes at 4°C, washed, and then incubated with fluorescein isothiocyanate (FITC)-labeled goat anti-mouse IgM (1:100, Becton Dickinson Corp., Mountain View, CA) for 45 minutes at 4°C. To determine specific cell populations within the mononuclear cell fraction that expressed SLe^x, mononuclear cells stained with antibody CSLEX1 were incubated also with various phycoerythrin-labeled monoclonal antibodies (10 μg/ml) for 45 minutes at 4°C. These antibodies were Mo2 (IgM, anti-CD14, Coulter Immunology, Hialeah, FL), which recognizes monoctyes; Leu 4 (IgG1, anti-CD3, Becton Dickinson) a T lymphocyte marker; and NKH-1 (IgG1, anti-CD56, Coulter Immunology) or Leu-11 (IgG1, anti-CD16, Becton Dickinson), both of which recognize natural killer cells and a small subset of T cells. Stained cells were fixed in 1% paraformaldehyde before fluorescence analysis by flow cytometry (FACStar with the Consort 30 software program, Becton Dickinson). Gating on either lymphocytes or monocytes was performed by forward and right angle light scatter characteristics, using standard methods. Quadrants were set based on the results of staining with appropriately labeled isotype-matched negative control antibodies, and 5000 to 10,000 events were collected.

Tissues and Immunohistochemistry

Human tissues were collected from surgical or autopsy cases. Autopsy specimens were frozen within 24 hours of death; systematic study has indicated that leukocyte antigens and other antigens are well preserved during this postmortem interval.[59,60] Tissues were categorized in to three groups: 1) noninflamed nonlymphoid; 2) inflamed nonlymphoid; or 3) lymphoid. Specific tissue diagnoses (and the number of specimens assessed) are described in Table 1 in Results. In addition, one case of dermal delayed hypersensitivity (30 U tuberculin at 72 hours) was examined.

Blocks of tissue were snap-frozen in liquid nitrogen; cryostat sections (6 μ) were cut and fixed in 2% paraformaldehyde. Immunohistochemistry was performed using an avidinbiotin-peroxidase method and diaminobenzidine as previously described,[19] with substitution of biotinylated anti-mouse IgM (Vector Laboratories, Burlingame, CA) for anti-mouse IgG where appropriate. The primary murine monoclonal antibodies used at appropriate dilutions were CSLEX1[48] (IgM, anti-SLe^x); EBM/11[61] (IgG1, anti-CD68, myeloid cells, notably monocytes/macrophages, Dako Corp., Carpinteria, CA); T3-4B5 (IgG1, anti-CD3, T lymphocytes, Dako Corp.); NKH-1 (IgG1, anti-CD56, natural killer cells and a small proportion of cytotoxic T lymphocytes, Coulter Immunology); B4–78 (IgG1, anti-CD20, B cells, donated by T. F. Tedder, Dana-Farber Cancer Institute, Boston, MA); H4/18[1] (IgG1, anti–E-selectin); and F8/86 (IgG1, anti-factor VIII related antigen, Dako Corp.). Also used were control isotype-matched antibodies of irrelevant specificities, at the same or greater concentrations than the test antibodies. Proportions of cells or vessels that stained were estimated visually and are noted in the text. In addition, the absolute numbers of SLe^x-positive leukocyte subpopulations in various tissues are indicated in Table 1, having been scored using the following arbitrary scale: −, absent; ±, rare/occasional; +, present in modest to moderate numbers; ++, present in large numbers. In inflamed dermal specimens (three cases each of psoriasis and chronic dermatitis, and one insect bite reaction), double-immunofluorescence studies were performed as follows: After paraformaldehyde fixation, sections were treated with 10% goat serum for 5 minutes and then incubated with both CSLEX1 and T3-4B5 for 16 to 18 hours at 4°C. They then were washed in phosphate-buffered saline and exposed to rhodamine-conjugated goat anti-murine IgG-specific immunoglobulin and FITC-conjugated goat anti-murine IgM-specific immunoglobulin (both from Southern Biotechnology Associates, Birmingham, AL), each at 1:18 in phosphate-buffered saline including 2% AB + normal human serum for 60 minutes. They then were washed again, mounted in 'Fluoromount' (Southern Biotechnology), and examined using an epifluorescence microscope. Controls included using single primary antibodies and a single mismatched secondary reagent, which did not yield fluorescence. In test-stained sections, at least 50 CD3-positive cells per case were examined for CSLEX1 binding.

Results

Peripheral Blood Leukocyte Expression of SLe^x

Flow cytometric analysis indicated that more than 99% of peripheral blood PMN expressed high levels of SLe^x (Figure 1). Similarly, a large majority of monocytes (>93%) stained strongly with the antibody CSLEX1 (Figure 1). These findings confirm those of Fukushima et al.[48] In addition, SLe^x was expressed at low levels on 20% to 40% of natural killer (NK) cells, identified by monoclonal antibodies (MAbs) directed against CD16 or CD56 (Figure 1). These results are largely similar to those of Ohmori et al.,[52] who found variable expression of SLe^x on approximately half of NK cells using antibody FH6, which is reported to recognize sialyl-Le^x-i(NeuAcα2,3Galβ1,4[Fucα1,3]GlcNAcβ1,3Galβ1,4[Fucα1,3]GlcNAc; the internal Fuc is not essential for FH6 binding).[49,52] It should be noted that staining of NK cells with antibody CSLEX1 did not produce discrete positive and negative populations. Thus, it is possible that a greater proportion of these cells than we have stated express this antigen, but at levels too low to be distinguished from background fluorescence. We also observed expression of SLe^x on approximately 5% to 15% of T lymphocytes, as marked by anti-CD3 antibody, with varying intensity of staining on the positive cells (Figure 1).

Expression of SLe^x in Noninflamed Tissues

In noninflamed tissues devoid of neutrophil or mononuclear cell infiltrates, the endothelial lining of vessels failed to express E-selectin. Certain cells in the dermis, appendix wall, or synovium were stained with anti-SLe^x antibody, most of these cells having the morphology of macrophages. The SLe^x-positive macrophages constituted only a small percentage of the total resident macrophage population identified by anti-CD68 antibody; on average, approximately 10% of tissue macrophages in noninflamed tissues (skin, appendix, or synovium) were stained with antibody CSLEX1.

Leukocyte Expression of SLe^x in Inflamed Tissues

In all cases of acute appendicitis, venular endothelium expressed E-selectin (Figure 2, Table 1). The leukocytic infiltrate contained large numbers of PMN, of which most, but not all, expressed SLe^x (Figure 2). In addition, substantial numbers of SLe^x-positive cells had the morphology of monocytes or macrophages. Roughly half the monocyte/macrophage population expressed SLe^x. The proportion of extravasated PMN and especially monocytes/macrophages expressing SLe^x was less than that identified on the related cells in the circulation by flow cytometry, suggesting down-regulation of this moiety on leukocytes during or after emigration.

Synovial tissue from patients with rheumatoid arthritis

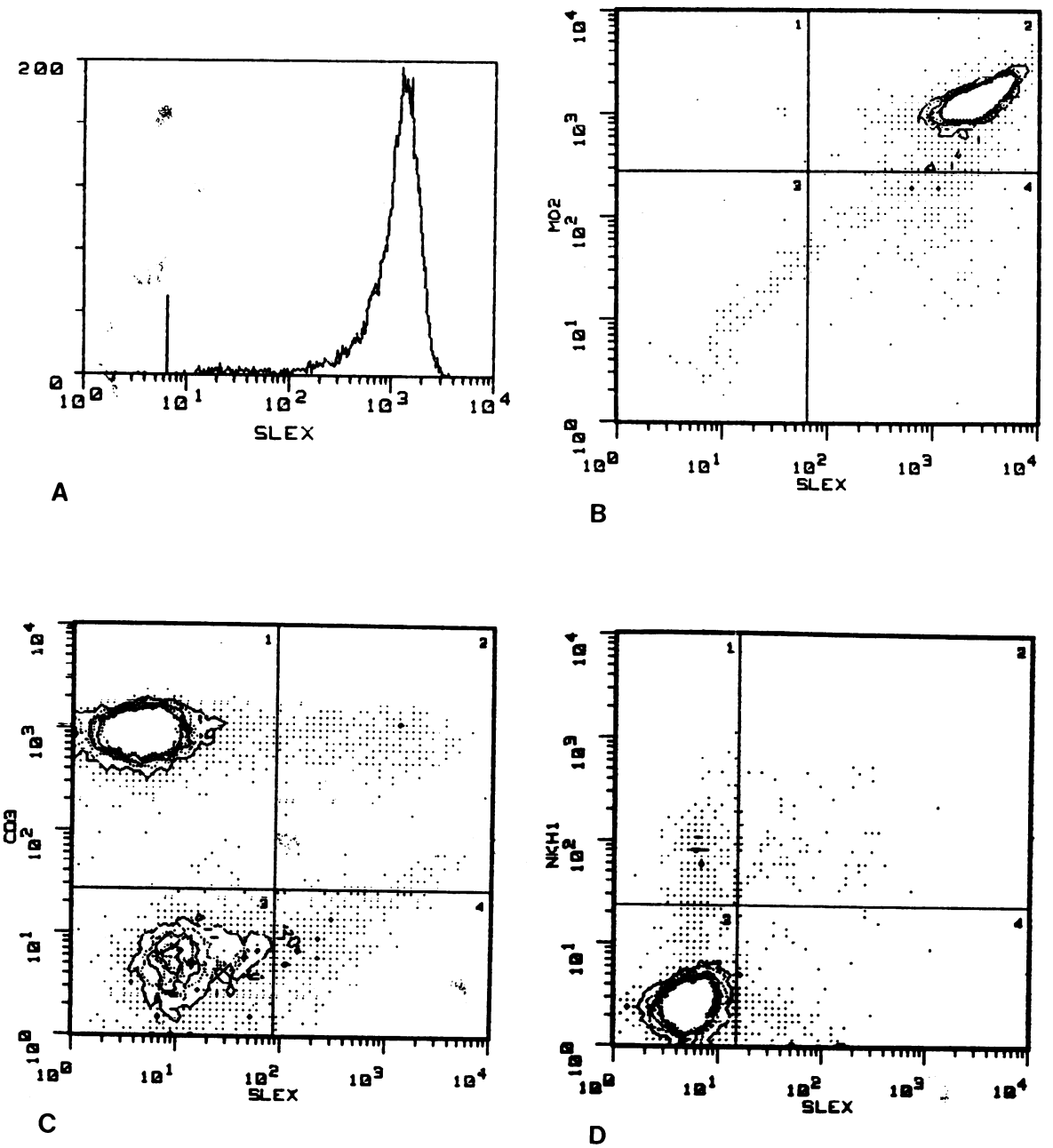

Figure 1. *Characterization of peripheral blood leukocyte expression of SLe^x by flow cytometry using one-color* (A) *or two-color* (B–D) *immunofluorescence. The double-staining results are displayed as plots of red fluorescence (different antibodies) versus green fluorescence (CSLEX1), and the upper right rectangles of these contain double-positive cells.* A: *Uniformly strong expression of SLe^x by isolated polymorphonuclear leukocytes using one-color analysis.* B: *Strong expression of SLe^x on mo2 (CD14)-positive peripheral blood mononuclear cells, i.e., monocytes.* C: *Approximately 5% of CD3-positive T lymphocytes express SLe^x, with varying intensity.* D: *About 25% of NKH1 (CD56)-positive cells (NK and related cells) in this individual express SLe^x, generally weakly.*

Table 1. *Staining of Venular Endothelium and Leukocytes for E-selectin and SLe^x in Various Tissues*

Tissue/Diagnosis	Number of cases studied	Endothelial E-selectin	Leukocyte SLe^x			Endothelial SLe^x
			PMN	MØ	Lymphocytes	
Non-inflamed						
Appendix	3	—	—	±	—	—
Synovium	4	—	—	±	—	—
Skin	3	—	—	±	±	—
Inflamed						
Appendicitis, acute	4	+(4)	++	+	±	+(2)
Synovium rheumatoid	4	+(2)	+	+	±	—
Skin						
Chronic dermatitis	4	+(3)	±	++	++	—
Insect bite	4	+(4)	+	+	+	+(1)
Psoriasis	3	+(3)	±	+	++	—
Lymphoid						
Lymph node						
Resting	5	—	—	+	±	+(3)
Reactive	4	+(2)	—	+	±	+(4)
Sarcoidosis	3	+(2)	—	+	±	+(2)
Peyer's patch	3	—	—	±	±	+(3)
Spleen	2	—	±	+	±	—
Thymus	2	—	—	+	±	+(2)
Tonsil	3	+(2)	—	+	±	+(2)

Figures in parenthesis indicate number of cases with positive endothelium. The areas of normal and inflamed appendix assessed were the wall and serosa rather than the mucosa. (Mø-monocyte/macrophages: PMN = polymorphonuclear leukocytes). Total numbers of SLe^x positive leukocytes scored using arbitrary scale—see methods.

showed aggregates and diffuse infiltrates of lymphocytes and macrophages, and relatively few PMN. Two of the four rheumatoid synovia contained vessels that expressed E-selectin, one weak and focal and the other strong and extensive. Antibody against SLe^x stained approximately 10% of macrophages in rheumatoid synovium. Because the macrophage population was greater in rheumatoid synovium, the number of SLe^x-positive macrophages was greater. Typically, about half of the extravasated PMN also stained with CSLEX1.

Inflamed skin (chronic dermatitis, psoriasis, delayed hypersensitivity, and insect bite reactions) showed accumulations of monocytes/macrophages and T lymphocytes. B cells and CD56-positive cells (natural killer and related cells) also were present sparsely, and in some instances a few PMN were observed. In most cases, endothelial cells of postcapillary venules expressed E-selectin. A variable portion of dermal monocytes/macrophages in these conditions were stained with CSLEX1, typically 20% to 60% (Figure 3). Also, some SLe^x-positive cells had the morphologic characteristics of lymphocytes (Figure 3); double immunofluorescence studies of seven cases of inflamed skin showed SLe^x to be present on 28% to 62% of CD3-positive cells, ie, T cells (mean, 41%). When present, a variable proportion of the extravasated PMN bound antibody CSLEX1.

Leukocyte SLe^x Expression in Lymphoid Tissues

In five resting lymph nodes, no endothelial cell expression of E-selectin was detected. In contrast, reactive nodes and those altered by sarcoidosis contained vessels that weakly or focally expressed E-selectin (Table 1, Figure 4). SLe^x was seen on some macrophages in all lymphoid organs studied. The fraction of the macrophage population positive for SLe^x in various lymphoid sites and conditions was typically 5% to 20%. Epithelioid cells and other macrophages (histiocytes) within sarcoid granulomas, however, were virtually all negative for SLe^x. Tonsil contained scattered interfollicular macrophages that were positive, but it was noted that follicular macrophages showed little or no expression of SLe^x. Follicular dendritic cells in tonsils did stain weakly with antibody CSLEX1 in some instances, whereas the superficial layer of tonsillar epithelium consistently showed strong staining. Thymic macrophages, predominantly medullary ones, bound antibody CSLEX1, as did a portion of macrophages in spleen and Peyer's patch. Most (>95%) T and B lymphocytes in secondary lymphoid tissues did not express SLe^x.

Putative Expression of SLe^x on Vascular Endothelium

No endothelial cell expression of SLe^x was seen in normal nonlymphoid tissues, or in most of the inflamed nonlymphoid tissues studied. In two of four cases of appendicitis, however, venular endothelial cells were focally decorated with antibody CSLEX1 (Figure 5). Studies on parallel sections indicated that many vessels expressed

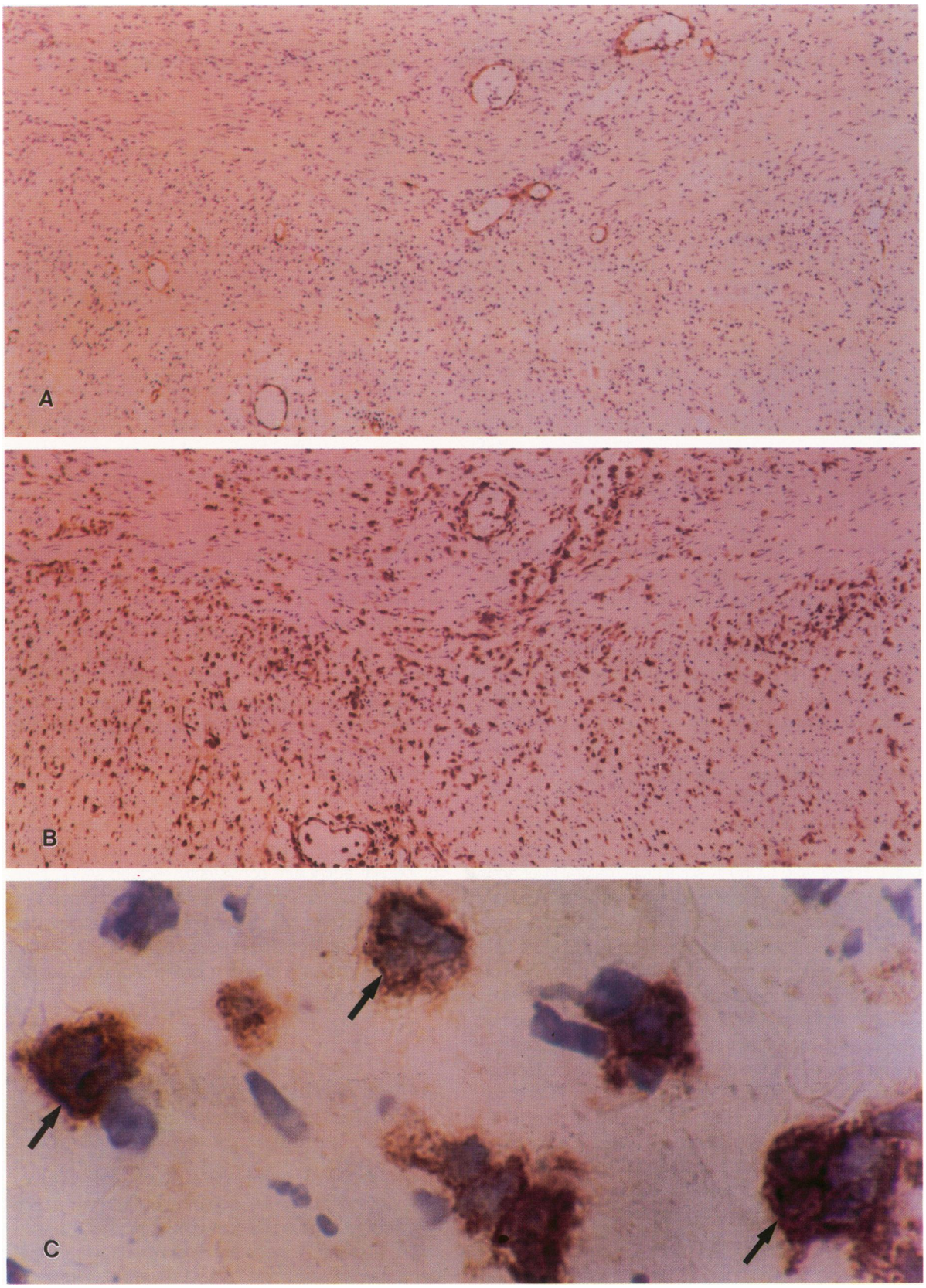
A
B
C

Figure 2. *Cryostat sections of acutely inflamed appendix (immunohistochemistry using chromogen diaminobenzidine, hematoxylin counterstain).* **A:** *Many vessels express E-selectin (×50).* **B:** *Parallel section stained for SLe^x demonstrates numerous positive infiltrating cells (×50).* **C:** *High power view shows that the SLe^x-positive cells are largely polymorphonuclear leukocytes (arrowed), as indicated by their lobulated nuclei (×2500).*

both endothelial SLe^x and E-selectin. Endothelial expression of SLe^x also was seen in lymphoid organs. In three of five resting nodes, 10% to 20% of venules were positive; 50% to 60% of venules were positive in two of three nodes obtained from patients with sarcoidosis. Endothelial staining with antibody CSLEX1 also was seen in Peyer's patch (range of venules positive, 10% to 30%), thymus, and tonsil. Most SLe^x-positive vessels in lymphoid tissues were high endothelial venules (Figure 4); however, venules with flat endothelium also stained. On high endothelial venules, staining with antibody CSLEX1 could be seen in the region of the endothelial cell plasma mem-

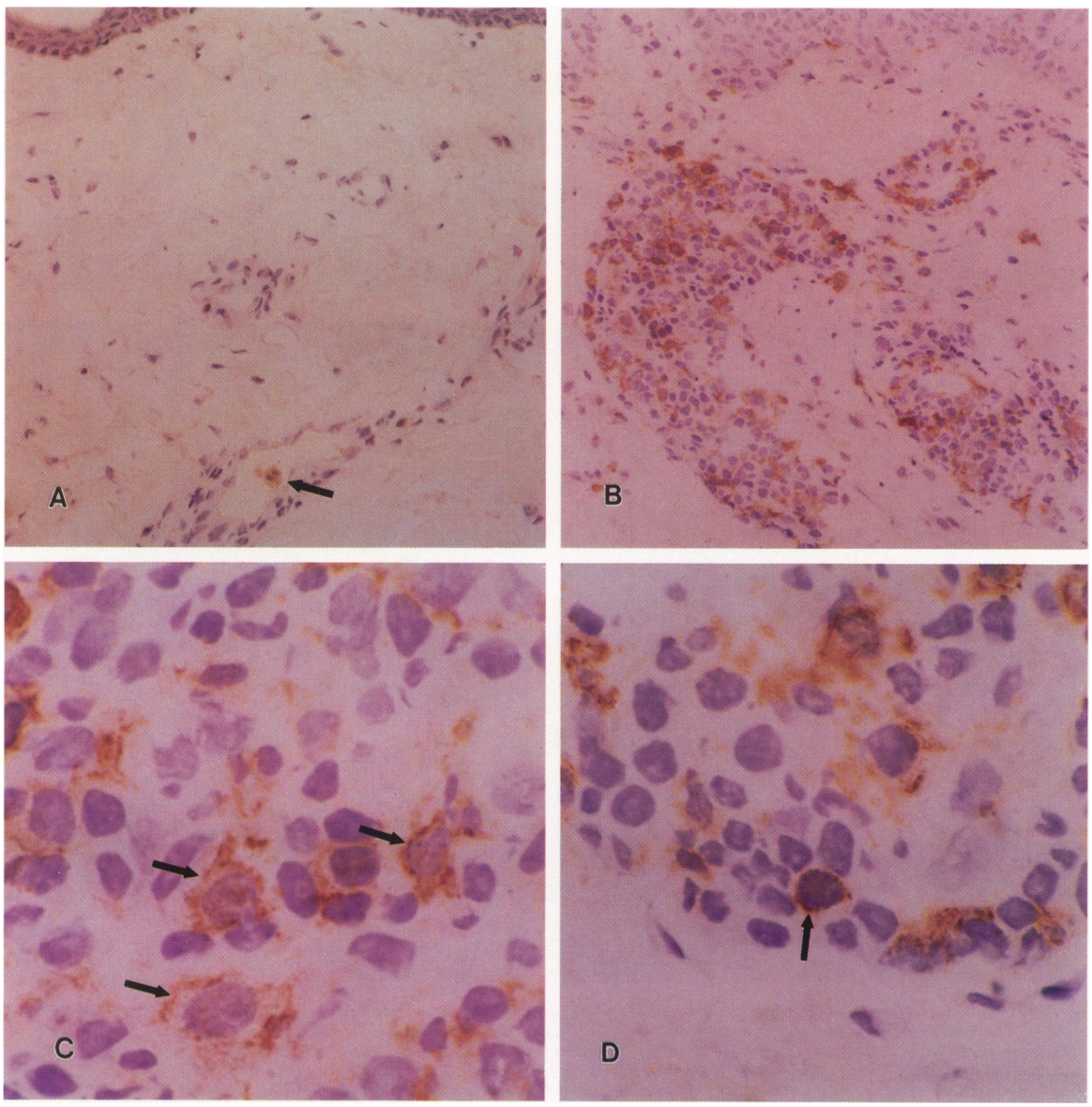

Figure 3. *Anti-SLe^x staining in normal skin* **(A)** *and dermal delayed hypersensitivity* **(B–D)** **A:** *Normal skin showing a weakly positive intravascular cell (arrowed, probably a monocyte), without major extravascular staining (×200).* **B:** *Delayed hypersensitivity reaction with numerous positive cells (×200).* **C:** *Higher power view of the delayed hypersensitivity reaction. Positive cells have the morphology of macrophages (some of these are arrowed) and show variable intensity of staining (×1200).* **D:** *In the same case of delayed hypersensitivity a positive leukocyte, which is arrowed, has a morphology comparable to that of a lymphocyte, in that it has a small round nucleus and little cytoplasm (×1200).*

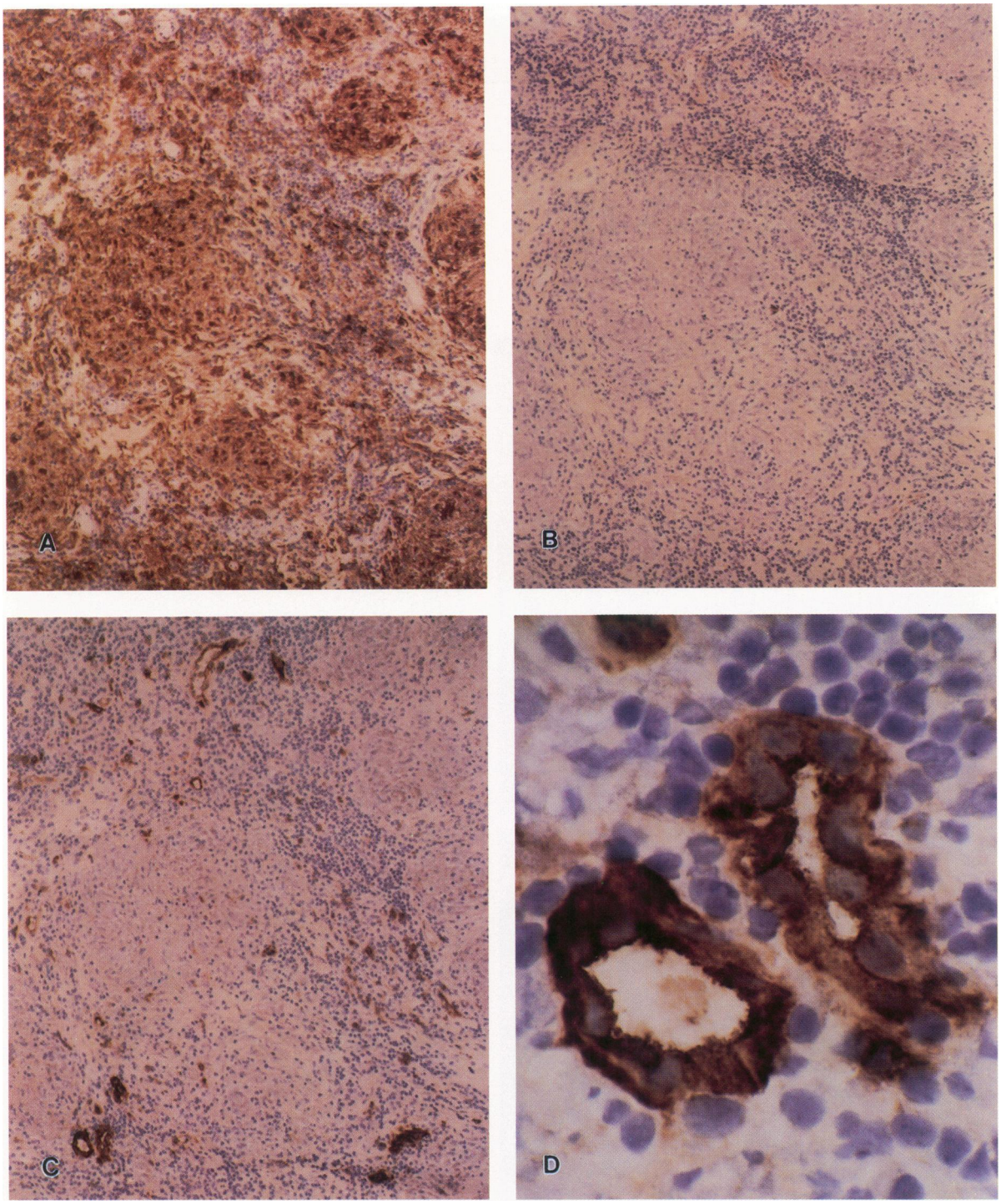

Figure 4. *Lymph node from subject with sarcoidosis.* **A**: *Macrophage population identified by anti-CD68—multiple granulomas are visible (×100).* **B**: *Parallel section assessed with anti-E-selectin antibody. This field shows little staining, one positive vessel being seen just to the right of the center of the panel (×100).* **C**: *Further parallel section stained with anti-SLe^x. In addition to scattered positive macrophages, e.g., to the right, positive venules are also visible (×100).* **D**: *Higher power view of venules expressing SLe^x. The vessel to the left of the panel, for example, shows staining in the regions of the luminal and abluminal endothelial plasma membranes, and in the endothelial cytoplasm. The staining within the lumen of this venule is likely to represent a tangential section through an intravascular leukocyte (×1200).*

brane, both on the luminal and abluminal sides, and also was seen in the cytoplasm (Figure 4). In lymphoid organs, E-selectin was sometimes expressed by high endothelial venules (Table 1); staining for SLe^x on endothelium in these tissues was more extensive than that for E-selectin.

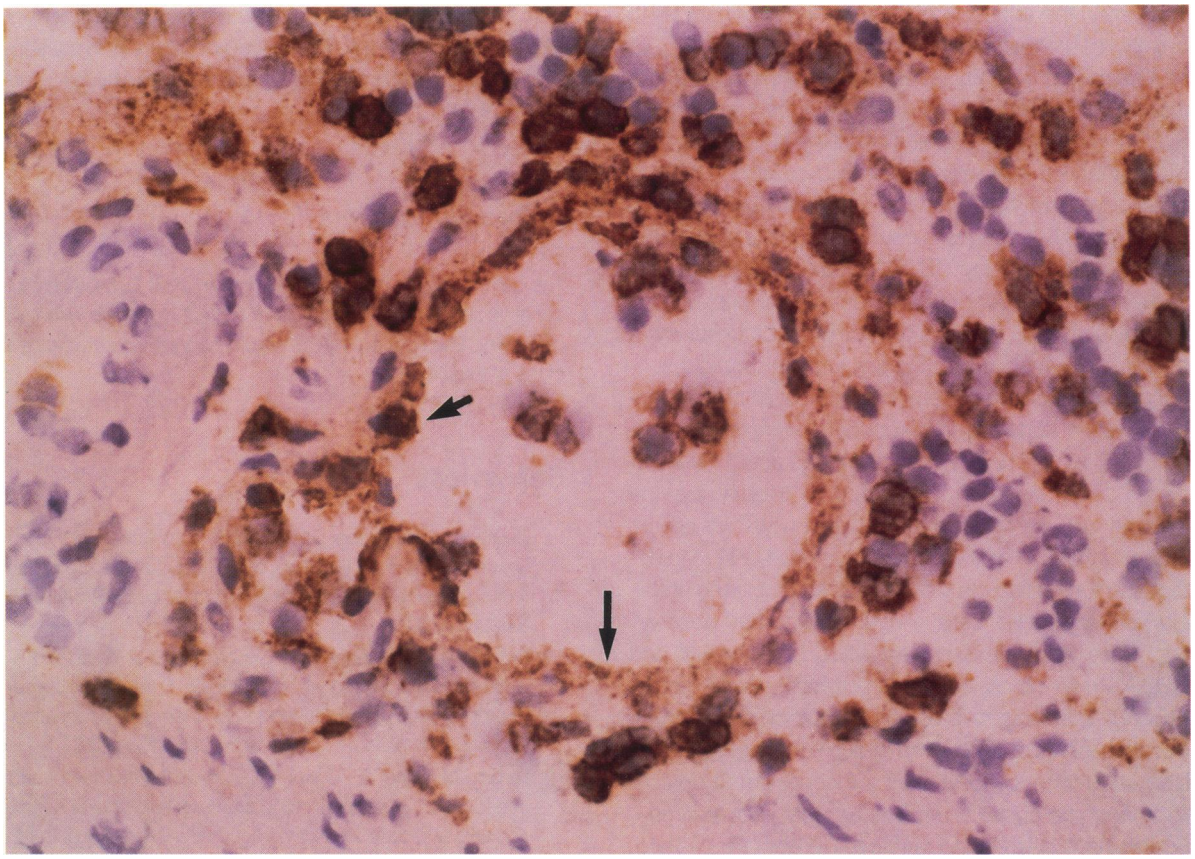

Figure 5. *Endothelial SLe^x in acute appendicitis. Numerous SLe^x-positive leukocytes are seen within and around this serosal vessel (×500). Some cells are in the process of extravasation (e.g. the one indicated by shorter arrow). In addition there is widespread staining of endothelium by anti-SLe^x, such as where arrowed (longer arrow).*

Discussion

This study assessed the relationship between the expression of the endothelial adhesion molecule E-selectin and a recently discovered ligand, the carbohydrate structure SLe^x,[7,43,44] at sites of inflammatory and immune processes and in lymphoid tissues. As described in previous studies,[49,51,54] isolated peripheral blood PMN and monocytes expressed SLe^x in abundance. In acute appendicitis, the endothelium of postcapillary venules expressed E-selectin, and a large number of extravasated PMN and monocytes/macrophages were positive for SLe^x. Other inflammatory conditions also showed E-selectin expression and accumulation of SLe^x-positive leukocytes (Table 1). In acute appendicitis, the proportion of infiltrating cells staining appeared diminished by comparison with intravascular leukocytes examined by flow cytometry. In certain other tissues, such as rheumatoid synovium or lymph nodes affected by sarcoidosis, a smaller proportion of extravascular mononuclear phagocytes were SLe^x-positive. These observations suggest that myeloid cells may lose SLe^x expression after leaving the circulation however, other explanations include that there is selective emigration of SLe^x-negative cells, or that SLe^x-negative cells are longer lived. Previous studies have noted increased SLe^x expression during malignant transformation of several cell types.[48–50,53,55] It is interesting to speculate that the regulation of expression of this structure may be linked to the migratory or differentiation state in various cell lineages.

Recently, several reports have shown that a subset of T cells can bind to E-selectin *in vitro*.[10–12] Using the antibody CSLEX1, we found that 5% to 15% of circulating T lymphocytes expressed SLe^x and a higher proportion of T cells in inflamed skin were found to be positive. It seems possible that this ligand mediates binding of certain T cells to E-selectin. In fact, preliminary studies suggest that a relatively high proportion of T cells that bind to E-selectin–transfected COS cells do express SLe^x (unpublished observations). Our findings concerning the expression of SLe^x on T cells are different from those of Ohmori et al,[52] who indicated that fewer than 1% of peripheral blood T cells expressed this structure. These investigators used a different antibody, FH6, which is described to recognize sialyl-Le^x-i.[52] The different results may be due to FH6 and CSLEX1, having distinct, although overlapping, specificities. In addition, the precise relationship between sLe^x and the carbohydrate antigen

involved in the adhesion of skin homing lymphocytes to E-selectin[11] remains to be determined.

Unexpectedly, CSLEX1 reacted with vascular endothelium in several tissues, indicating the presence of SLe^x. It was found on endothelial cells in inflamed appendices but not in normal appendices; also CSLEX1-positive vessels were found in secondary lymphoid tissues. Several explanations could account for endothelial staining with antibody CSLEX1. First, endothelial cells could synthesize SLe^x under certain conditions. SLe^x in lymphoid tissues was visible within endothelial cytoplasm, rather than just on the surface, which might suggest its production by these cells. Cultured human umbilical vein endothelial cells appear to express SLe^x-i, as detected by antibody FH6[62]; this expression was reported to be essentially unaffected by treatment of the cultures with interleukin-1β or interferon-γ.[63] Using the antibody CSLEX1, however, we have been unable to detect SLe^x on resting or cytokine-activated cultured umbilical vein endothelial cells *in vitro* (unpublished observation). Possible differences in specificity between FH6 and CSLEX1 may be important in interpreting these results. Second, regarding the presence of SLe^x on endothelium, it could be a residual of transmigration of leukocytes, possibly shed from leukocytes as a mechanism of detachment from the endothelium. Interestingly, activated neutrophils have been found to release SLe^x *in vitro*.[7] Third, SLe^x-bearing glycoproteins could be adsorbed by E-selectin directly from serum. The levels of circulating SLe^x in normal individuals and in subjects with nonmalignant diseases, however, appear to be very low.[64] In lymphoid tissues, E-selectin staining assessed with antibody H4/18 was typically much more focal than endothelial SLe^x staining. It is unlikely that SLe^x bound to E-selectin would block binding of the anti–E-selectin antibody H4/18, because this antibody binds to a site on E-selectin within the complement regulatorylike repeats rather than the lectin domain.[7] Possibly the mechanisms whereby SLe^x is present on endothelium in various situations (eg, in lymphoid tissue *versus* inflamed nonlymphoid tissue) are different. The potential *in vivo* function of SLe^x on endothelium deserves further attention. If SLe^x on endothelial cells represents material that has bound to E-selectin, it might inhibit leukocyte adhesion and extravasation at some stage in inflammation. Additionally, it is not known whether SLe^x on endothelium, whatever its source, may function as a ligand for circulating platelets that express R selectin,[57] or conceivably for leukocytes that express L-selectin.

The results of these studies are consistent with the conclusion that the demonstrated *in vitro* adhesive role of PMN and monocyte SLe^x, ie, as a ligand for endothelial E-selectin, is relevant to the evolution of inflammation *in vivo*. They have disclosed SLe^x expression on a significant fraction of circulating T lymphocytes, suggested that SLe^x is lost from leukocytes during or after extravasation, and shown binding of CSLEX1 to endothelial cells in certain settings.

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