A New Method for Studying the Selective Adherence of Blood Lymphocytes to the Microvasculature of Human Retina

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Purpose. To develop a sensitive and reproducible technique for measuring the adherence of blood lymphocytes to vessel walls exposed in sections of human retina and for examining the role of lymphocyte and vascular adhesion molecules in these events.

Methods. Cryostat sections of human retina were overlaid with blood lymphocytes from healthy subjects, and experimental conditions were sought by which preferential attachment of the cells occurred to blood vessel walls in the retinal sections. Adherent lymphocytes were identified by staining with methyl green-thionine, and transected blood vessels were identified by their structure and by staining of basement membranes with periodic acid–Schiff. The adherence of enriched preparations of CD4+ (T-helper) and CD8+ (T-cytotoxic) lymphocytes, of interleukin-2 (IL-2)–activated cells, and of lymphocytes from patients with ocular Behçet’s disease was examined. The distribution of adhesion molecules on retinal vessel walls was determined by immunohistochemistry, and the contribution of leukocyte integrins to lymphocyte binding was studied by blocking experiments with monoclonal antibodies.

Results. The optimal selectivity of blood lymphocyte attachment to retinal vessel walls occurred when purified lymphocytes were suspended in culture medium with 10% fetal calf serum and overlaid onto retinal sections for 30 minutes at 23°C with gentle agitation. Under these conditions, 92% of the lymphocytes that adhered to the section were confined to the retinal microvasculature, and CD4+ T cells were more adherent than CD8+ T cells (P < 0.01). Prior exposure of normal lymphocytes to IL-2 enhanced their binding to retinal blood vessels, and lymphocytes from patients with Behçet’s disease showed supranormal vascular adherence (P < 0.005).

Many transected vessels stained positively for CD31; PECAM (mean 62%), CD54; ICAM-1 (mean 73%), CD62E; E-selectin (mean 35%), CD62P; P-selectin (mean 61%), and CD106; VCAM-1 (mean 42%). However, these vascular adhesion molecules occupied <20% of the area of the blood vessel walls. Lymphocyte adhesion to the retinal vessels was more dependent on CD29 (the common chain of the β1 integrins) expression than either CD11a/CD18 or CD49d.

Conclusions. This technique allows measurements to be made of lymphocyte adherence to vascular and nonvascular structures of retina ex vivo. Extension of this approach to the study of leukocyte adherence to sections of pathologic retina may be of clinical and experimental applicability in understanding mechanisms of retinal inflammation. Invest Ophthalmol Vis Sci. 1997;38:2608–2618.

Leukocytes are considered to play prominent pathogenic roles in several of the retinal vasculitides, either by inducing vascular occlusions or infiltrating the retina to initiate tissue damage.¹ The T lymphocyte is the principal infiltrating leukocyte of the retina in patients with various forms of posterior uveitis,² and this cell is important in the initiation, progression, and adoptive transfer of experimental autoimmune uveitis.³,⁴

Lymphocyte entry into tissue is a multistep process controlled by a sequential interaction of cell adhesion molecules with counterreceptors expressed on various components of blood vessel walls.⁵ The initial adhesion to endothelium is dependent on lectin–carbohydr-
lymphocytes to blood vessel walls in sections of retinal

Woodruff1 established a technique that permitted the

The study was conducted in three stages. First, the

Experimental Design

Isolation of Lymphocytes

Mononuclear leukocytes were isolated from 40 ml heparinized blood (10 U/ml) by our standard method.16 Briefly, blood was diluted with an equal volume of 0.9% NaCl and layered onto Lymphoprep (Nycomed, Birmingham, UK) at a ratio of 1 vol Lymphoprep to 2 vols of diluted blood. After centrifugation at 500g for 30 minutes, the mononuclear cell layer was carefully removed and washed twice with Dulbecco’s minimum essential medium (DMEM). Lymphocytes were enriched by suspending mononuclear leukocytes in 2 ml 30% (vol/vol) Percoll solution
(Pharmacia, Biotech, Milton Keynes, UK) and layering onto a Percoll gradient whose dilutions ranged from 40% to 70%. After centrifugation at 400g for 30 minutes, lymphocytes were collected from the resulting 50–60 interface, and the cells were washed twice with DMEM. The purity of the lymphocytes was >95% and the viability of cell preparations >95%, as assessed by Trypan blue exclusion.

Preparation of CD4+ and CD8+ Lymphocytes

Blood lymphocytes were first depleted of their B lymphocytes by incubation with magnetic beads coated with anti-B cell antibodies (anti-CD19, Dynabeads, Dynal, Wirral, UK) at a cell to bead ratio of 1:1 with constant mixing at 4°C for 30 minutes. For the enrichment of CD4+ (T-helper) and CD8+ (T-cytotoxic) subsets, lymphocytes were mixed with Dynabeads coated with either anti-CD8 or anti-CD4 antibodies as described in the above procedure. The percentage of total T cells (CD3) and their CD4+ and CD8+ subpopulations in the initial and final cell preparation was determined using a FACScan flow cytometer (Becton Dickinson, Oxford, UK).

Lymphocyte Activation With IL-2

Lymphocytes, resuspended at 1 X 10^6 cells/ml in DMEM plus 10% fetal calf serum, were incubated with recombinant IL-2 (range, 0.02 to 20.0 U/ml) for 24 hours at 37°C. The cells were then washed in DMEM, resuspended in medium, and applied to retinal sections. Control lymphocytes were incubated for 24 hours in the absence of IL-2.

Adherence Assay

Enriched preparations of lymphocytes were suspended in DMEM with 10% fetal calf serum and 25 mM Hepes, and 50 µl was gently pipetted onto each section with gentle agitation on an orbital shaker (Lackham, Life Sciences Int'l, Basingstoke, UK; 35 rpm/2-cm radius) for 30 minutes over a temperature range of 7°C to 37°C. Nonadherent lymphocytes were removed by gently immersing the slide in phosphate-buffered saline. The sections with their adherent lymphocytes were fixed for 30 minutes in 2% glutaraldehyde in phosphate-buffered saline, and the bound primary antibodies were detected by a 30-minute incubation with bridging antibody (rabbit antimouse immunoglobulin diluted 1:10, DAKO), followed by a further incubation for 30 minutes with a 1:20 dilution of avidin peroxidase complexes (DAKO). Visualization of positive staining was achieved with the use of the substrate naphthol-AS-MX-phosphate and Fast Red (Sigma-Aldrich).

Expression of Adherence Results

To compare the adherent properties of distinct preparations of lymphocytes to blood vessel walls in sections from different retinas, results were expressed as the number of lymphocytes bound per mm² of vessel wall. This was achieved by measuring with an eyepiece graticule the total cross-sectional area of all transected vessels (excluding the lumen) and counting the number of lymphocytes bound to every transected vessel (complete or fragmented). Therefore, the lymphocyte adherence to vessel walls (mm²) equals the total number of lymphocytes adherent to vessel walls divided by the total cross-sectional area of transected blood vessel walls (mm²).

Nonspecific adherence was evaluated by measuring the area of nonvascular tissue and counting the number of adherent lymphocytes that this tissue supported. Therefore, lymphocyte adherence to nonvascular tissue (mm²) equals the total number of lymphocytes adherent to nonvascular sites divided by the total cross-sectional area of nonvascular sites (mm²).

The percentage of lymphocytes bound to the section that selectively adhered to vessel walls was calculated by dividing the number of lymphocytes bound to vessel walls by the number of lymphocytes bound to the whole section, and multiplying by 100.

Immunohistochemical Staining of Adhesion Molecules on Blood Vessel Walls

Retinal tissue sections were stained using the avidin peroxidase–anti-avidin peroxidase method. Briefly, sections were fixed for 10 minutes with 1% paraformaldehyde in phosphate-buffered saline and treated for 20 minutes in a humid chamber at room temperature with normal rabbit serum (Sigma-Aldrich Co., Poole, UK) diluted 1:10 in Tris-buffered saline (pH 7.6) to block nonspecific binding of the second antibody. After washing with Tris-buffered saline, the sections were treated for 30 minutes with a panel of mouse monoclonal antibodies directed against CD54 (RR1/1.1.1, kindly donated by R. Rothlein, Boehringer-Engelheim, Ridgefield, CT), CD106, CD62E, and CD31 (all purchased from Serotec, Oxford, UK), CD62P (Biodesign International, Kennebunk, ME, and an IgGl isotype control (DAKO, High Wycombe, UK). The sections were then washed for 2 minutes in Tris-buffered saline, and the bound primary antibodies were detected by a 30-minute incubation with bridging antibody (rabbit antimouse immunoglobulin diluted 1:10, DAKO), followed by a further incubation for 30 minutes with a 1:20 dilution of avidin peroxidase–anti-avidin peroxidase complexes (DAKO). Visualization of positive staining was achieved with the use of the substrate naphthol-AS-MX-phosphate and Fast Red (Sigma-Aldrich).
Inhibition of Leukocyte–Retinal Vessel Adhesion

To assess the contribution of leukocyte adhesion molecules in the attachment of lymphocytes to retinal blood vessels, lymphocytes were treated with saturating concentrations of monoclonal antibodies against the adhesion molecules CD11a (Serotec), CD18 (DAKO), CD29 (β chain common to the β1 integrins), and CD49d (both Serotec). Isolated lymphocytes (1 × 10⁶ cells) were incubated for 45 minutes at 23°C with 10 μl of antibody diluted in DMEM with 25 mM Hepes and 10% fetal calf serum to a final volume of 100 μl. The lymphocytes were then pelleted by centrifugation, resuspended in the same antibody dilution to 10⁸ cells/50 μl medium, and applied to the retinal sections. In all experiments, lymphocytes were also incubated with an indifferent mouse IgG1 antibody as a negative control.

Statistical Evaluation of Results

Results are expressed as the mean ± standard deviation. The Wilcoxon signed rank test for paired data was used to test the significance of differences between the means of test and control groups. Coefficients of variation were calculated for lymphocyte adhesion to cross-sectional areas of transected blood vessel walls in serial retinal tissue sections.

RESULTS

Selectivity of Lymphocyte Adhesion to Retinal Vessel Walls

The selective adherence of overlaid lymphocytes for retinal vessel walls occurred when 1 × 10⁶ cells were suspended in 50 μl DMEM with 10% fetal calf serum and applied to retinal sections for 30 minutes with gentle agitation (Fig. 1). Although only 0.01% of the overlaid lymphocytes bound to the total section, >96% were confined to the retinal vasculature. Alteration of any of these parameters generally resulted in an increase in the number of lymphocytes adhering to nonvascular tissue. When the assay was performed at 7°C, few of the overlaid lymphocytes bound to the vessel walls, but increasing the temperature to 23°C resulted in a sixfold increase in attachment and a specificity of adhesion of >96% (in other words, the percentage of lymphocytes that adhered to the retinal section but were confined to vessel walls). At 37°C, the selective adherence of lymphocytes was reduced (mean 81% attachment) because more lymphocytes bound to nonvascular tissue.

Since Stamper and Woodruff⁴ reported that fixation of lymphoid tissue before assay preserved tissue structure, sections of retina or overlaid lymphocytes were prefixed with glutaraldehyde or paraformaldehyde. Figure 2 shows that pretreatment of either sections or lymphocytes with paraformaldehyde produced a marked reduction in the number of adherent lymphocytes, and that fixation of lymphocytes with glutaraldehyde also reduced their adherence to vessel walls. The number of lymphocytes adhering to vessel walls in untreated and glutaraldehyde-treated sections was similar (see Fig. 2), but the selectivity of lymphocyte binding to the vasculature was reduced (P < 0.05) by glutaraldehyde fixation (82%) when compared with untreated sections (96%).

In the next stage of our study, we investigated the variability in the adherence properties of vessel walls in serial tissue sections and in sections obtained from different retinas. When lymphocytes from one healthy subject were overlaid onto eight serial sections (7-μm thickness), the mean number of cells binding to vessel walls was 779 ± 152 cells/mm² blood vessel wall, and the coefficient of variation was 15.5%. Figure 3 shows the adherence of lymphocytes from one subject to sections obtained from six retinas. The level of adherence ranged from 361 cells/mm² to 1093 cells/mm² (mean 662 ± 265 cells/mm²), but the specific adherence for blood vessels was always >96%. From these findings, we decided that all comparative adherence analyses of lymphocytes prepared from different subjects or of lymphocytes subjected to different treatments would be undertaken on sections prepared from the same block of retinal tissue. In addition, each adherence result would be the mean value derived from reading eight serial sections.

Vascular Adherence of T-Lymphocyte Subpopulations, of Activated Lymphocytes, and of Lymphocytes From Patients With Ocular Behçet’s Disease

To determine if the adherence of T lymphocytes differed between CD4+ or CD8+ cells, enriched preparations of these subpopulations (>75%) were examined for their ability to bind to retinal blood vessels. Figure 4 shows the results of three experiments in which CD4+, CD8+, and unfractionated lymphocytes from three normal subjects were added to sections prepared from three retinas. In all experiments, the binding of the CD4+ cells to vessel walls was similar to that of unseparated lymphocytes, but the CD8+ cells were always less adherent than the CD4+ cells (P < 0.01). Lymphocyte migration across the blood–retinal barrier is a property of activated rather than resting cells,¹⁷ and therefore we also investigated the adherence properties of IL-2–stimulated lymphocytes. In the experiment illustrated in Figure 5, activation of unfractionated lymphocytes with 0.02 and 0.2 U/ml IL-2 produced a 27% and 25% increase in adhesion, respectively (P < 0.05). The finding that the adherence of lymphocytes treated with high concentrations of IL-2 (200 U/ml) was similar to that of untreated cells suggests that there is a critical relation between...
FIGURE 1. Selectivity of lymphocyte attachment to blood vessel walls in tissue sections of human retina. Serial sections of retina were either untreated or overlaid with blood lymphocytes as described in the text. The transected blood vessels in an untreated section (A) have no associated lymphocytes, but after incubation with blood lymphocytes (B), the same vessel now supports the binding of several lymphocytes. (C) In a section of another retina, it is the blood vessel wall that selectively supports the attachment of overlaid lymphocytes. Blood vessel walls were identified by staining with periodic acid–Schiff and lymphocytes by staining with methyl green-thionine. Adherent lymphocytes appear as small round cells with intensely staining dark-blue nuclei lying above the plane of the section, and blood vessels are surrounded by a magenta-stained basement membrane. (Magnification, ×400)

IL-2 receptor occupancy and the generation of intracellular signals that promote cellular adhesiveness. Similar results were obtained in two further experiments. The above experiments demonstrate that T-lymphocyte attachment to retinal vessel walls is preferentially mediated by CD4+ cells and that adhesion is enhanced after cell activation.

Features common to Behçet’s disease include a high preponderance of activated lymphocytes in the circulation18 and the infiltration of the retina by blood lymphocytes.19 A comparative study was undertaken of the adherent properties of blood lymphocytes from 13 patients with Behçet’s disease and 13 healthy age- and sex-matched control subjects. In the experiments illustrated in Figure 6, lymphocytes from Behçet’s patients exhibited a greater adhesion to vessel walls than the paired control cells (mean 48% increase; P < 0.005), and all preparations showed a similar degree of binding specificity for the retinal vasculature (>96%). Only one patient (experiment 4) did not have active disease, but his lymphocytes were also more adherent than the corresponding control cells.

To determine whether the increased adhesiveness of lymphocytes from patients with Behçet’s disease was a feature common to autoimmune disorders, binding studies were also undertaken on lymphocytes from five
Lymphocyte Adherence to Retinal Vessel Walls

SECTION PHE- FIXED
LYMPHOCYTES PRE- FIXED
GLUT
PFA

FIGURE 2. Effect of fixation on lymphocyte adhesion to retinal vessel walls. Lymphocyte or serial retinal sections were either untreated (−) or fixed before the adherence assay with either 2% glutaraldehyde (GLUT) or 1% paraformaldehyde (PFA) in phosphate-buffered saline for 30 minutes at room temperature as indicated in the figure. At the end of the assay, all sections with adherent lymphocytes were fixed for 30 minutes in 2% glutaraldehyde–phosphate-buffered saline. Results are expressed as the number of lymphocytes adhering to vascular (white blocks) or nonvascular (dark blocks) tissue. Each result represents the mean value from reading eight serial sections (7-μm thickness from one retina), and vertical lines denote the standard deviation of the mean. Lymphocytes were overlaid onto the sections for 30 minutes at 23°C with gentle agitation. Prefixation of lymphocytes with GLUT or PFA markedly reduced their binding to vessel walls. Lymphocyte attachment was also impaired after prefixation of sections with PFA. GLUT treatment of sections enhanced the number of lymphocytes adhering to nonvascular tissue.

The next stage of the investigation determined if the abnormal adherence of Behçet's lymphocytes for vessel walls could be reproduced with sections from nonocular tissue. Blood lymphocytes from four patients with active Behçet's disease and four healthy controls were simultaneously overlaid onto sections of retina and human brain. The patients' lymphocytes were always more adherent than control cells in their binding to retinal vessel walls, whereas supranormal adherence of Behçet's lymphocytes to cerebral vessel walls was seen in only two of four experiments (data not shown).

Adhesion Molecules and the Binding of Lymphocytes

Vessel walls in retinal tissue from seven donor eyes were examined by immunohistochemistry for their expression of CD31, CD54, CD62E, CD62P, and CD106.

patients with active rheumatoid arthritis and two patients with Sjögren's syndrome. The lymphocytes from these patients behaved similarly to those of control cells in their attachment to retinal vessel walls (mean 1863 ± 398 versus 2024 ± 872 cells/mm² vessel wall).

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Adhesion Molecules and the Binding of Lymphocytes

Vessel walls in retinal tissue from seven donor eyes were examined by immunohistochemistry for their expression of CD31, CD54, CD62E, CD62P, and CD106.

Most of the vessels in these sections were positive for CD31 (mean 62%), CD54 (mean 73%), and CD62P (mean 61%), and a minority expressed CD62E (mean 35%) and CD106 (mean 42%) (Table 1A). Some of the adhesion molecules were present on the luminal

FIGURE 3. Variability in the adhesiveness of retinal vessel walls. Tissue sections from six retinas were overlaid with blood lymphocytes from one healthy subject. Adhesion is expressed as the number of lymphocytes adhering to vascular (white blocks) or nonvascular (dark blocks) tissue. Each block represents the mean result of eight serial sections; vertical lines denote the standard deviation of the mean. Lymphocytes were overlaid onto sections for 30 minutes at 23°C with gentle agitation. The selectivity of lymphocyte adhesion for vessel walls in sections from all retinas was >96%.

FIGURE 4. Adherence properties of CD4- and CD8-enriched lymphocytes. Unfractionated lymphocytes (dark blocks), CD4-enriched lymphocytes (striped blocks), and CD8-enriched lymphocytes (white blocks) from three healthy subjects were overlaid onto serial retinal sections from one retina. Adhesion is expressed as the number of lymphocytes bound per unit area of vessel wall. The mean (± standard deviation) percentage of CD4 and CD8 cells in the enriched preparations was 78% and 74%, respectively. In each experiment, the CD4-enriched cells were more adherent than the CD8 cells (*P < 0.01).
activated against CD11a, CD18, CD29, or CD49d. Figure 7 shows that the anti-CD29 and anti-CD18 antibodies produced a mean 71% inhibition ($P < 0.001$) and 18% inhibition of adhesion ($P < 0.05$), respectively. The anti-CD49d antibody did not modify adhesion, and although the anti-CD11a induced a mean 17% inhibition of adhesion, this effect was not significant. The findings demonstrate that CD29 is an important integrin for promoting lymphocyte attachment to vessel walls in retinal sections.

**DISCUSSION**

This study shows that blood vessels in sections of human retina support the attachment of overlaid lymphocytes. Although only a tiny proportion of the lymphocytes adhered to the sections (1:10,000), >96% of the cells bound were confined to blood vessel walls. The high selectivity of lymphocytes for vessel walls was reproduced in sections obtained from several retinas. Adherent lymphocytes were seen as small, intensely staining cells in sections stained with methyl green-thionine, and blood vessels were recognized by staining of their basement membrane with periodic acid–Schiff. Lymphocyte binding was an active process in that prefixation of the cells or retinal tissue with paraformaldehyde or glutaraldehyde impaired their interaction with vessel walls, and occasionally there was considerable variability in their distribution from one retina to another. Examination of serial sections revealed that CD54 was often coexpressed with CD31 and CD62E, but overall no consistent pattern was established regarding the simultaneous expression of adhesion molecules.

At an early stage of the study, it was apparent that the expression of an adhesion molecule in a blood vessel was confined to a small part of its total area. Consequently, four of the retinal samples described in Table 1A were reanalyzed and the results presented as the percentage of total area of vessel walls expressing adhesion molecules. This was achieved by measuring with an eyepiece graticule the total cross-sectional area of every fragmented and complete transected blood vessel wall (excluding the lumen) that was exposed in the retinal section. Using a similar procedure, measurements were made of the areas of vessel walls stained by antiadhesion molecule antibodies. Table 1B shows that only small areas of blood vessel walls were occupied by the adhesion molecules investigated in the study. For example, although CD54 was identified on most blood vessels, its expression was confined to only 19% of their total surface area.

To assess the contribution of lymphocyte adhesion molecules in promoting adhesion, lymphocytes from healthy subjects were treated, before their introduction into the adhesion assay, with antibodies di-
TABLE 1. Distribution of Adhesion Molecules on Retinal Blood Vessel Walls

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<tr>
<th>Retina</th>
<th>IgGl</th>
<th>CD31</th>
<th>CD54</th>
<th>CD62E</th>
<th>CD62P</th>
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<td>A. Percentage of vessels expressing adhesion molecules</td>
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<td>B. Percentage of total area of vessel expressing adhesion molecules</td>
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Retina tissue from seven eyes were examined for their expression of vascular adhesion molecules. In (A) results (mean ± SD) are presented as the percentage of blood vessels expressing adhesion molecules, and in (B) results from four of the samples are presented as the percentage of total area of retinal vessel walls occupied by these same molecules. Serial sections from each of the seven retinas were also treated with a control antibody of the same isotype (IgG-1) as that of the anti-adhesion molecule antibodies. ND = not done.
both activated T cells and soluble IL-2 receptors in the circulation, and because T lymphocytes make up the main population of infiltrating lymphocytes in the posterior chamber of the eye. Due to variations in the adherence properties of vessel walls between different retinas, care was taken to ensure that lymphocytes from one control subject and one patient with Behçet's disease were always allocated to every other section prepared from the same block of retinal tissue. Furthermore, each result was the mean adherence value from reading eight sections. A similar procedure would be necessary for the introduction of pathologic tissue into the adherence assay and for the study of leukocytes from patients with other forms of ocular disease, either in point-prevalent or longitudinal studies.

The additional observation that blood lymphocytes from patients with rheumatoid arthritis and with Sjogren's syndrome bound to retinal vessel walls in similar numbers to those of healthy controls suggests that the supranormal adherence properties of Behçet's lymphocytes may not be a general characteristic of lymphocytes from patients with autoimmune disorders. That the enhanced binding of lymphocytes from patients with Behçet's disease to vessel walls was more apparent with retinal tissue than with brain tissue could arise from the preferential interaction of lymphocytes with a distinct group or combination of vascular adhesion molecules on retinal vessels or from a degree of organ selectivity. Addressing the latter consideration fully would require an extensive comparative examination of the ability of Behçet's lymphocytes to adhere to vessel walls in sections from several organs (e.g., lung, kidney, skin).

By immunohistochemistry, we demonstrated that CD54, which is constitutively expressed on resting endothelial cells and upregulated by the action of inflammatory cytokines, was present on the majority of vessel walls exposed in the retinal sections. High levels of CD54 are present in the eyes of animals with experimentally induced uveitis, and increased expression of CD54 appears to precede retinal inflammatory cell infiltrates. CD54 is the ligand for the leukocyte integrins CD11a/CD18 and CD11b/CD18, and evidence for a pathogenetic role in intraocular inflammation is provided by the demonstration that antibodies directed against CD54 or CD11a/CD18 prevent or inhibit experimentally induced uveitis. Many blood vessels in our sections were also stained by antibodies directed against CD106 and CD62E. Closer examination of the retinal vessels revealed that all the adhesion molecules, including CD54, were confined to small areas (<20%) of exposed blood vessel walls. Such a limited expression of vascular adhesion molecules would be in keeping with the normally restricted passage of lymphocytes across the blood-retinal barrier. Adhesion molecules are highly expressed on vessel walls in areas of inflammatory lesions, as seen in multiple sclerosis and rheumatoid arthritis, and similar findings are likely to apply to the retinal vasculature in inflammatory disorders of the eye, particularly because lymphocytes with a high expression of CD11a/CD18 are located adjacent to CD54+ cells in the retinas of patients with posterior uveitis. Thus, in comparison with normal retina, blood vessel walls exposed in tissue sections from patients with retinal inflammation are likely to support the adhesion of larger numbers of overlaid lymphocytes. Whether the selective accumulation of leukocyte populations within retinal inflammatory infiltrates arises from a recognition of distinct adhesion molecules at the level of the blood-retinal barrier could be investigated by combining the in situ adherence assay with immunostaining techniques.

Lymphocyte-endothelial interaction is governed by leukocyte integrins such as CD11a/CD18 and CD49d recognizing corresponding ligands (CD54 and CD106, respectively) on the endothelial surface. In our antibody blocking studies, lymphocyte adherence to retinal vessel walls was dependent on the expression of CD18 but not that of CD11a and CD49d. Moreover, lymphocyte attachment was highly dependent on CD29, whose expression is increased on subsets of CD4+ memory cells and also on CD4+ T cells that undergo transendothelial migration. Because CD29 is the β chain common to the β1 family of leukocyte integrins that recognize collagen, laminin, and fibronectin, it is unclear whether in our assay lymphocyte binding to vessel walls is caused by attachment to endothelial cells or by recognition of either the extracellular matrix or traces of its proteins expressed on endothelial cells. This question could be addressed by functional blocking studies with antibodies directed against endothelial adhesion molecules and by performing the current adhesion assay in parallel with the study of lymphocyte adherence to monolayers of retinal-derived endothelial cells and to purified proteins of the extracellular matrix. An important supplement to these investigations would be the ultrastructural localization of extracellular matrix proteins in retinal vessel walls. All of the β integrins and most of the α subunits are highly expressed on human retinal vessel walls, introducing the intriguing consideration that these molecules may also have a prominent role in promoting lymphocyte adhesion.

Application of the frozen-section assay to experimental autoimmune uveitis would be advanta-
Lymphocyte Adherence to Retinal Vessel Walls

Key Words

adherence, lymphocytes, microvasculature, retina, Behçet’s disease

References


