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Cytokine Production (IL-1α, IL-1β, and TNFα) and Endothelial Cell Activation (ELAM-1 and HLA-DR) in Reactive Lymphadenitis, Hodgkin's Disease, and in Non-Hodgkin's Lymphomas

An Immunocytochemical Study

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Cryostat sections of 58 lymph nodes were immunostained with a polyclonal rabbit serum against IL-1α, and with monoclonal antibodies directed to IL-1α (Vmp18), IL-1β (Vhp20 and BRbC3), and tumor necrosis factor α (TNFα) (B154.7). Furthermore the presence of cytokine-containing cells was correlated with the expression of endothelial leukocyte adhesion molecule (ELAM-1; 29F2) and of human leukocyte antigen (HLA-DR) (OKIa-1) by endothelial cells. Cells containing IL-1 and/or TNFα were detected mainly in pathologic conditions characterized by reactive or neoplastic expansion of the lymph node paracortex. Cells positive for IL-1 were detected in 16 of 21 cases of Hodgkin's disease, in 4 of 4 cases of T-NHL, and in 5 cases of diffuse or mixed lymphadenitis. Interleukin-1α was detected in macrophages, interdigitating reticulum cells (IDRCs), endothelial cells, and neoplastic Hodgkin's and Reed–Sternberg (H-RS) cells. Cells positive for IL-1β were much fewer and consisted mainly of macrophages. Hodgkin's Reed–Sternberg cells were negative for IL-1β even after in vitro stimulation with bacterial endotoxin. Tumor necrosis factor α (TNFα) was present in macrophages and H-RS cells. Endothelial leukocyte adhesion molecule-1 expression by endothelial venules was detected in 17 of 20 cases of Hodgkin's disease, in 2 of 4 cases of T-NHL, and in 5 of 5 cases of diffuse lymphadenitis. In these pathologic conditions, HLA-DR antigens also were expressed frequently by endothelial cells. Cytokine-containing cells and ELAM-1–positive high endothelial venules (HEV) were extremely rare in lymph nodes involved by follicular lymphadenitis (12 cases) or B-NHL (16 cases). In cases of reactive or neoplastic B-cell proliferations, HLA-DR–positive HEVs still were present often. Our results indicate that IL-1/TNFα production at tissue level is often associated with ELAM-1 expression by HEVs, but is less well correlated with expression of HLA-DR antigens by endothelial cells. (Am J Pathol 1990, 137:1163–1171)

Interleukin 1 (IL-1) and tumor necrosis factor α (TNFα) are pleiotropic molecules that play a pivotal role in inflammatory and immune reactions (see Dinarello,[1] Oppenheim et al,[2] and Beutler and Cerami[3] for review). There are two molecular forms of IL-1, IL-1α, and IL-1β that differ in the amino acid sequence but retain the capacity to bind the same receptor.[4–6] Cells containing IL-1 or TNFα can be demonstrated on tissue sections by immunocytochemical methods.[7–12] IL-1 has been detected in interdigitating re-

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ticulum cells (IDRCs), in activated Langerhans' cells, and in epithelioid macrophages of granulomas.[7,8,11,12] IL-1β and TNFα were demonstrated in epithelioid macrophages of sarcoid and tuberculous granulomas and in scattered cells of the lymphoid tissue.[9,11] IL-1 and TNFα also have been detected in Reed–Sternberg (RS) cells of Hodgkin's disease (HD).[7,10]

Cytokines are powerful inducers of 'endothelial cell activation,' as described by Pober.[13] In fact, they can induce quantitative changes in the level of expression of specific gene products (ie, proteins) that allow endothelial cells to perform new functions. Molecules whose production is increased during endothelial cell activation include endothelial leukocyte adhesion molecule (ELAM-1), intercellular adhesion molecule (ICAM-1), chemotactic factors, IL-1, IL-6, prostaglandins, and growth factors (see Pober[13] and Mantovani and Dejana[14] for review). We focused our attention on ELAM-1 as an indicator of vascular activation because it is absent on normal vessels, is mainly expressed by activated high endothelial venules (HEV), and can be demonstrated with immunocytochemistry.[15–16] *In vivo* and *in vitro* experiments indicate that ELAM-1 supports granulocyte adhesion to vascular endothelium and probably is involved in extravasation.[17,18] Interferonγ (IFNγ) is another molecule active on endothelial cells; IFNγ is supposed to modulate the antigen-presenting function through upregulation of MHC class II antigens (HLA-DR).[19,20] It has been suggested that IL-1 and TNF induce endothelial activation through a pathway distinct from that of IFNγ.[13,14,18]

The aim of the present study was to investigate the relationship between production of IL-1 and TNFα and vascular activation *in vivo*. For this purpose, lymph node sections were immunostained to correlate the presence of cells containing IL-1/TNFα with the expression of activation antigens ELAM-1 and HLA-DR by vascular endothelial cells. The study was done on 58 lymph nodes from patients with reactive lymphadenitis, Hodgkin's disease, and non-Hodgkin's lymphoma.

Materials and Methods

Tissues and Cell Suspensions

Fresh fragments of 58 lymph nodes, removed for diagnostic purpose, were embedded in overfreezing cryotome tissue (Miles, Elkhart, IN) compound, snap frozen in liquid nitrogen, and stored at −80°C until sectioning. The diagnosis of Hodgkin's disease, B- or T-cell non-Hodgkin's lymphoma (NHL), and control reactive lymphadenitis were supported by conventional histology and immunocytochemistry. Non-Hodgkin's lymphomas were classified according to the Kiel classification.

Lymph node cell suspensions were obtained by gently scraping the tissues with a scalpel. Peripheral blood leukocytes (PBL) were obtained from normal volunteers and isolated by centrifugation on a Lymphoprep Discontinuous Gradient (Nyegaard, Oslo, Norway). Peripheral blood leukocytes and lymph node cells were suspended in RPMI-1640 medium containing 1% human AB serum, 100 U/ml penicillin, and 100 μg/ml streptomycin. A fraction of the cells was used to prepare cytocentrifuge smears; another fraction was cultured in 50 ml polypropylene test tubes at a concentration of 2×10^6/ml with 20 μg/ml LPS from *Escherichia coli* 055.B5 (Sigma Chemical Co, St. Louis, MO) for 18 hours in an atmosphere containing 5% CO_2 at 37°C. At the end of incubation, LPS-stimulated cells were cytocentrifuged. Cytosmears were wrapped individually in aluminum foil and stored at −80°C until immunostaining.

Immunostaining Procedures

Cryostat sections and cytocentrifuge smears were fixed in acetone for 10 minutes at room temperature and immunostained for IL-1α, IL-1β TNFα, ELAM-1, HLA-DR, and monocyte/macrophage antigens. Interleukin-1α was demonstrated with Vmp18, a murine monoclonal antibody to the synthetic peptide 199-208 of the murine IL-1α, which corresponds to 196-205 in the human sequence, and with a rabbit polyclonal serum (Genzyme, Boston, MA). Vmp18 (IgG1) was obtained following a previously described procedure[21] by immunizing BALB/c mice with the peptide-KLH conjugate, fusing lymph node cells with P3X63-Ag8.653 myeloma cells, screening for recognition of the immunizing peptide in solid-phase radioimmunoassay, and cloning of positive hybridomas. Interleukin-1β was demonstrated with Vhp20, a monoclonal IgG2a antibody to the synthetic IL-1β peptide 163-171,[21] and with BRhC3, a mouse monoclonal IgG1 antibody obtained by immunization with recombinant IL-1β and recognizing an epitope within the fragment in position 133-147 (provided by M. Bigio and R. Rossi, Sclavo, Siena).[22] Tumor necrosis factor α was recognized by the monoclonal antibody B154.7, whose specificity was previously described.[23] B154.7 was donated by B. Perussia, Wistar Institute, Philadelphia, PA. Endothelial leukoctye adhesion molecule 1 was recognized by monoclonal antibody 29F2. 29F2 was produced from a fusion of splenocytes from a mouse immunized with cytokine-activated human umbilical vein endothelial cells. Specificity was confirmed by binding to COS cells transfected with cDNA encoding human ELAM-1 (Pigott et al, manuscript in preparation). Monoclonal antibodies Vmp18, Vhp20, BRhC3, and B154.7 were protein A purified IgG from ascitic fluids; 29F2 was obtained as culture supernatant. Monocyte/macrophages were demonstrated

Table 1. *Immunoreactivity for IL-1α, IL-1β, TNFα, ELAM-1, and HLA-DR in Lymph Nodes Involved by Reactive Lymphadenitis*

Case	Age/sex	Histology	Mac*	TNFα	IL-1α	IL-1β	Endothelium	
							HLA-DR	ELAM-1
1	10/M	Follicul.	++	–	–	–	0†	0†
2	12/F	Follicul.	+	–	–	–	3	0
3	11/F	Follicul.	+++	–	–	–	0	0
4	7/F	Follicul.	+	–	–	–	0	0
5	15/M	Follicul.	++	–	–	–	126	0
6	24/M	Follicul.	+	–	En	–	0	7
7	25/M	Follicul.	+	–	NT	–	19	3
8	23/M	Follicul.	++	–	Neg	–	130	3
9	28/M	Follicul.	+++	–	–	–	23	2
10	26/F	Follicul.	++	–	–	–	92	7
11	32/M	Follicul.	++	–	–	–	0	0
12	24/M	Follicul.	++	–	–	–	25	2
13	52/M	Diffuse	++	Mac	Mac/IDR/En	Mac/IDR	NT	41
14	25/M	Diffuse	++	NT	Mac/IDR/En	–	3	21
15	28/M	Diffuse	+++	Mac	Mac/IDR	–	18	17
16	16/M	Mixed	++	Mac	Mac/IDR	–	0	18
17	57/F	Mixed	++	NT	Mac	Mac	2	4

Cases 6–12 were obtained from HIV+ patients.

Follicul., follicular hyperplasia; diffuse, paracortical hyperplasia; mixed, combined follicular and paracortical hyperplasia; Mac, macrophages; En, endothelial cells; IDR, interdigitating reticulum cells; NT, not tested.

* Approximate evaluation of the number of Dako-mac+ macrophages (+ = 0–10 macrophage/field at 400×; ++ = 10–50; +++ = > 50.

† Number of positive blood vessels in 10 microscope fields at ×150.

by Dako-mac (Dakopatts, Denmark) and HLA-DR antigens with OKIa-1 (Ortho, Raritan, NJ).

Cryostat sections and cytosmears were preincubated with normal horse or goat serum to prevent nonspecific binding and were incubated with an optimal dilution of the primary antibody (Vmp18, Vhp20, BRhC3, B154.7, rabbit anti–IL-1α, 1:100 v/v; Dako-mac, OKIa-1, 29F2, 1:10 v/v) for 30 minutes. The slides were incubated sequentially with biotin-conjugated horse anti-mouse immunoglobulin antibodies or with biotin-conjugated goat anti-rabbit immunoglobulin antibodies, followed by avidin-biotin peroxidase complex (PK 4002; Vector Laboratories, Burlingam, CA). Each incubation step lasted 30 minutes with 5-minute TRIS-buffered saline (TBS) washes between each step. The sections finally were incubated with 0.03% H_2O_2 and 0.06% 3,3′-diaminobenzidine (BDH Chemicals, Poole, United Kingdom) for 3 to 5 minutes. Then slides were washed for 5 minutes in running tap water, counterstained with hematoxylin for 5 minutes, and mounted in Canadian balsam. Endogenous peroxidase was seen only in eosinophils. In some experiments endogenous peroxidase was inhibited by pretreatment with 0.01% v/v H_2O_2. In all experiments a routine control was included in which only the primary antibody was omitted.

The specificity of the immunostaining for IL-1α and IL-1β was supported by the observation that unstimulated PBLs were unreactive, that LPS-stimulated PBLs contained 80% to 90% of markedly positive monocytes, and that each couple of reagents, although directed against different epitopes of the molecules, gave comparable patterns of immunoreactivity in terms of number and morphology of positive cells.[11] The specificity of the immunostaining for TNF (B154.7) was shown in a previous study demonstrating that B154.7 and B154.2, two anti-TNFα mAbs directed against non–cross-reactive antigens,[23] were both reactive with about 30% of LPS-stimulated monocytes, and were unreactive with unstimulated PBL.[11] In each experiment, cytosmears of unstimulated and LPS-stimulated PBL were included as negative and positive controls for IL-1/TNF immunostaining. The specificity of the immunostaining for ELAM-1 was strongly supported by the observation that the reactivity for 29F2 was confined to the endothelium of high endothelial venules.

Results

Cryostat sections of 17 control reactive lymph nodes were immunostained to investigate the distribution of cells containing IL-1α (Vmp18 and rabbit polyclonal serum), IL-1β (Vhp20 and BRhC3), and TNFα (B154.7). Furthermore the activation status of vascular endothelial cells was investigated through the use of monoclonal antibodies directed against ELAM-1 (29F2) and HLA-DR (OKIa-1). Cells containing IL-1/TNFα were extremely rare in lymph nodes characterized by activation of the B-cell compartment (cases 1 to 12). On the contrary, IL-1–positive and/or TNF-positive cells were frequently detected in cases showing diffuse or mixed lymphadenitis with expansion of the T-cell dependent areas (Table 1). IL-1–positive cells were located in the paracortex and consisted of macrophage/

IDRCs and of some HEVs (Figure 1a). Cells positive for TNFα were characterized by macrophage morphology (Figure 1a).

Blood vessels with activated endothelial cells were detected in 13 of 17 lymph nodes. The number of positive vessels was extremely variable from case to case and was estimated by counting the number in 10 microscope fields at 150× (Table 1). High endothelial venules expressing HLA-DR antigens were particularly numerous in follicular lymphadenitis of HIV-positive patients (cases 6 to 12) as previously reported.[24] Endothelial leukocyte adhesion molecule 1 was mostly detected in paracortical HEVs of diffuse and mixed lymphadenitis (cases 13 to 17). Endothelial leukocyte adhesion molecule-1–positive HEVs showed focal distribution and were often associated with clusters of IL-1–positive cells (Figure 1b). The intensity of ELAM-1 staining was variable even in HEVs present in the same area. Immunostaining of serial sections (cases 13 and 14) revealed that IL-1α and ELAM-1 could be present on endothelial cells of the same vessel.

In Hodgkin's disease, cells containing IL-1 were detected in 16 of 21 cases and consisted of IDRC, macrophages, a variable number (10% to 50%) of Hodgkin's (Figure 2a) and Reed–Sternberg cells, and of some HEVs (Table 2). Interleukin-1–positive cells were mainly observed in the cellular areas of nodular sclerosis and in mixed cellularity type. Immunoreactivity for IL-1α was more widespread than for IL-1β as judged by the number of positive cases (16 *versus* 6) and by number and type of positive cells.

Hodgkin's Reed–Sternberg cells were significantly positive for IL-1α, and were negative for IL-1β. This observation was confirmed in cytosmears prepared from lymph node cell suspensions (6 cases). Rosetting H-RS cells were positive for IL-1α (Figure 2b) and were negative for IL-1β even after *in vitro* stimulation with bacterial endotoxin (LPS) for 18 hours. The effectiveness of the treatment was proved by the observation that a fraction of lymph node macrophages became positive for IL-1β after LPS stimulation. Immunoreactivity for TNFα was observed in 4 of 21 cases in tissue sections, in 2 of 6 cases in unstimulated cytosmears, and in 3 of 4 cases after LPS-stimulation. Immunoreactivity for TNFα was confined also to macrophages and H-RS cells.

Evidence of endothelial cell activation was obtained in 19 of 20 cases of HD. It is interesting to note that the highest numbers of ELAM-1–positive HEVs were observed in cases of nodular sclerosis, whereas the highest numbers of HLA-DR–positive HEVs were present in cases of mixed cellularity. High endothelial venules reactive for ELAM-1 and/or for HLA-DR antigens were focally distributed throughout the section and were present frequently in areas rich in H-RS cells (Figure 2c). By comparison with immunostained serial sections, it was established that HEVs could be double negative, double positive for ELAM-1/HLA-DR, or single positive for either antigen (Figures 3a, b).

Twenty cases of non-Hodgkin's lymphomas (16 B-cell type and 4 T-cell type) were immunostained with the same panel of antibodies. In B-NHL, macrophages were scattered regularly throughout the tumour, but were much less numerous than in Hodgkin's disease and in T-NHL. Cytokine-containing cells were observed in only four cases of B-NHL and consisted of some macrophages and/or endothelial cells stained for IL-1α. As expected, the low content of IL-1–positive/TNF-positive cells was associated with virtual absence of ELAM-1 in HEVs. HLA-DR-positive HEVs were detected in eight cases, four of which also contained IL-1–positive cells; HLA-DR–positive vessels often were located in the interfollicular T-cell areas of nodular centroblastic/centrocytic (Cb/Cc) lymphoma. The association between IL-1–positive cells and HLA-DR–positive HEVs is probably fortuitous. On the other hand, it might indicate that IL-1 is part of a cytokine circuit, which also may upregulate HLA-DR in vascular endothelium.

In T-NHL the pattern of immunoreactivity was similar to that of reactive lymphadenitis with paracortical hyperplasia, and of Hodgkin's disease. Neoplastic T lymphocytes had no detectable IL/TNF. Macrophages were numerous and often were stained for IL-1α, IL-1β, and, to a lesser extent, for TNFα. Endothelial cell activation was prominent and characterized by the presence of many HEVs stained for ELAM-1 and/or for HLA-DR.

Discussion

In the present study we demonstrated that the appearance of cells containing IL-1/TNF frequently is associated with expression of ELAM-1 antigen by endothelial cells of HEVs. Interleukin-1/TNF production and endothelial cell activation occur in the T-dependent areas and are most marked in those pathologic conditions involving the lymph node paracortex. In contrast, cells stained for IL-1/TNF are ex-

Figure 1. *Cryostat section of a diffuse lymphadenitis with marked paracortical activation immunostained for IL-1α with Vmp18* **(A)** *and for ELAM-1 with 29F2* **(B)**. *The lymph node paracortex is populated by lymphocytes and by numerous cells with IDR/macrophage morphology, most of which are immunoreactive for IL-1α. A possible paracrine effect of IL-1α on blood vessels is supported by the expression of ELAM-1 in two HEVs present in the same area. (ABC-peroxidase, counterstained with hematoxylin, ×250).* **Figure 2.** *Cryostat section* **(A)** *and cytocentrifuge smear* **(B)** *of a lymph node involved by Hodgkin's disease immunostained for IL-1α with Vmp18. Rosetting Hodgkin's cells show diffuse cytoplasmic staining (×1000).* **(C)** *Section of a lymph node involved by Hodgkin's disease immunostained for ELAM-1 with 29F2. The high endothelium of two blood vessels is markedly positive. (ABC-peroxidase, counterstained with hematoxylin, ×250).*

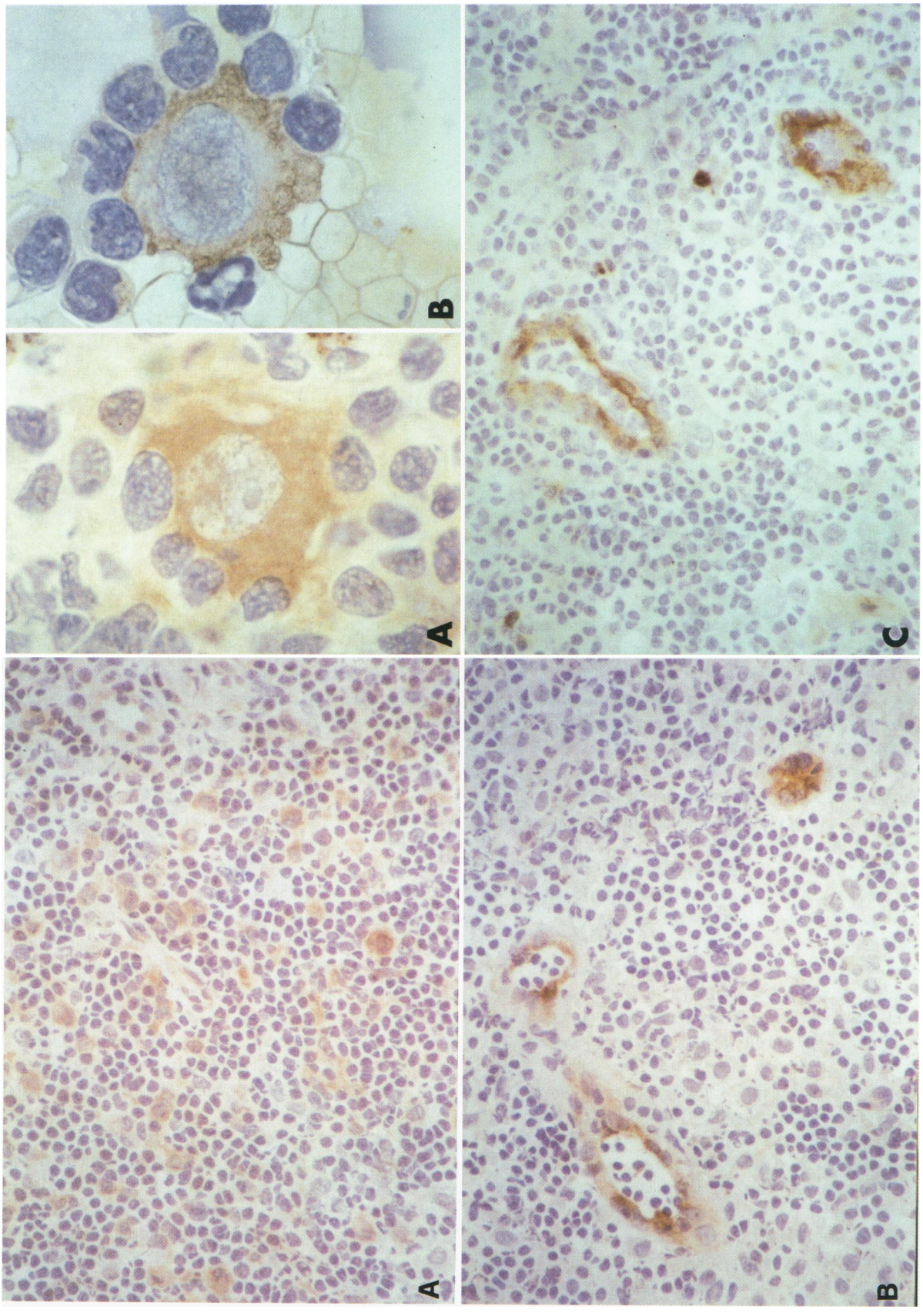
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Table 2. *Immunoreactivity for IL-1α, IL-1β, TNFα, ELAM-1, and Class II MHC Antigens in Lymph Nodes Involved by Hodgkin's Disease*

Case	Age/sex	Histology	Mac*	TNFα	IL-1α	IL-1β	Endothelium	
							HLA-DR	ELAM-1
1	59/F	HD-LP	+	–	Mac	–	9†	1†
2	36/F	HD-LP	+++	–	Ep	–	9	0
3	16/F	HD-NS	+++	–	Mac	–	0	55
4	22/M	HD-NS	+++	–	–	–	0	11
5	19/F	HD-NS	+++	Mac/H	Mac/H	–	0	0
6	19/M	HD-NS	++	–	Mac/H	–	0	2
7	25/F	ND-NS	+++	–	Mac/H	–	2	36
8	25/M	HD-NS	+++	Mac/H	–	–	4	125
9	15/F	HD-NS	++	–	Mac/H/En	Mac	0	40
10	26/M	HD-NS	+	Mac	Mac/H/En	Mac	2	1
11	26/F	HD-NS	+	–	–	–	NT	NT
12	61/F	HD-NS	++	–	Mac/H	–	25	11
13	45/F	HD-NS	+++	–	NT	NT	0	28
14	8/M	HD-MC	+++	–	Mac/H/En	Mac	5	2
15	33/M	HD-MC	+++	–	Mac/En	–	0	11
16	26/M	HD-MC	+++	–	Mac/H	–	58	5
17	42/F	HD-MC	++	–	Mac/H	–	28	0
18	6/M	HD-MC	+++	Mac/H	Mac/H	Mac	82	8
19	28/M	HD-MC	NT	NT	–	–	8	2
20	13/F	HD-MC	+++	–	Mac	Mac	1	1
21	36/M	HD-MC	+++	NT	Mac/H	Mac	98	20

LP, lymphocyte predominance; NS, nodular sclerosis; NT, not tested; MC, mixed cellularity; Mac, macrophages; Ep, epithelioid macrophages; H, Hodgkin's/Reed–Sternberg cells; En, endothelial cells.

* Approximate evaluation of the number of Dako-mac+ macrophages (+ = 0–10 macrophage/field at 400×; ++ = 10–50; +++ = > 50).

† Number of positive blood vessels in 10 microscope fields at ×150.

tremely rare in reactive and neoplastic proliferations of the B-cell compartment; in these conditions, ELAM-1 is low or absent, but a marked expression of HLA-DR often is found on paracortical HEVs. These findings correlate with previous reports in which cytokine production and endothelial cell activation in lymphoid tissues were investigated as isolated events.[7,10,15] Our observations support the view that during lymph node reactions involving B-cell or T-cell areas, at least two distinct patterns of endothelial cell activation are induced.

In the lymph node paracortex, ELAM-1–positive HEVs showed a zonal distribution and often were located close to areas rich in IL-1–positive/TNF-positve cells. However only some HEVs were stained and the intensity of the staining was variable, even in the same area. It has been shown previously that expression of ELAM-1 by activated endothelial cells is a transitory event lasting a few hours *in vitro*[25] and *in vivo*.[18] Thus it can be speculated that persistent stimulation with IL-1/TNF may result in activation/deactivation of ELAM-1 synthesis by endothelial cells and in asynchronous expression of the antigen by HEVs. Alternatively it might be proposed that IL-1/TNF are only active over a short-range distance.

The presence of IL-1 and TNFα in H-RS cells has been demonstrated previously in tissue sections[7,10] and in Hodgkin's cell lines.[10,26] In the present report we provide the additional information that H-RS tissue cells and LPS-stimulated H-RS cells contain mainly IL-1α. Our finding may be related to the recent report that Hodgkin's cell lines are prone to synthesize only one type of IL-1.[26] In that study, however, it was shown that of three different cell lines, one produced IL-1α, the other IL-1β, and a third was inactive in IL-1 secretion. In previous investigations we observed that tissue IDRCs and activated Langerhans' cells contain only IL-1α, whereas LPS-stimulated monocyte/macrophages and epithelioid macrophages are positive for both forms of the molecule.[11,12] It was demonstrated that IDRC/Langerhans' cells are characterized by striking functional similarities with H-RS cells. In fact, H-RS cells, IDRCs, and Langerhans' cells all can present antigen in a HLA-DR–restricted manner[27] and establish close membrane interactions with CD4 T lymphocytes having activated phenotypes.[28,29] Furthermore Langerhans' cells, IDRCs, and H-RS cells all are capable of prostaglandin synthesis through prostaglandin H synthase.[30] IL-1α is the cell-membrane–associated form of IL-1[31] and is involved in T-lymphocyte activation. The high and selective content of IL-1α in IDRCs and H-RS cells might be an additional similarity, perhaps related to a common functional pathway.

In Hodgkin's disease, ELAM-1 was expressed to a similar extent in cases of nodular sclerosis and of mixed cellularity. In contrast, HLA-DR rarely was present on HEVs of nodular sclerosis but was strongly expressed in mixed cellularity. Because it was clearly established both *in vivo* and *in vitro* that expression of HLA-DR by endothelial cells is upregulated by interferon and is poorly influenced by IL-1/TNFα,[13,20] our results may provide circumstantial ev-

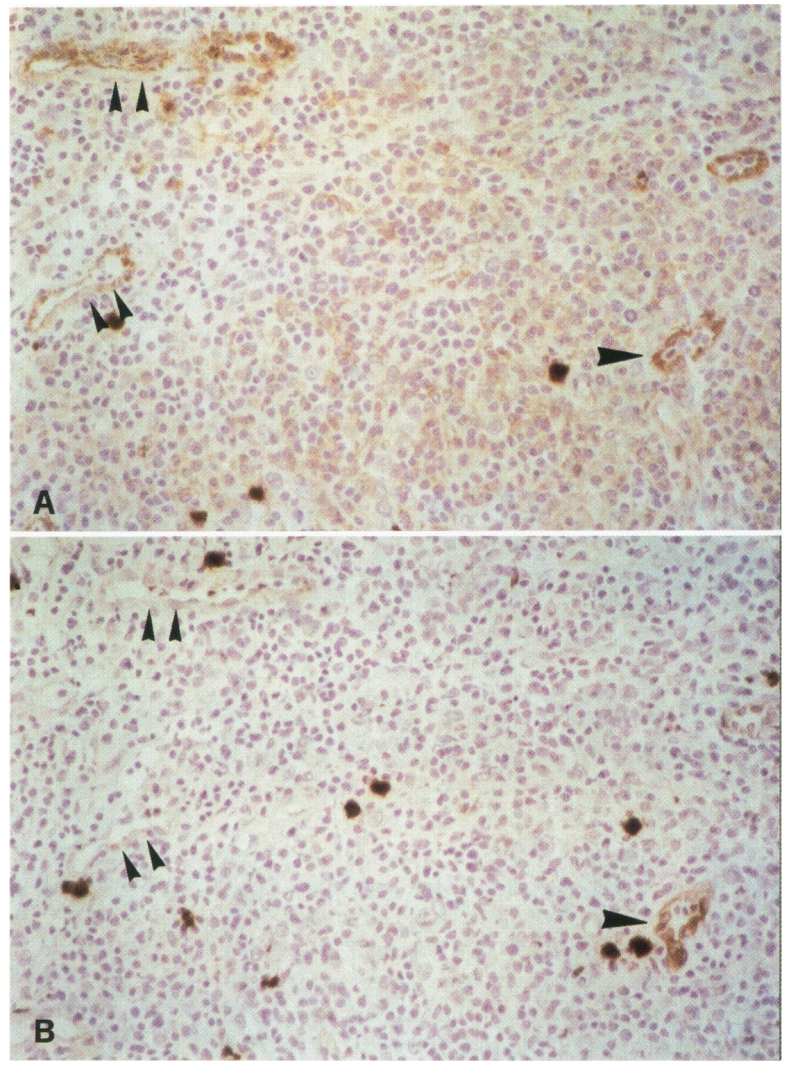

Figure 3. *Two serial sections of a lymph node involved by Hodgkin's disease were immunostained for HLA-DR with OKIa-1* (A) *and for ELAM-1 with 29F2* (B). *On the right side, the same blood vessel is HLA-DR+/ELAM-1+ (single arrow). On the left side, two blood vessels are HLA-DR+/ELAM-1- (double arrows). (ABC-peroxidase, counterstained with hematoxylin, ×100).*

idence that in most cases of mixed cellularity IFNγ is produced. Therefore it is possible that the histologic subtypes of Hodgkin's disease reflect production of different groups of cytokines within the lesion. The recent demonstration that IFNγ is produced in a Hodgkin's cell line (SUP-HD1)[32] but not in others may support this view. Furthermore the concept that HD is a cytokine-secreting tumor is now receiving several experimental confirmations. In fact, IL-5 mRNA, a potent eosinopoietic factor, was recently demonstrated in H-RS cells using *in situ* hybridization.[33] Furthermore macrophage colony-stimulating factor, a molecule able to modulate proliferation and/or differentiation of macrophages, was identified in culture supernatants of Hodgkin's cell lines.[34] These findings may account for some histologic features of HD lesions, such as rich eosinophil infiltrate and high content of macrophages. Furthermore they suggest that molecules other than IL-1/TNFα also might be responsible for vascular activation and ELAM-1 expression in tissues involved by HD.

Interleukin 1 and/or TNF were not detected in neoplastic B or T lymphocytes, whereas they were demonstrated previously in cultured lymphoid cell lines and in activated peripheral blood lymphocytes.[35,36] In a recent study, presence of IL-1α and TNFα was investigated in 35 B NHL using Northern blot analysis.[37] IL-1α was found in a single case, and TNFα was observed in 12 cases, mainly of lymphoplasmacytic type. In our series there was no case of lymphoplasmacytic lymphoma, and this may account for our negative results. Furthermore it is still possible that some IL-1/TNF was present in neoplastic lymphocytes but that it was below the threshold of sensitivity of our method. Absence of ELAM-1 in B NHL might provide indirect evidence that IL-1/TNFαs, if present at all, were in fact present in minimal amounts.

The significance of ELAM-1 expression in HEVs deserves a comment. Endothelial leukocyte adhesion molecule 1, ICAM-1,[38] and inducible cell adhesion molecule 110 (IN-CAM 110),[39] also described as vascular cell adhe-

Table 3. *Immunoreactivity for IL-1α, IL-1β, TNFα, ELAM-1, and Class II Antigens in Lymph Nodes Involved by non-Hodgkin's Lymphoma*

Case	Age/sex	Histology	Mac*	TNFα	IL-1α	IL-1β	Endothelium	
							HLA-DR	ELAM-1
B-cell type								
1	60/M	WDLL	+/++	–	–	–	0†	0†
2	58/M	Cb/Cc nod.	++	–	–	–	0	0
3	54/F	Cb/Cc nod.	+	–	–	–	6	0
4	54/F	Cb/Cc nod.	++	NT	En	–	12	0
5	68/M	Cb/Cc diff.	NT	–	–	–	0	0
6	49/M	Cb/Cc diff.	+	–	–	–	36	0
7	58/F	Cb diff.	++	–	–	–	1	0
8	48/M	Cb diff.	++	NT	Mac	–	9	0
9	55/M	Cb diff.	++	–	NT	–	1	0
10	67/M	Immunobl.	+	–	En	–	1	0
11	58/F	Immunobl.	++	–	–	–	0	0
12	63/M	Immunobl.	++	–	–	–	0	4
13	51/M	Immunobl.	++	–	–	–	0	0
14	52/F	Immunobl.	+	–	–	–	0	0
15	15/M	Burkitt-type	++	–	Mac	–	9	0
16	12/F	Burkitt-type	+	–	–	–	0	0
T-cell type								
17	14/M	Lymphobl.	++	–	Mac/En	Mac/En	5	0
18	52/M	T-zone	+++	–	Mac	Mac	98	23
19	65/M	T-zone	++	–	Mac/En	Mac	60	10
20	58	Lymphoepith.	+++	Ep	Ep	Ep	25	0

WDLL, well-differentiated lymphocytic lymphoma; Cb/Cc, centroblastic/centrocytic; Immunobl., immunoblastic; Lymphobl., lymphoblastic; Mac, macrophages; En, endothelial cells; Ep, epithelioid macrophages; NT, not tested.

* Approximate evaluation of the number of Dako-mac+ macrophages (+ = 0–10 macrophage/field at 400×; ++ = 10–50; +++ = > 50).

† Number of positive blood vessels in 10 microscope fields at ×150.

sion molecule 1),[40] all are capable of mediating leukocyte adhesion to endothelial cells. However *in vitro* studies demonstrated that ELAM-1 primarily supports the adhesion of granulocytes,[17] whereas ICAM-1 and INCAM 110 are involved in the adhesion of lymphocytes and monocytes.[38–40] Thus expression of ELAM-1 in HEVs is expected to be associated with the presence of granulocytes in the cellular infiltrate. This seems to be the case in HD and T NHL, in which a high degree of cellular pleomorphism is typical of the lesion.

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