Focal topographic changes in inflammatory microcirculation associated with lymphocyte slowing and transmigration

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West, Charles A., Chufa He, Mei Su, Timothy W. Secomb, Moritz A. Konerding, Alan J. Young, and Steven J. Mentzer. Focal topographic changes in inflammatory microcirculation associated with lymphocyte slowing and transmigration. Am J Physiol Heart Circ Physiol 281: H1742–H1750, 2001.—Microcirculation is the primary mechanism for delivering lymphocytes to inflammatory tissues. Blood flow within microvessels ensures a supply of lymphocytes at the blood-endothelial interface. Whether the structure of the inflammatory microcirculation facilitates lymphocyte transmigration is less clear. To illuminate the microcirculatory changes associated with lymphocyte transmigration, we used intravital videomicroscopy to examine the dermal microcirculation after application of the epicutaneous antigen oxazolone. Intravascular injection of fluorescein-labeled dextran demonstrated focal topographic changes in the microcirculation. These focal changes had the appearance of loops or hairpin turns in the oxazolone-stimulated skin. Changes were maximal at 96 h and coincided with peak lymphocyte recruitment. To determine whether these changes were associated with lymphocyte transmigration, lymphocytes obtained from efferent lymph of draining lymph nodes at 96 h were fluorescently labeled and reinjected into inflammatory microcirculation. Epifluorescence intravital video microscopy demonstrated focal areas associated with lymphocyte slowing and occasional transmigration. In contrast, focal loops and lymphocyte slowing were rarely observed in the contralateral control microcirculation. Results suggest that structural adaptations in inflammatory microcirculation represented by focal topographic changes may contribute to regulation of tissue entry by recirculating lymphocytes.

Lymphocyte migration from the microcirculation into extravascular tissue is a central process in host defense. In normal circumstances, most recirculating lymphocytes remain in the blood. A generally accepted estimate is that <10% of recirculating lymphocytes migrate out of the blood and percolate through noninflamed tissue (14, 15, 30). This baseline lymphocyte migration is believed to provide an immune surveillance function. In inflammatory conditions, such as acute transplant rejection, lymphocyte traffic out of the bloodstream can increase more than 10-fold (39). These migratory lymphocytes accumulate in perivascular tissue and are responsible for amplifying lymphocytic inflammation.

Attempts to define the mechanisms regulating lymphocyte exit from the inflammatory microcirculation have focused on receptor and counter-receptor interactions between lymphocytes and vascular endothelial cells (10, 27, 28, 32, 33). Evidence for cell adhesion molecules providing a general mechanism for regulating tissue entry has been suggested by the heterogeneity of receptor expression in tissues, inducible expression of some of these molecules by inflammatory cytokines, and functional evidence for participation of these molecules in discriminating receptor and counter-receptor interactions (reviewed in Refs. 7 and 36). Although in vitro perfusion chamber experiments have confirmed the function of cell adhesion molecules under conditions of flow (18, 26), the relative contribution of microcirculatory hemodynamics to lymphocyte transmigration in vivo is less clear. Histological observations in pathological conditions, such as psoriasis, have suggested significant structural changes in the microcirculation (3, 5). Although these changes have potential functional implications for lymphocyte transmigration, lymphocyte flow patterns in antigen-stimulated tissue have been difficult to obtain. A major obstacle in direct examination of inflammatory microcirculation has been the thickened tissue and inflammatory edema present at peak lymphocyte recruitment (19, 37, 38).

In this report, we studied physiological changes in the microcirculation associated with lymphocyte recruitment after stimulation with the epicutaneous antigen oxazolone. Epilumination intravital videomicroscopy used to examine the inflammatory microcirculation at peak lymphocyte recruitment demonstrated focal topographic changes in the antigen-stimulated microcirculation associated with lymphocyte slowing and transmigration.
These results suggest that microcirculatory adaptations in inflammatory tissue contribute to regulation of tissue entry by recirculating lymphocytes.

METHODS

Animals. Randomly bred sheep ranging in weight from 25 to 35 kg were used in these studies. If there was any gross or microscopic evidence of dermatitis, sheep were excluded from the analysis. Sheep were given free access to food and water. Care of animals was consistent with guidelines of the American Association for Accreditation of Laboratory Animal Care (Bethesda, MD).

Antigen stimulation. The sheep ear and neck region was sheared bilaterally, and the lanolin was removed with an equal mixture of ether (JT Baker; Phillipsburg, NJ) and ethanol (AAPER; Shelbyville, KY). The antigen, a 5% solution of 4-ethoxymethylene-2-phenyl-2-oxazolin-5-one (oxazolone; Sigma; St. Louis, MO) (11), was sprayed onto the ear and a localized region of the neck as a 4:1 oxazolone/olive oil mixture using a syringe and 23-gauge needle (16). A vehicle-only control was applied to the contralateral skin.

Lymph duct cannulation. The prescapular lymph node, with a lymphatic drainage basin including the ear and neck (13), was used for all effenter lymph duct cannulations. After general endotracheal anesthesia and sterile surgical preparation, an incision was placed in the jugular furrow 5 cm cephalad to the suprasternal notch (12, 17). The effenter lymph duct was cannulated with a heparin-bonded polyurethane catheter (Solo-Cath, CBAS-C35; Setters Life Sciences, Somerville, NJ) or 250-ml sterile plastic bags (Abbott; North Chicago, IL). Each collection bag or tube contained 200 international units of heparin, 2,000 international units of penicillin (Cellgro, Mediatech; Herndon, VA), and 2,000 µg of streptomycin (Cellgro).

Fluorescent cell tracers. Lymph cells were labeled with succinimidyl esters of the mixed isomer preparation of 5-(and-6)-carboxytetramethylrhodamine [5(6)-TAMRA,excitation 540 nm/emission 565 nm; Molecular Probes, Eugene, OR]. The dye was aliquoted as a 0.1 M solution in anhydrous dimethylsulfoxide and stored at −70°C. Before labeling, the lymph cells were washed three times in DMEM with 2,000 mg/l glucose (Sigma) and resuspended in PBS containing 25 µl of the stock 5(6)-TAMRA fluorescent dye. The cells were incubated for 15 min at room temperature and washed in cold DMEM. The cells were resuspended in room-temperature PBS at 0.7 to 5.0 × 10^7 cells/ml before injection of 5 ml over a 12-s injection period.

Fluorescent plasma marker. FITC-dextran (Sigma) 500,000 molecular weight was dissolved in saline (15 mg/ml) and not exposed to light before injection. The FITC-dextran was injected into the arterial cannula using a 20-ml syringe.

Arterial cannulation. Similar cannulation techniques were used for both the antigen-stimulated and control carotid arteries. The common carotid artery was exposed using an incision in the jugular furrow 7 cm cephalad to the suprasternal notch. A 5-0 monofilament (Prolene, Ethicon; Somerville, NJ) purse-string suture was placed in the carotid adventitia. A heparin-bonded polyurethane catheter (Solo-cath, CBAS-C35; Setters Life Sciences) was passed through a 14-gauge catheter (Insysite intravascular catheter; B-D Infusion Therapy Systems, Sandy, UT) into the carotid artery. The catheter was secured using the purse-string suture and surgical glue.

The catheter was tunneled through subcutaneous tissue to the dorsum of the neck and secured. The catheter was fitted with a sub-nose adapter and flushed with heparinized saline (100 U/ml) (Elkins-Sinn; Cherry Hill, NJ). The carotid cannulas were used for both injection and hemodynamic monitoring.

Monoclonal antibodies. The sheep T lymphocyte monoclonal antibodies (MAB) anti-CD4 MAB 17D (24), and the anti-CD8 MAB IL-A51 (9) have been previously described. The anti-CD29 MAB FW4 has also been previously reported (23, 25). The anti-CD40 MAB M2/61 was produced and characterized as previously described (20–22). The anti-CD72 MAB DU-87, characterized by immunohistochemistry and flow cytometry, demonstrated staining of a subset of B lymphocytes. Affinity-purified MAB or cultured supernatants were used at fivefold saturating concentrations in all experiments.

Immunofluorescence and flow cytometry. Cells were washed twice with PBS containing 2.5% fetal calf serum and 0.02% sodium azide. Approximately 10^6 cells were incubated on ice for 30 min with an excess concentration of monoclonal antibody. Cells were washed twice and stained with fluorescein-conjugated goat Fab’/2-anti-mouse IgG antibody (Southern Biotechnology; Birmingham, AL) diluted 1:10. The cells were incubated on ice for another 30 min. After three washes, cells were fixed in 1% paraformaldehyde and analyzed by flow cytometry using a Coulter Epics XL flow cytometer with Expo 2.0 software (Miami, FL). The flow cytometry data were collected at room temperature and exported to the Microsoft Excel (Redmond, WA) spreadsheet for data analysis using WinList 4.0 (Verity; Topsham, ME). The flow cytometry experiments were calibrated daily using Sphero Rainbow Calibration Particles (SpheroTech; Libertyville, IL).

Intravital microscopy system. The basic microscope body was the Nikon substage metallurgical/industrial microscope fitted with a CF infinity-connected optical system with custom extenders to accommodate the additional length of the microscope window (MicroVideo Instruments, Arrow, MA). The microscope system is mounted on a floor-based adjustable height microscope stand with rotational axes in two directions to permit gentle apposition to the ear. The custom-designed epillumination system had a super-high-pressure mercury lamphouse for the delivery of light through the optical system as bright field/dark field or fluorescence illumination. The fluorescent filter block used in these experiments was an orange (DM 560 nm) filter. The Nikon epiparamatic objectives were ×10 and ×20 magnification.

An intravital microscopy window machined from titanium (MicroSurg, Boston MA) was directly attached to the microscope stand to limit vibration. The window’s 0.59” internal diameter cylinder was designed to accommodate the Nikon optical system. The tissue surface of the window was composed of a 0.635” flange surrounding the window lens. Within the flange, two concentric 2.5-mm vacuum galleries provided tissue apposition to the lens surface without compression of the tissue and with minimal circulatory disturbances. The window lens (1.275” × .059”) was custom designed with ultraviolet-grade fused silica (Kreischer Optics; McHenry, IL). The surfaces were optically polished to retical quality (20:10 scratch/dig per MIL-0-13830A). Mounted to the microscope window was a custom-designed machined acrylic stage that supported the ventral surface of the ear and ensured stable tissue apposition.

The camera was a Dage-MTI CCD-72 series high resolution charge-coupled device (CCD) imager and high performance analog processor with 768 × 493 active elements and 570 TVL resolution (Dage-MTI, Michigan City, IN). The
microscope light level camera gain and offset were set to use the full dynamic range of the video analog contrast settings. The image was intensified using a GenIIsys optically coupled image intensifier with 106 dynamic range (Dage-MTI).

**Image analysis.** The intravital videomicroscopy images were recorded on a Panasonic model AG-675OA S-VHS recorder (Secaucus, NJ) (30 frame/s) with horizontal resolution of 400 lines. Time-base correction was performed using a TBC III board (VT2500, Digital Processing Systems; Florence, KY). Video of the recorded images was processed through a M-Vision 1,000 PCI bus frame grabber (Mutech; Waltham, MA) in a Pentium III (700 MHz, 256 megabyte RAM) computer running the MetaMorph Imaging System 4.0 (Universal Imaging; Brandywine, PA) under Microsoft Windows NT. Image stacks were routinely created from 12-s to 5-min video sequences. The image stacks were processed with standard MetaMorph filters. After routine distance calibration and thresholding, the “stacked” image sequence was measured using the MetaMorph object tracking and integrated morphometry applications.

Topographic changes in the microcirculation were assessed by intravital microscopy after fluorescent plasma marker injection. Representative images at the peak of the fluorescence increase were captured and exported for further analysis. A ×400 200-μm image grid overlay was used to define a microscopic field. Two observers blinded to the treatment condition assessed topographic changes within each microscopic field. Interobserver variation was small and repeat sampling demonstrated highly significant reproducibility. The images of the topographic changes, defined by both plasma markers and dark field microscopy, were digitally combined with the cell migration path to provided spatial correlation.

**Morphometric analysis including apparent cell volume was obtained from individual images within the stack. Apparent cell volume was defined as the volume of a sphere having equatorial cross-sectional area equal to that of the cell. Data were logged into a Microsoft Excel 2000 spreadsheet by dynamic data exchange to facilitate further analysis.**

**Tracking cell movement.** Fluorescence labeling permitted identification of relevant cells for tracking and an objective measure of cell location. After routine distance calibration,
Metamorph object tracking (Universal Imaging) was used to determine the x-y centroids of the lymphocytes and track their displacement through the planes in the source image stack. For displacement reference, the algorithm used the location of the cell at its first position in the track. Each cell was imaged as a disk covering many pixels. The disk was imaged with high contrast and its position was determined with subpixel accuracy. The image of the cell was tracked using a cross-correlation, centroid-finding algorithm to determine the best match of the cell position in successive images. The resulting measures included the x and y coordinates, velocity of the cell, and absolute angle of each segment of the cell path. Absolute angles were measured from an arbitrary zero point at the 9 o’clock position. Angles were measured as ±180°. Lymphocyte transmigration was defined as a cell migrating out of the vessel path and remaining stationary for the remainder of the recording period.

Statistical analysis. The migratory data were based on multiple comparisons of paired data by Student-Newman-Keuls or Mann-Whitney test for nonparametric analysis of variance. Data were expressed as means ± SD. Significance level for the sample distribution was defined as P < 0.05.

RESULTS

Topographic changes in microcirculation. Microcirculation of the sheep ear is composed of a superficial venous plexus (SVP) and a superficial arterial plexus (SAP) (Fig. 1). Histological examination of the oxazolone-stimulated ear demonstrated neutrophil infiltration into the tissues between 24 and 48 h, with a peak accumulation of lymphocytes near the SVP at 96 h after antigen stimulation (38). Dark-field intravital microscopy of the ear at 96 h demonstrated multiple occurrences of highly characteristic structures in the SVP, consisting of distended, tortuous vascular loops (Fig. 2, A and B). The prominence of these focal areas directly correlated with the time course of lymphocyte recruitment (38). Also, similar qualitative changes in the control ear were infrequently observed (not shown). To quantify the changes in the antigen-stimulated and control microcirculations, fluoresceinated dextran was used as a plasma marker (Fig. 2, C and D). With the use of a ×200 400-μm image grid to define a microscopic field, an analysis of 42 microscopic fields in six sheep demonstrated that the observed topographic changes were significantly more prevalent in the antigen-stimulated ear than in the contralateral control ear (3.84 ± 0.86 vs. 0.69 ± 0.61 changes per field; P < 0.001).
Time-location map clusters. To determine whether the focal topographic changes observed in the SVP were associated with lymphocyte recruitment, lymphocytes were obtained from the prescapular lymph node draining the oxazolone-stimulated ear and neck region. Long-term efferent lymph cannulations were maintained in 12 sheep. Flow cytometry of the lymphocytes obtained from the efferent lymph demonstrated that most cells were CD4\(^+\) or CD8\(^+\) T lymphocytes with substantially fewer B lymphocytes or monocytes as reflected by CD40 and CD72 staining (Fig. 3). These efferent lymphocytes were labeled with fluorescent dye and injected into the feeding arterial circulation of the ear. Although focal topographic changes could be visualized in dark-field illumination (Fig. 4A), epifluorescence videomicroscopy was used to track the movement of the injected lymphocytes in the ear microcirculation (Fig. 4, B–E). The location of the fluorescent lymphocyte centroid was recorded on each frame (30 frames/s) with the cumulative overlay reflecting the migratory path of the lymphocyte (Fig. 4F). The path of the lymphocyte represented intravascular migration based on 1) the entry and exit velocities of the labeled cells and 2) digital overlays of cells flowing through a given vascular segment.

To avoid tracking errors, simple time-location maps were used (Fig. 5, A and B). The x-y locations of the fluorescent lymphocytes were recorded on each frame (33-ms intervals; 360 total frames) throughout the typical 12-s injection period. The coordinates were then combined to create the time-location map for each injection. The time-location maps of the oxazolone-stimulated, microcirculation-demonstrated discrete clusters (Fig. 5A) were not seen in the control microcirculation (Fig. 5B). When digitally overlaid on dark-field images of the microcirculation, these cluster regions provided a map of the focal topographic changes in the microcirculation. Clusters defined the site of periodic lymphocyte transmigration, which supports the physiological importance of these regions (Fig. 5A).

Cluster regions also provided a functional measure of focal topographic changes in the microcirculation.
With the use of the ×200 400-μm image grid to define a microscopic field, the frequency of cluster regions was assessed over the 6-day inflammatory response (Fig. 6). Tissue edema present 24 h after antigen stimulation limited the reliable assessment of lymphocyte movement. With resolution of the tissue edema, the frequency of lymphocyte “tethering” in the microcirculation increased. The number of cluster regions per microscopic field was maximal 96 h after oxazolone stimulation. At 144 h after antigen stimulation, cluster regions were infrequently observed. The time course of the topographic changes closely correlated with the actual recruitment of lymphocytes into the antigen-stimulated tissue (38).

Lymphocyte velocity. Lymphocyte velocity in a typical cluster region was calculated as the linear distance traveled during the recording intervals (Fig. 7). Because the cluster regions were preselected for areas of lymphocyte slowing, and few cluster regions were observed in the control circulation, velocity comparisons were limited to the entry and exit segments of the microcirculation after the ipsilateral carotid artery injection of 10⁷-labeled lymphocytes. Velocities were calculated as the linear displacement of the cell between frames (33-ms recording interval). Ten sequential lymphocytes (each represented by a different symbol) and their aggregate mean velocity (line), are shown.

Fig. 5. Time-location map of the oxazolone-stimulated (A) and control (B) microcirculations after a single injection of fluorescently labeled lymphocytes. The maps represent the continuous acquisition of 360 images, obtained at 33-ms intervals, subsequent to the ipsilateral carotid injection of 10⁷-labeled lymphocytes. Lymphocyte position in each image was plotted on an arbitrary 200 μm × 200 μm grid. A: continuous viewing of the video sequence suggested that cell movement was from right to left. The arrow shows a lymphocyte that appeared to migrate out of the microcirculation and persisted without movement for the remainder of the recording period. B: time-location map of the identical region of the contralateral control ear after ipsilateral carotid injection of the same number of labeled lymphocytes.

Fig. 6. Time course of the frequency of cluster regions observed in the SVP of the antigen-stimulated sheep ear. The ear was stimulated with epicutaneous oxazolone at time 0 and observed by intravital microscopy from 48 h to 144 h. Each time point represents 3–5 sheep. With the use of a ×200 400-μm image grid to define a microscopic field, time-location maps based on the continuous acquisition of 360 images were used to define the cluster regions. The mean number of cluster regions is shown per microscopic field. Error bars reflect one standard deviation.

Fig. 7. Cell velocity changes as individual lymphocytes pass through cluster regions of the microcirculation. Videomicroscopy recordings of a cluster region of the microcirculation were obtained after the ipsilateral carotid artery injection of 10⁷-labeled lymphocytes. Velocities were calculated as the linear displacement of the cell between frames (33-ms recording interval). Ten sequential lymphocytes (each represented by a different symbol) and their aggregate mean velocity (line), are shown.
cluster. Also, the entry segments in many time-location map clusters were abbreviated relative to the exit segments suggesting that the cells were entering from the SAP. A sample of 16 clusters in four sheep demonstrated comparable velocities of the entry (mean, $2.21 \pm 0.8 \mu m/ms$) and exit (mean, $2.83 \pm 0.63 \mu m/ms$) segments. A typical cluster demonstrated nearly a 10-fold reduction in lymphocyte velocity (mean, $0.235 \pm 0.169 \mu m/ms$) in the region of maximal slowing. Lymphocyte velocity was largely independent of the estimated cell volume (Fig. 8).

**Angle of the migratory path.** The migratory path of individual lymphocytes (Fig. 4F) suggested that the focal slowing was associated with “hairpin turns” or loops in the SVP. To investigate this possibility, the angle of the migratory path was examined in 24 clusters in six sheep. For each cluster, the angle of sequential segments of the migratory path was calculated as illustrated in Fig. 9. Because the longer entry and exit segments disproportionately reflected the two-dimensional vascular network of the ear, an abbreviated entry and exit path consisting of only three points was examined. With the use of this approach, the mean the angle between the entry and exit segments of the 24 clusters was $10 \pm 16^\circ$.

**DISCUSSION**

In this study, intravital microscopy demonstrated focal topographic changes in oxazolone-stimulated sheep skin. The prominence of these focal topographic changes was temporally associated with lymphocyte migration into the oxazolone-stimulated skin; the changes were maximal ~4 days after antigen stimulation. The injection of fluorescent plasma markers and fluorescently labeled lymphocytes suggested that these topographic changes were inflammation-associated adaptations of preexisting vascular loops. Their association with lymphocyte slowing and transmigration suggested the function of these adaptive changes. In contrast to the oxazolone-stimulated ear, the contralateral control microcirculation demonstrated infrequent vascular loops and few regions with demonstrable lymphocyte slowing. These results suggest that structural adaptations in the inflammatory microcirculation, represented by these focal topographic changes, may contribute to the regulation of tissue entry by recirculating lymphocytes.

The nature of the focal topographic changes observed in the oxazolone-stimulated skin remains unclear. The interval of several days between the administration of the antigen and the observed changes suggests that cellular proliferation, and not simply protein synthesis, is required for the changes in the microcirculation. This speculation is supported by the anatomic observations in normal skin and human disease states (2–4, 29). Several authors have noted the existence of “looping” vessels in the normal human papillary dermis (4, 6, 29). These vessels appear to be more prominent in diseases such as psoriasis (5). Also consistent with these histologic observations, intravital microscopic examination of the control microcirculation suggested low-level, intermittent perfusion of superficial capillaries in the normal skin. The fleeting perfusion of these noninflamed vessels precluded their sustained visualization by either dark-field or epifluorescence intravital microscopy; however, their location was consistent with the larger vessels seen in the oxazolone-stimulated skin. The looping vessels in the antigen-stimulated skin reflected a similar periodicity (~80 μm), but were substantially more prominent.

Although the inflammatory process was stimulated by oxazolone, the anatomic separation of the epidermis

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Fig. 8. Mean velocity of lymphocytes passing through the cluster regions of the microcirculation as a function of their apparent cell volume. After the ipsilateral carotid artery injection of $10^7$-labeled lymphocytes, intravital microscopy recordings were obtained in a representative cluster region. Mean velocity was calculated as the mean velocity of each cell during the 200-ms interval of maximal slowing. The cross-sectional area of each cell was measured by digital morphometry. The apparent cell volume was defined as the volume of a cell that would have that equatorial cross-sectional area.

Fig. 9. The difference in the entry and exit path angle of migratory lymphocytes. The absolute angle reflected the path taken by individual lymphocytes from frame to frame. Angles were arbitrarily measured from the 9 o'clock position and ranged from 0 to ±180 degrees. In this sequence of eight consecutive lymphocytes, the entry path (open triangle) and exit path (open square) were distinguished from lymphocytes in the cluster region (solid circle) by cell velocity.
and the focal vascular changes makes it unlikely that epicutaneous antigen was the direct cause of these changes. Similarly, the microvascular changes appeared to facilitate lymphocyte recruitment suggesting that migrating lymphocytes were not responsible for the initial changes in the microvessels (8). More likely, we suspect that dendritic cells or other resident immune surveillance cells were responsible for triggering the initial changes in the microvasculature (34, 35). It is plausible that lymphocytes, after their initial recruitment, contributed to the evolution and regulation of the microvascular changes (7).

It is theoretically possible that these focal topographic changes were unique to sheep; that is, lymphocyte recruitment into the sheep ear involves a mechanism distinct from other organs or species. We consider this possibility unlikely because the histological pattern and time course of the response to oxazolone was identical to that reported in other species (1, 16, 31).

Several anatomic advantages of the sheep model facilitated these studies. First, the sheep ear provided a simplified vascular network composed of a dorsal and ventral plexus separated by an avascular ear cartilage. The ventral microcirculation provided consistent control for histologic studies and the contralateral ear provided a control for intravital microscopy studies. Second, the anatomy of the sheep ear ensured that it was readily accessible to intravital microscopy while the animal was spontaneously ventilating. General anesthesia and surgical manipulation was unnecessary for serial intravital microscopy. Third, recirculating lymphocytes obtained from the draining prescapular efferent lymph, and reflecting the same inflammatory time course, were available for injection studies. Sheep have unusually large efferent lymph ducts that can be cannulated for weeks in unanesthetized animals (38, 39). In the present studies, the prescapular lymph node drained the same area of oxazolone stimulation. By directly cannulating the prescapular efferent lymph duct, we were able to readily obtain “migratory” lymphocytes stimulated by the same antigen as the microcirculation. These lymphocytes were fluorescently labeled and reinfected with a minimum of ex vivo manipulation.

The major experimental limitation of these studies was that the marked structural changes in the oxazolone-stimulated skin precluded direct comparisons of comparable segments of the contralateral control microcirculation. As a result, the lymphocyte velocity comparisons were made within the antigen-stimulated microcirculation. A second problem was that reliable motion analysis was restricted to lymphocyte velocities <3 μm/ms. As lymphocyte velocities increased, the tracking of nonlinear migratory paths of individual lymphocytes was increasingly more problematic. As a result of these two limitations, we developed an empirical approach to identifying areas of structural interest. This empirical approach, based on the x-y coordinates of the labeled lymphocytes, identified the focal topographic changes as “clusters” on lymphocyte time-location maps. Lymphocyte velocities within these time-location map clusters were compared with entry and exit velocities. The validity of this approach was supported by the absence of clusters in the control microcirculation and the presence of transmigrated lymphocytes within the clusters.

The focal topographic changes observed in this study suggest the possibility that structural adaptations in the microcirculation participate in the regulation of tissue entry by recirculating lymphocytes. The nature of the structural changes has implications for both local microhemodynamics and adhesion molecule expression. For example, the increased blood flow through the looping inflammatory vessels would likely increase lymphocyte delivery to inflammatory microcirculation, but would also increase shear rates at the endothelial surface. Observed changes in the vascular loop may contribute to hemodynamic modifications required for lymphocyte transmigration. Finally, it is plausible that areas of increased delivery and lymphocytes slowing would coincide with endothelial cell expression of appropriate cell adhesion molecules. In future work, we will explore the structural and functional implications of these specialized regions of the microcirculation.

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