Ocular findings in systemic cutaneous basophil hypersensitivity

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SCBH was induced by immunizing guinea pigs with a protein antigen and challenging 1 week later with a large intraperitoneal dose of the same antigen. Animals developed a delayed-onset, erythematous skin rash and dermal infiltration by basophils and eosinophils. The uveal tracts of these animals were infiltrated by eosinophils, as were several other internal organs. The eye is affected in SCBH, and as in other forms of ocular cell-mediated hypersensitivity, the eosinophil is a prominent cellular component of these reactions.

Key words: delayed hypersensitivity, cutaneous basophil hypersensitivity, eosinophil, basophil, uveal tract, systemic cutaneous basophil hypersensitivity

Two types of delayed cutaneous hypersensitivity reactions can be distinguished in animals and man with the use of clinical and microscopic criteria.1-3 "Classic" delayed hypersensitivity (DH) is favored when guinea pigs are immunized with antigens in mycobacteria-containing adjuvants. DH skin reactions are erythematous, indurated, and infiltrated by large numbers of mononuclear cells. Cutaneous basophil hypersensitivity (CBH) is induced when mycobacteria-containing adjuvants are not used during immunization, and these skin reactions are typically erythematous and nonindurated and contain large numbers of basophilic leukocytes in addition to lymphocytes. Both DH and CBH reactions are delayed in onset for several hours after challenge, and both types of reactions are mediated by sensitized T lymphocytes. However, DH is a long-lasting state of hypersensitivity effected by lymphocytes with relatively high avidity for antigen, whereas CBH is often a transient state of hypersensitivity mediated by lymphocytes of lower average avidity.

Studies of cell-mediated hypersensitivity reactions in guinea pig eyes have also differentiated two types of ocular responses which correspond to DH and CBH in the skin.4,5 Animals primed for DH develop highly cellular reactions in their corneas and uveal tracts which contain large numbers of mononuclear cells. Those primed for CBH develop corneal reactions containing large numbers of basophils, but the limbus and uveal tracts of these animals are heavily infiltrated by eosinophils. To further evaluate the ocular features of these reactions, we studied the eyes of guinea pigs primed for systemic cutaneous basophil hypersensitivity (SCBH), a systemic expression of cell-mediated hypersensitivity in which guinea pigs are immunized for CBH and challenged intraperitoneally with a large dose of antigen.6

Materials and methods

Immunization and elicitation of SCBH. Thirty male and female Hartley strain guinea pigs, 400 to
Fig. 1. Delayed onset, erythematous rash elicited 24 hr after intraperitoneal challenge of guinea pig primed for SCBH with OA.

Table I. Differential counts of cells infiltrating guinea pig uveal tract in systemic delayed hypersensitivity

<table>
<thead>
<tr>
<th>Time after challenge (hr)</th>
<th>No. of eyes</th>
<th>Mononuclears</th>
<th>Neutrophils</th>
<th>Eosinophils</th>
<th>Basophils</th>
</tr>
</thead>
<tbody>
<tr>
<td>24</td>
<td>7</td>
<td>78.2 ± 8.0*</td>
<td>3.7 ± 2.7</td>
<td>18.9 ± 4.1</td>
<td>0</td>
</tr>
<tr>
<td>48</td>
<td>16</td>
<td>83.0 ± 5.5</td>
<td>5.9 ± 5.2</td>
<td>11.1 ± 1.4</td>
<td>0</td>
</tr>
<tr>
<td>72</td>
<td>10</td>
<td>85.3 ± 2.6</td>
<td>3.2 ± 1.1</td>
<td>12.0 ± 2.3</td>
<td>0</td>
</tr>
</tbody>
</table>

*Mean percent of cells counted ± S.E.M.

600 gm, were immunized in the footpads with 100 µg of either ovalbumin (OA) or bovine gamma globulin (BGG), both obtained from Miles Laboratories, Elkhart, Ind., in 0.1 ml of incomplete Freund's adjuvant (IFA) (Difco Laboratories, Detroit, Mich.). Five to 7 days later, SCBH was induced by an intraperitoneal challenge injection of 50 mg of the antigen used for immunization in 2 ml of saline. Animals were observed at time intervals ranging from 1 to 72 hr after challenge and sacrificed at 24, 48, or 72 hr. Skin reactions were more readily observed in animals after shaving and depilating with Nair (Carter-Wallace Inc., Cranbury, N. J.), but depilation itself did not affect the development of SCBH reactions.

Histology. Eyes and other tissues were removed from animals sacrificed with pentobarbital anesthesia and immediately placed in fixative. Tissues were either fixed in Karnovsky's mixture of glutaraldehyde and paraformaldehyde2 for 5 hr and then transferred to 0.1M sodium cacodylate buffer, pH 7.4, or else placed in Helly's fluid according to the protocol of Askenase.8 The tissues fixed by the former method were embedded in JB-4 plastic embedding medium (Polysciences, Warrington, Pa.), sectioned at 1 µm, and stained with Giemsa's reagent (Fisher Scientific, Pittsburgh, Pa.). Those fixed in Helly's fluid were embedded in paraffin, sectioned at 5 µm, and stained with Giemsa's reagent. A certain number of tissues were fixed initially in 1.5% glutaraldehyde prior to transferring them to Karnovsky's fixative and processing them for JB-4 embedding. Differential cell counts were performed on 1 µm sections of uveal tract by means of a method previously described.4-5 Mean percentages ± the standard error of the mean were calculated for several eyes removed at 24, 48, and 72 hr time intervals (Table I).

Controls. The eyes and other organs of several control animals were examined microscopically with both methods of tissue processing. Control
animals were treated in one of the following three ways: (1) not sensitized, (2) sensitized but not challenged, (3) challenged intraperitoneally but not sensitized.

Results

Appearance of SCBH reactions. Guinea pigs primed for SCBH with either OA or BGG and challenged 5 to 7 days later with an intraperitoneal injection of the same antigen used for sensitization developed a delayed-onset, erythematous, macular, and very slightly papular rash (Fig. 1). The rash was detectable 6 hr after challenge but was best observed at 24 hr and was somewhat faded by 48 hr. The rash began on the flanks and nape of the neck and then extended to the abdomen, legs, and back. Initially, macular skin lesions were discrete but later became confluent. The eyelids were often diffusely erythematous, especially when the rash was severe and diffuse. The conjunctivae were often slightly injected; however, this was not a consistent finding and could not be correlated with the severity of the rash. Of the 30 animals primed for SCBH, 28 developed a diffuse rash involving all areas of the skin, whereas only two animals had no perceptible rash at the time of sacrifice.

Histology of SCBH skin reactions. The papillary and reticular dermis of the erythematous skin reactions were infiltrated by moderate numbers of inflammatory cells. A consistent finding at 24, 48, and 72 hr was the presence of both mononuclear cells and neutrophils. In general, larger numbers of neutrophils were observed at 24 hr, whereas mononuclear cells predominated at 48 and 72 hr. Eosinophils and basophils (Fig. 2) were consistently found at all time intervals. In some tissue sections, especially those taken at 48 and 72 hr, eosinophils were more prominent than basophils. The number of basophils and eosinophils varied considerably in skin taken from different animals, and in some sections only an occasional eosinophil or basophil could be identified among a background of mononuclear cells and neutrophils. In other skin reactions, eosinophils and basophils were seen throughout the dermis and often appeared in collections within and outside of vessels. The venules of the upper dermis were often dilated and compacted with large numbers of erythrocytes and leukocytes, many of which were basophils and eosinophils. Fixed-tissue mast cells were observed throughout the dermis. They were easily distinguishable from basophils because of their round nuclei and small, darkly staining granules. Basophils, which are granulocytes, have segmented nuclei and large, oval or pear-shaped granules. Both basophils and mast cells appeared intact and did not show evidence of degranulation. Little or no edema was observed in the skin, and the deeper dermis and subcutis showed little or no inflammation.

Histology of eye reactions. Ocular inflammation was far more prominent in the choroid and ciliary body than in any other area of the eye. The most striking finding was the large number of eosinophils which infiltrated the uveal tract (Fig. 3). Unlike the skin, basophils were either totally absent or only rarely observed. Eosinophils were seen throughout the choroid and ciliary body but seemed to have a predilection for the outer...
Fig. 3. Eosinophils (e) infiltrating the uveal tract of guinea pig primed for SCBH. (Giemsa; x1080.)

layers of the choroid, where they were frequently lined up near the sclera. Eosinophils were sometimes observed within choroidal blood vessels, especially in eyes removed at 24 hr. The number of eosinophils observed correlated with the intensity of the overall uveal inflammation. Large numbers of eosinophils and other inflammatory cells were present at 24, 48, and 72 hr.

Mononuclear cells (mainly lymphocytes and lymphoblasts) were observed in all sections of the uveal tract and were the predominant cell at all three time intervals. Mast cells were present in large numbers throughout the uveal tract and, as in the skin, showed no evidence of degranulation. Choroidal blood vessels were often filled with large numbers of erythrocytes and leukocytes (especially eosinophils and neutrophils). Endothelial cell hypertrophy was sometimes observed; however, this was not as striking as the endothelial changes seen when sensitized animals are challenged with ocular injections of protein antigens.\(^4,5\) In previous studies,\(^4\) autoradiography was used to demonstrate endothelial cell hyperplasia; however, such studies were not performed in the present series of experiments.

Differential counts of cells infiltrating the uveal tract (Table I) indicated that mononuclear cells were the most numerous inflammatory cell at 24, 48, and 72 hr after intraperitoneal challenge, comprising 78% to 85% of the total cell counts. Eosinophils were numerous at all three time intervals, accounting for 18% of the infiltrate at 24 hr, 11% at 48 hr, and 12% at 72 hr. Neutrophils were observed in relatively small numbers at all three time intervals (3% to 6%), and no basophils were seen in the uveal tract for any of the cell counts. The counts for any one cell type did not change significantly during the three time intervals according to Student's \(t\) test \((p < 0.05)\).

The conjunctiva showed only mild evidence of inflammation microscopically. An occasional eosinophil, neutrophil, or mononuclear cell could however, often be found. The cornea, limbus, and other areas of the eye were free of inflammatory cell infiltration or other pathologic changes in all animals.

**Histology of other tissues.** Eosinophils were prominent in the microvasculature of the internal organs; however, they were found less consistently in lung, thymus, spleen, and lymph nodes than in the uveal tract. Some sections of lung contained large numbers of eosinophils, but since these cells are sometimes found in normal guinea pig lung,\(^6\) their significance is uncertain. Eosinophils were found in the red pulp of spleen and in the medulla of the thymus and lymph nodes. Although eosinophils in these organs were most consistently observed in animals sacrificed 24 hr after challenge, they were also seen in tissues removed at 48 and 72 hr.
Controls. The three groups of control animals lacked hyperacute inflammatory cell infiltration of the uveal tract. Fixed tissue mast cells were consistently seen in large numbers throughout the uveal tract (especially the choroid), but as in other animals, no degranulation was observed. An occasional leukocyte was present in the uveal tract, but in no control eyes were cell observed in large numbers; therefore no differential cell counts were performed on controls. The most frequently observed infiltrating cell in control eyes was the lymphocyte, but an occasional plasma cell or neutrophil could also be found. Eosinophils were also observed occasionally, and almost every section had one or two eosinophils in the choroid.

Discussion

Delayed hypersensitivity, or cell-mediated immunity, is the major immunologic mechanism in defense against many types of infection, in contact allergy, and in the rejection of tissue grafts and tumors. Delayed hypersensitivity responses are presumed to be important in many diseases affecting the eye either through local tissue damage or as part of a broader systemic process. They may be important in the pathogenesis of sympathetic ophthalmia, Vogt-Koyanagi-Harada syndrome, and Behcet's disease. Delayed hypersensitivity responses are known to be depressed in atopic conditions, herpes zoster, leprosy, sarcoidosis, and certain rheumatic disorders.

Although eosinophils are generally considered a hallmark of allergic reactions involving humoral immunity, they have only recently been associated with delayed hypersensitivity responses. They are especially prominent in delayed hypersensitivity reactions involving the eye, although they may be seen within internal organs in a systemic form of delayed hypersensitivity, SCBH. In human ocular disease, eosinophils are associated with sympathetic ophthalmia, cicatricial pemphigoid, and atopic and vernal conjunctivitis. In many cases of sympathetic ophthalmia, eosinophils are concentrated in the inner choroid just under the choriocapillaris. In the guinea pig, however, eosinophils seem to have a predilection for the outer layers of the choroid, a variation which may be species-related and perhaps determined by different patterns of uveal blood flow in man and in guinea pigs. In a recently described animal model of lens-induced uveitis in the rat, probably mediated by immune complexes, eosinophils are also a prominent feature.

Our finding of large numbers of eosinophils in the uveal tract in a systemic form of delayed hypersensitivity is not surprising considering our earlier observations of locally induced delayed reactions in the guinea pig cornea and uveal tract. Although milder in intensity, the microscopic picture of the systemic reaction is qualitatively similar to the locally induced reactions at the limbus and in the uvea. The absence of basophils contrasts with the microscopic picture seen in the skin and the central cornea in CBH reactions. We previously suggested that certain local tissue factors, perhaps related to the microvasculature, could influence the composition of the inflammatory cell infiltrate. A second reason for the paucity of basophils in the uveal tract could be the abundance of mast cells throughout the uvea. Mast cells secrete eosinophil chemotactic factor of anaphylaxis (ECF-A), a substance chemotactic for eosinophils which is preformed in mast cells and basophils. Mast cells and basophils are believed to have similar functions, and there is a well-known inverse relation between basophil and mast cell frequency in various species. Perhaps the already present large population of mast cells obviates the need for an influx of basophils.

The role of the eosinophil and the reason for its prevalence in ocular delayed hypersensitivity is uncertain. Eosinophils phagocytose immune complexes and are thought to cause tissue injury in certain diseases. Recent investigations have identified a number of enzymes within eosinophil granules which are capable of inactivating the products of mast cell/basophil secretion. For example, arylsulfatase, histaminase, and phospholipase D, which are found in eosinophils, can inhibit slow-reacting substance of anaphylaxis, histamine, and platelet-acti-
vating factor, respectively. These findings, coupled with the lag of eosinophil influx by several hours following initial mediator release, suggest a role for eosinophils in modulating or limiting allergic inflammation. The tissue eosinophilia which accompanies delayed hypersensitivity responses in the eye and in other organs could be secondary to the activation and secretion of mast cells in these tissues. Although the mechanism for mast cell mediator release does not appear to be the extrusion of granules (as in IgE-mediated anaphylaxis), a model in which a small quantum of intact or dissolved granule material flows to the cell surface, fuses with the plasma membrane, and is discharged into the extracellular space has been proposed. The actual mechanism for eosinophil chemotaxis in uveal hypersensitivity reactions is not presently known and will require further investigation.

REFERENCES


