Cellular and Molecular Dynamics in Exercise-Induced Urticarial Vasculitis Lesions

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Background: Based on the histologic findings of fully developed lesions, leukocytoclastic vasculitis has been regarded as the histologic criterion for differentiating urticarial vasculitis from urticaria. Nevertheless, because the early lesions have not been examined histologically, the key biological events leading to the development of leukocytoclastic vasculitis remain unknown. To address this issue, urticarial vasculitis lesions induced by physical exercise were sequentially examined histologically and immunohistochemically in a patient over the course of 24 hours. Serum levels of various cytokines also were determined in parallel.

Observations: At 3 hours after exercise challenge, the number of identifiable mast cells decreased and the first cell type that appeared around the vessels was the eosinophil. The serum tumor necrosis factor α level was strikingly increased as compared with that before challenge. Intense expression of E-selectin was also induced at 3 hours. The deposition of eosinophil peroxidase was observed at 3 hours and reached maximum deposition at 10 hours. The eosinophil peroxidase deposits preceded the prominent influx of neutrophils and the subsequent deposits of neutrophil elastase.

Conclusion: The extracellular deposition of eosinophil granule proteins, in addition to the deposition of immune complexes and a variety of cytokines from the infiltrating cells, appears to be one of the key biological events that determines whether urticarial lesions resolve without causing vasculitis or develop into vasculitis.


Urticarial vasculitis is characterized clinically by urticarial lesions that last for more than 24 hours and resolve with purpura or hyperpigmentation, and histologically by leukocytoclastic vasculitis. Based on the histologic findings of fully developed lesions, the presence of vessel damage, including swelling of endothelial cells, fibrinoid necrosis, a predominantly neutrophilic perivascular infiltrate with nuclear debris, and extravasated red blood cells, has been regarded as the histologic criterion for differentiating this disorder from urticaria. However, because skin biopsy specimens of urticarial vasculitis are routinely prepared from the fully developed lesions and the early lesions have not been examined histologically, the key biological events leading to the development of leukocytoclastic vasculitis remain unknown. This is probably due to the paucity of patients in whom characteristic lesions of urticarial vasculitis can be reproduced by physical challenge, despite sporadic case reports of urticarial vasculitis induced by administration of drugs and by exposure to a cold stimulus.

The importance of the timing when taking biopsy specimens in a dynamic process such as vasculitis has been emphasized by several investigators. Zax et al clearly demonstrated that different histologic patterns can be seen depending on the timing of biopsy specimens obtained from a single patient with leukocytoclastic vasculitis. They argued that it is the natural course of leukocytoclastic vasculitis to be initially a predominantly neutrophilic process and to evolve into a lymphocytic process. Nevertheless, it can be argued that because these investigators examined 1-day-old lesions even at the earliest time point, their study did not provide insight into the initiating biological event that is responsible for the development of leukocytoclastic vasculitis. It is therefore not sur-
PATIENT, MATERIALS, AND METHODS

A 54-year-old woman presented with a 2-year history of recurring erythematous wheals and purpuric lesions over the lower extremities. She noticed that the eruption appeared after physical exercise. The individual lesions persisted longer than 24 hours but disappeared after less than 72 hours. The eruption tended to resolve with slight pigmentation. Examination revealed urticarial lesions and purpura on her legs. She also noticed low-grade fever, mild arthralgia, and fatigue. She had sicca symptoms. Relevant laboratory values were as follows: white blood cell count, 5.3 × 10^9/L; with a differential count of 0.66 neutrophils, 0.01 basophils, 0.04 monocytes, and 0.29 lymphocytes; erythrocyte sedimentation rate, 87 mm/h; serum total protein, 84 g/L (reference range, 65-85 g/L); increased γ-globulin, 27.4 g/L; positive antinuclear antibody, 1:1280 in homogeneous pattern; and rheumatoid factor, 211 × 10^3 IU/L (reference range, up to 25 × 10^3 IU/L). C3 and C4 values, DNA antibody, and urinalysis results were within normal limits. Antibodies to Ro (SS-A) and La (SS-B) were positive; anticytoplasmic antibodies were absent. Serum chemistry studies revealed normal values except for a zinc sulfate turbidity test result of 36.8 U (reference range, 4-12 U) and a thyrom turbidity test result of 44.9 U (reference range, 0-5 U). Results of a Schirmer test were positive. Skin biopsy of the purpuric lesion on the leg demonstrated leukocytoclastic vasculitis. A diagnosis of urticarial vasculitis associated with Sjögren syndrome was made, based on the clinical, laboratory, and histopathologic findings.

The patient was admitted to the hospital because the lesions spread distantly to involve the extremities and the wheals increased in size. After admission, these lesions spontaneously resolved over the course of a week, leaving slight pigmentation. After complete resolution occurred, challenge by physical exercise was performed to determine whether exercise could reproduce characteristic lesions of urticarial vasculitis. Exercise was done by walking up and down steps for 15 minutes at room temperature. After obtaining informed consent, biopsy specimens were taken before and 3, 10, and 24 hours after exercise challenge. At the same time points, serum samples were taken for determination of the levels of cytokines. The biopsy specimens were fixed with formalin and stained with hematoxylin-eosin and toluidine blue (pH 4.2). The hematotoxylin-eosin sections were examined for histologic assessment and to enumerate eosinophils and neutrophils. Mast cells were enumerated by counting stained cells in the toluidine blue-stained sections: only those with obvious nucleus and with apparent cytoplasmic granules were counted in high-power field at ×400 magnification. The numbers of these cells were recorded as those in perivascular lesions that were defined as the unit area including the vascular wall of the vessel in the center of each field. For each specimen at least 5 random fields, with the aid of an ocular grid, were counted per section. The other portions of the biopsy specimens were immediately frozen with liquid nitrogen and stored at −80°C until used. Cryostat sections, 6-µm thick, were air dried, fixed in acetone for 10 minutes, and stained by the streptavidin biotin-staining procedure as described previously. T lymphocytes were enumerated by counting positively stained cells in anti–CD3-stained sections, as described above. The following monoclonal antibodies were used: anti–CD3 (anti-Leu4, Becton Dickinson, Mountain View, Calif), anti–E-selectin (BBIG-E6, British Biotechnology, Oxon, England), anti–intercellular adhesion molecule-1 (ICAM-1) (BBIG-11, R&D Systems, Oxon), anti–vascular cell adhesion molecule-1 (VCAM-1) (BBIG-V, British Biotechnology). Anti–eosinophil peroxidase (EPO) (SF25.5, Nichirei Inc, Tokyo, Japan), anti-human neutrophil elastase (NP57, DAKO, Glostrup, Denmark).

Sections were counterstained with Mayer hematoxylin. The specificity of the staining was confirmed by the control staining of adjacent sections without the primary monoclonal antibody. For each biopsy, at least 4 sections were stained with each monoclonal antibody. The following grading system was used for evaluation of cell adhesion molecule expression and extracellular protein deposition: (1) intensity of endothelial staining of cell adhesion molecule was graded as absent, weak, moderate, or strong; and (2) extent of extracellular deposition of EPO and neutrophil elastase was graded as absent, faint, moderate, extensive, or enormous. Direct immunofluorescence microscopic study was performed to detect immunoreactant deposition (IgG, IgM, IgA, C3, C4, and C1q) in the upper dermal vessels in each specimen. The intensity was evaluated by the same criteria for that of cell adhesion molecule staining. The serum levels of tumor necrosis factor α (TNF-α) and interferon γ (IFN-γ) were sequentially measured by commercially available radioimmunoassay system (TNF-α, Medgenix, Fleurus, Belgium; IFN-γ, Centocor, Malvern, Pa), as performed previously. The serum levels of interleukin (IL)-5 and IL-8 were determined by commercially available enzyme-linked immunosorbent assay system (IL-5, Immunotech SA, Marseille, France; IL-8, Toray Industries Inc, Tokyo). All assays were performed according to the manufacturer’s instructions, as described previously.
RESULTS

CLINICAL AND LABORATORY FINDINGS

Within 3 hours after exercise challenge, some urticarial lesions developed on the patient’s legs. At 10 hours, the lesions increased in number and became partly purpuric. At 24 hours, palpable purpura were observed. These purpura remained palpable for more than 24 hours and disappeared at 72 hours, leaving pigmentation.

The differential white blood cell counts were determined before and 24 hours after exercise challenge:

- Before challenge: $4.8 \times 10^9/L$ with $0.66$ neutrophils, $0.01$ eosinophils, $0.04$ monocytes, and $0.29$ lymphocytes.
- 24 hours after challenge: $4.6 \times 10^9/L$ with $0.52$ neutrophils, $0.02$ eosinophils, $0.01$ basophils, $0.02$ monocytes, and $0.43$ lymphocytes.

HISTOPATHOLOGIC FINDINGS

In normal skin before exercise challenge, minimal perivascular lymphocytic infiltrates were observed. At 3 hours after challenge, vascular dilatation with red blood cells was observed in the upper dermis. The infiltrate around the vessels was mainly composed of eosinophils (Figure 1, A), although a scant perivascular infiltration of T lymphocytes was observed. At 10 hours, a large number of neutrophils with slight nuclear fragments and extravasated red blood cells were seen. Occasional eosinophils that were perivascular at 3 hours appeared to extend into the surrounding tissue at 10 hours (Figure 1, B), at which time total leukocyte numbers were dramatically increased and the infiltrate around the vessel consisted mainly of neutrophils and T lymphocytes. The specimen obtained from palpable purpura at 24 hours showed neutrophils with numerous nuclear fragments, extravasated red blood cells, and T lymphocytes (Figure 1, C). Fibrin deposition was seen in the upper dermal vessels, indicating that leukocytoclastic vasculitis was induced by exercise challenge. Figure 2 summarizes the results of quantitative analysis of the dermal cellular infiltrates at various time points before and after exercise challenge. The number of the mast cells observed in the toluidine blue-stained sections was maximal before challenge and gradually declined afterward, suggesting the degranulation of mast cells.

IMMUNOHISTOCHEMICAL FINDINGS

Table 1 summarizes the results of the immunohistochemical analyses. At 3 hours, intense E-selectin expression on the upper dermal vessels was induced, although the expression was not seen before exercise challenge. The intensity of the expression of E-selectin gradually declined at 10 and 24 hours. After exercise challenge, VCAM-1 expression was marginally observed on vascular endothelial cells. Constitutive ICAM-1 expression on vascular endothelial cells was observed before challenge. Slight up-regulation of ICAM-1 was observed at 3 hours and remained at a similar level at 10 and 24 hours.

Table 1. Significant deposition of EPO was primarily present near blood vessels at 3 hours. A large amount of extracellular deposition of EPO was observed at 10 hours (Figure 3), at which time identifiable, intact eosinophils decreased in number and neutrophils were abundantly seen; however, the deposition of EPO was hardly seen at 24 hours. In contrast, moderate amount of deposition of neutrophil elastase was seen around the vessels at 10 hours; the most extensive cellular and extracellular neutrophil elastase deposits were observed at 24 hours and extended into the dermal interstitium.

Figure 1. Histologic findings (hematoxylin-eosin, original magnification ×200). A, Eosinophil infiltration around vessel at 3 hours; B, neutrophils, nuclear fragments, and lymphocytes around vessel at 10 hours; and C, numerous nuclear fragments and fibrinoid deposition around vessel at 24 hours.
DIRECT IMMUNOFLUORESCENCE STUDY

Direct immunofluorescence study revealed deposits of IgM and C3 in the upper dermal vessels before exercise challenge; however, the intensity of the deposition of IgM and C3 decreased at 10 and 24 hours. Before exercise challenge, deposits of the immunoreactant were already detected in the dermal vessels, and at 3 hours they were preferentially observed in the vessels without endothelial damage, rather than in those with endothelial damage. Immunoreactant deposits were minimally detected in the lesions at 10 and 24 hours.

SERUM CYTOKINE LEVELS

The serum concentrations of cytokines are shown in Table 2. The level of TNF-α increased strikingly at 3 hours but gradually declined. A substantial increase in IFN-γ level was also noted before exercise challenge as compared with controls but showed no essential change after challenge. Serum IL-5 and IL-8 levels were essentially unchanged throughout the study.

COMMENT

Although stimulated neutrophils could be the final effector cells responsible for endothelial damage in urticarial vasculitis, it remains unknown whether
produce factors that activate eosinophils. Although mast cells are the only cells shown to store TNF-α, they are not yet universally accepted as the key activator of eosinophils, although some authors have suggested that they may be the first or one of the first cell types to participate in the inflammatory cascade before the recruitment of neutrophils. Extensive deposition of eosinophil granule proteins may contribute to an early event that is needed for complete evolution of the vasculitis lesion. Toyoda et al demonstrated that free eosinophil granules in urticarial lesions were characteristic of the long-duration wheals among patients with chronic idiopathic urticaria. There appears to exist a correlation between the deposition of eosinophil granule proteins and vessel injury in urticarial vasculitis. The possibility that eosinophil granule proteins may act on endothelial cells directly is likely because a major contribution of eosinophil granule proteins to tissue damage has been reported in bullous pemphigoid, delayed pressure urticarial lesions, and eosinophilic vasculitis. However, it remains inconclusive that the discharge of potentially toxic granule proteins from eosinophils is one of the crucial factors in determining whether urticarial lesions resolve without causing vasculitis or develop into leukocytoclastic vasculitis lesions, because at present we are unable to obtain sequential biopsy specimens from controls with urticarial lesions that are reproduced by clinical challenge and that resolve without developing vasculitis. Nevertheless, in view of their known potential to activate complements, mast cells, and neutrophils, it is possible that these proteins may not act alone but in combination with other infiltrating cells and their granule proteins to initiate or take part in a cascade that leads to vessel damage.

In summary, this study demonstrates a dynamic series of cellular and molecular events that occur during the early phase of exercise-induced urticarial vasculitis lesions. A sequence of events beginning as a wheal-and-flare response and resulting in the development of leukocytoclastic vasculitis would be (1) immune complex deposition; (2) activation of mast cells and subsequent release of proinflammatory mediators such as TNF-α; (3) the influx of eosinophils and extracellular deposition of eosinophil granule proteins; (4) the persistent activation of mast cells; (5) the influx of neutrophils and subsequent release of potent proteolytic enzymes; and (6) damage of vascular vessels. Induction of endothelial adhesion molecule expression by release of a variety of cytokines from these infiltrating cells would provide a mechanism for ordered accumulation of eosinophils, neutrophils, and lymphocytes in the urticarial vasculitis lesions. Based on the results of our time-course analyses of exercise-induced urticarial vasculitis lesions, we suggest that extensive extracellular deposition of eosinophil granule proteins is one of the key biological events in the development of urticarial vasculitis.

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Table 2. Time Course of Serum Cytokine Levels

<table>
<thead>
<tr>
<th>Cytokines</th>
<th>0†</th>
<th>3</th>
<th>10</th>
<th>24</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNF-α, pg/mL</td>
<td>3.2</td>
<td>25.0</td>
<td>17.0</td>
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<tr>
<td>IFN-γ, IU/mL</td>
<td>0.25</td>
<td>0.29</td>
<td>0.14</td>
<td>0.20</td>
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<tr>
<td>IL-5, pg/mL</td>
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<td>24.0</td>
<td>23.0</td>
<td>15.5</td>
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<tr>
<td>IL-8, pg/mL</td>
<td>7.5</td>
<td>10.5</td>
<td>7.9</td>
<td>6.3</td>
</tr>
</tbody>
</table>

†Before challenge.

*The lower limits of sensitivity of the assay were 2.0 pg/mL for tumor necrosis factor (TNF-α), 0.03 IU/mL for interferon (IFN)γ, 10.0 pg/mL for interleukin (IL)5, and 3.0 pg/mL for IL-8. Serum samples obtained from 10 healthy volunteers served as controls: the TNF-α, IFN-γ, IL-5, and IL-8 levels in the controls were below the lower detection limit.

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