Histopathology of delayed hypersensitivity reactions in the guinea pig uveal tract

Mitchell H. Friedlaender, Edward L. Howes, Jr., Joan M. Hall, Hedy Krasnobrod, and Mary Ann Wormstead

Two distinct patterns of delayed-onset, cell-mediated hypersensitivity have been induced in the guinea pig eye with the same soluble protein antigen and two different methods of immunization. The predominant histologic feature of these reactions was the large number of eosinophils which infiltrated the uveal tract and the limbus. Skin test reactions in the same animals contained very few eosinophils and were typical of cutaneous basophil hypersensitivity or classic delayed hypersensitivity of the tuberculin type. It is suggested that local factors play a role in determining the character of immune expression in different tissues.

Key words: delayed hypersensitivity, cutaneous basophil hypersensitivity, eosinophil, basophil, mast cell, uveitis, histopathology

Delayed hypersensitivity reactions are characterized histologically by perivascular infiltration of mononuclear cells. Recent investigations of these reactions in the skin of man and various laboratory animals have distinguished two forms of delayed hypersensitivity on clinical and histologic grounds. Classic delayed hypersensitivity (DH) is induced by sensitization with mycobacteria or with other antigens administered in mycobacteria-containing adjuvants. A second form of delayed hypersensitivity, cutaneous basophil hypersensitivity (CBH), is induced by a variety of immunizing procedures which avoid the use of mycobacterial adjuvants. Both are effected by lymphocytes, but skin reactions of CBH are relatively nonindurated and infiltrated by large numbers of basophilic leukocytes in addition to mononuclear cells.

Two distinct histologic pictures of delayed hypersensitivity can be produced in the guinea pig cornea when animals are primed for CBH and DH. In CBH-primed guinea pigs, large numbers of basophils and eosinophils infiltrate the cornea and limbus after intracorneal challenge. In DH-primed animals the infiltrate consists mainly of mononuclear cells and neutrophils. In order to determine the cellular components of DH reactions in the uveal tract, we induced CBH and DH in guinea pigs and studied the inflammatory response following intravitreal challenge.

Materials and methods

Animals, immunization, and challenge. Hartley strain guinea pigs weighing 400 to 500 gm were used throughout. For induction of CBH, animals were sensitized with 100 μg of ovalbumin (OA) (Miles Laboratories, Inc., Elkhart, Ind.) or 100 μg of bovine gamma globulin (BGG) (Miles) in 0.1 ml of incomplete Freund's adjuvant (IFA) (Difco Laboratories, Detroit, Mich.) divided between the hind footpads. For induction of DH, animals were sensitized with 50 μg of OA in 0.1 ml of incomplete Freund's adjuvant (IFA) (Difco Laboratories, Detroit, Mich.) divided between the hind footpads. Animals were challenged with 10 μg of OA on the fourth day after sensitization.

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were sensitized with hind-footpad injections of 10 μg of OA or BGG in 0.1 ml of complete Freund's adjuvant (CFA) containing 3 mg/ml heat-killed Mycobacterium tuberculosis.

Animals primed for CBH and DH were skin-tested at 7 days or at 3 to 4 weeks after immunization with 10 to 25 μg of OA or BGG in 0.1 ml of saline on shaved, depilated flank skin. Eye testing was carried out simultaneously in these same animals by anesthetizing the eye with topical proparacaine and injecting 10 to 25 μg of OA or BGG in 0.025 ml of saline intravitreally through a 30-gauge needle introduced at the pars plana. Control injections of appropriate doses of both antigens were performed intravitreally and intradermally in unsensitized animals.

**Evaluation of reactions.** Reactions were examined 1, 6, 24, and 48 hr after testing. Eyes were observed for conjunctival injection, chemosis, and corneal clouding. Skin tests were measured for erythema and induration. Animals were sacrificed at 24 and 48 hr. Eyes and skin test sites were removed and bisected with a razor blade. Half of each specimen was fixed in Karnovsky’s fixative and processed for Epon embedding. Some tissues were embedded in JB-4 plastic embedding medium (Polysciences, Inc., Warrington, Pa.). Both Epon- and JB-4-embedded tissues were sectioned at 1 μm and stained with Giemsa's reagent for light microscopy. The other half of each specimen was fixed in Helly’s fluid, processed for paraffin embedding, and stained with Giemsa's reagent following the protocol of Askenase. The latter technique was useful for screening large numbers of specimens but was less satisfactory for cell counts and differentiation of cell types than were the 1 μm sections.

Differential cell counts were performed in six guinea pigs sensitized with OA in IFA and six sensitized with OA in CFA. Counts were made in three separate regions of the choroid in 1 μm sections of eyes removed 48 hr after challenge. Differential counts were also carried out in the ciliary body and iris and at the limbus. At least 100 infiltrating cells were counted in each area of the eye except in a few specimens where several sections contained fewer than 100 cells. Differential counts were performed on each 1 μm Giemsa-stained section by two different observers. The results of both groups were found to be nearly identical and were averaged. Counting both plastic- and paraffin-embedded sections, a total of 59 guinea pigs (118 eyes) primed for CBH were studied histologically, and 25 animals (50 eyes) primed for DH were evaluated.

**Migration-inhibition factor assays and hemolytic antibody titers.** Migration-inhibition factor (MIF) assays were performed on mineral oil-induced peritoneal exudate cells obtained from guinea pigs 7 days or 6 weeks after sensitization with OA. Cells were washed in RPMI 1640, packed in capillary tubes, and cultured in Sykes-Moore chambers in the presence of 50 to 100 μg of OA and 15% normal guinea pig serum. Macrophage migration was measured at 24 hr and percent inhibition was determined by a comparison with cell migration in cultures without antigen, using the following formula:

\[
1 - \frac{\text{migration with antigen}}{\text{migration without antigen}} \times 100
\]

Hemolytic antibody (HA) titers to OA and BGG were measured at the time of sacrifice, by a modification of the technique of Hübner and Genegoian.

**Results**

**Eye reactions in animals sensitized with antigens in IFA.** Animals sensitized 7 days previously with OA or BGG in IFA developed delayed-onset reactions of mild ocular injection when challenged with appropriate doses of specific antigen. No gross reactions occurred in unsensitized or nonspecifically sensitized controls.

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**Fig. 1.** CBH-choroid. Numerous inflammatory cells present. (×480.)

**Fig. 2.** CBH-choroid. Eosinophils dominant cell in field (×1,200.)

**Fig. 3.** CBH-skin. Several basophils and a mast cell (smaller, dark granules) are seen. (×1,200.)

**Fig. 4.** DH-choroid. Mononuclear infiltrate more pronounced. (×300.)

**Fig. 5.** DH-choroid. Early plasma cell, several eosinophils, and a mast cell (small, dark granules) are seen. (×1,200.)

**Fig. 6.** DH-choroid. Endothelial cell hypertrophy. (×1,200.)
Table I. Differential counts of infiltrating cells of delayed-type hypersensitivity reactions in guinea pigs primed for CBH*

<table>
<thead>
<tr>
<th>Location</th>
<th>Mononuclears</th>
<th>Neutrophils</th>
<th>Basophils</th>
<th>Eosinophils</th>
</tr>
</thead>
<tbody>
<tr>
<td>Choroid</td>
<td>50.4 ± 3.81</td>
<td>2.0 ± 0.69</td>
<td>0.06 ± 0.07</td>
<td>47.2 ± 4.0</td>
</tr>
<tr>
<td>Ciliary Body</td>
<td>40.7 ± 3.7</td>
<td>1.2 ± 0.42</td>
<td>0.04 ± 0.04</td>
<td>58.4 ± 4.0</td>
</tr>
<tr>
<td>Iris</td>
<td>53.7 ± 3.9</td>
<td>0.46 ± 0.24</td>
<td>0.00</td>
<td>15.1 ± 3.6</td>
</tr>
<tr>
<td>Limbus</td>
<td>46.4 ± 9.5</td>
<td>5.2 ± 1.8</td>
<td>0.62 ± 0.44</td>
<td>46.1 ± 5.8</td>
</tr>
<tr>
<td>Skin</td>
<td>41.6 ± 5.5</td>
<td>1.0 ± 0.44</td>
<td>51.4 ± 5.0</td>
<td>7.2 ± 1.5</td>
</tr>
</tbody>
</table>

*Reactions were elicited in 6 guinea pigs (12 eyes) primed for CBH with 100 fig of OA in IFA and challenged intravitreally and intradermally at 7 days with 10 to 25 fig of OA. Differential cell counts were performed in the iris, ciliary body, limbus and skin and in three different regions of the choroid.

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<th>Basophils</th>
<th>Eosinophils</th>
</tr>
</thead>
<tbody>
<tr>
<td>Choroid</td>
<td>69.9 ± 5.31</td>
<td>9.9 ± 5.2</td>
<td>0.27 ± 0.14</td>
<td>18.8 ± 3.2</td>
</tr>
<tr>
<td>Ciliary Body</td>
<td>63.1 ± 4.7</td>
<td>2.8 ± 1.3</td>
<td>0.04 ± 0.04</td>
<td>33.4 ± 5.8</td>
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<tr>
<td>Iris</td>
<td>91.9 ± 2.1</td>
<td>1.2 ± 0.60</td>
<td>0.00</td>
<td>6.4 ± 2.2</td>
</tr>
<tr>
<td>Limbus</td>
<td>60.5 ± 6.0</td>
<td>13.8 ± 5.4</td>
<td>1.3 ± 0.67</td>
<td>19.1 ± 5.3</td>
</tr>
<tr>
<td>Skin</td>
<td>59.7 ± 0.5</td>
<td>20.4 ± 6.9</td>
<td>17.7 ± 4.9</td>
<td>1.9 ± 0.76</td>
</tr>
</tbody>
</table>

*Reactions were elicited in 6 guinea pigs (12 eyes) primed for DH with 10 fig of OA in CFA and challenged intravitreally and intradermally at 3 to 4 weeks with 10 to 25 fig of OA. Differential cell counts were performed in the iris, ciliary body, limbus and skin in three different regions of the choroid.

Histologically, the inflammatory reaction at 24 or 48 hr involved the entire uveal tract, the corneoscleral limbus, and the conjunctiva. The distribution of cells was somewhat variable. Some areas of the choroid contained large collections of inflammatory cells (Fig. 1), whereas other areas were relatively spared. Moderate numbers of mononuclear cells infiltrated the iris, the ciliary body, and the choroid.

Differential cell counts of inflammatory cells infiltrating the choroid, iris, ciliary body, and limbus revealed a characteristic distribution of cell types in different regions of the eye (Table I). Mononuclear cells, including large and small lymphocytes, lymphoblasts, and monocytes, accounted for 40% to 50% of the inflammatory cells infiltrating the choroid, ciliary body, and limbus. Although the total number of cells counted in the iris was smaller than that in other areas of the uveal tract, the percentage of mononuclear cells infiltrating the iris was nearly twice as high. Eosinophils were a prominent finding throughout the uveal tract (Fig. 2) and at the limbus. In the choroid and ciliary body and at the limbus, eosinophils comprised 46% to 58% of the inflammatory cell infiltrate. Although eosinophils were most prominent in the ciliary body (58%), they were much less frequent in the iris, accounting for only 15% of the cells (p < 0.001). In the choroid, eosinophils were widely distributed but seemed to have a predilection for the outermost layers of the uveal tract where they often were lined up just inside the sclera. Eosinophils could be easily differentiated from neutrophils because of their larger, eosinophilic, cytoplasmic granules.

Large numbers of mast cells were seen throughout the uveal tract, especially in the choroid. Between 0 and 50 intact mast cells were encountered per 100 inflammatory cells in the uveal tract. The number of fixed tissue mast cells in general was directly proportional to the area of tissue examined during the process of counting 100 cells. Neutrophils were occasionally observed in the uveal tract and were slightly more numerous at the limbus, where they accounted...
for 5% of the inflammatory cells. Basophils were exceedingly rare, and plasma cells were almost never observed.

Ocular testing was also performed at 3 to 4 weeks after immunization with antigens in IFA, at a time when CBH reactivity had largely disappeared and been replaced by "late" reactions. Such reactions elicited little or no response grossly; histologically, only small numbers of mononuclear cells and fewer eosinophils were present in the uveal tract and at the limbus.

Skin reactions in animals sensitized with antigens in IFA. Skin responses in these animals were typical of CBH. These reactions were erythematous with little or no induration and reached peak intensity 24 hr after challenge.

Histologically, skin reactions differed markedly from their ocular counterparts (Fig. 3). Large numbers of mononuclear cells and basophils infiltrated the papillary and reticular dermis (Table I). Basophils were the most frequently observed cells, accounting for 51% of the infiltrate, and 42% of the cells counted were mononuclear. Eosinophils, common in ocular reactions in these same animals, accounted for only 7% of the cells in the skin. Neutrophils were rare, comprising only 1% of the infiltrate.

Eye reactions in animals sensitized with antigens in CFA. Animals challenged 3 to 4 weeks after sensitization with OA or BGG in CFA developed delayed-onset reactions characterized by moderate conjunctival injection and chemosis, and occasional corneal clouding. Reactions were comparable whether elicited at 7 days or 3 to 4 weeks after immunization, although the former were generally less intense.

Histologically, the uveal tracts of these animals contained larger numbers of inflammatory cells than the previous group. The thickness of the choroid in most sections was twice that of animals sensitized with antigen in IFA (Fig. 4). Mononuclear cells, including lymphocytes, lymphoblasts, monocytes, plasma cells, and plasma cell precursors, were the most prominent cells encountered (Fig. 5). Mononuclear cells comprised 60% to 70% of the inflammatory infiltrate in the choroid and ciliary body and at the limbus (Table II). In the iris, 92% of the cells counted were mononuclear. As in CBH-primed animals, eosinophils were commonly seen in guinea pigs primed for DH. Again, eosinophils were most numerous in the ciliary body where they comprised 33% of the inflammatory cell infiltrate. Eosinophils accounted for 19% of the inflammatory cells in the choroid and at the limbus and only 6% of the cells in the iris.

Neutrophils were more commonly seen in DH reactions than in CBH, especially at the limbus where they accounted for 14% of the inflammatory cell infiltrate. Basophils were only rarely seen. Mast cells were encountered less often in DH reactions than in CBH, accounting for 0 to 16 cells per 100 infiltrating cells. This seemed to reflect the smaller area of tissue in which 100 cells could be found in these highly cellular reactions.

Skin reactions in animals sensitized with antigens in CFA. Guinea pigs sensitized with antigens in CFA developed typical DH skin responses when challenged 3 to 4 weeks after sensitization. Reactions were erythematous and indurated and reached peak intensity at 48 hr.

Histologically, the predominant cells in DH skin reactions were mononuclear. Lymphocytes, lymphoblasts, plasma cells, and monocytes accounted for 60% of the infiltrating cells, and neutrophils comprised 20% (Table II). Basophils were somewhat more common than usually seen in DH reactions, accounting for 18% of the total infiltrate. In contrast to the ocular DH reactions in the same animals, eosinophils were rarely observed in the skin.

Vessel alterations in eye and skin reactions. Blood vessels in the uveal tract, at the limbus, and in the dermis exhibited characteristic changes which have been pointed out previously in DH reactions in the skin. These include hypertrophy and hyperplasia of endothelial cells and pericytes and thickening of the basal lamina of some vessels (Fig. 6). These changes were considerably more striking in DH than in CBH reactions.
**MIF assays and HA titers.** In guinea pigs sensitized with OA or BGG in IFA, peritoneal exudate cells migrated without evidence of inhibition when cultured either with or without antigen. This failure of macrophage migration has been noted previously in CBH-primed guinea pigs except when certain potent antigens such as hemocyanin and vaccinia virus are used. In guinea pigs primed for DH, good inhibition was obtained after 24 hr when OA was added to the chamber. The average percent inhibition was 52% when compared to migration without antigen.

HA titers to OA and BGG were completely negative in animals primed for CBH 7 days after sensitization. In guinea pigs primed for classic DH, antibody titers were present in dilutions greater than 1:256 at 3 to 4 weeks.

Thus CBH-primed animals are believed to manifest a state of delayed-type hypersensitivity which develops prior to the onset of antibody formation. Animals primed for DH and tested 3 to 4 weeks later exhibit in vitro evidence of both humoral and cellular immunity.

**Discussion**

Delayed hypersensitivity reactions have long been studied in ocular tissues of experimental animals. Frequently, sensitization is accomplished by injecting antigens in mycobacteria-containing adjuvants, a technique which primes animals for classic DH. The delayed-onset inflammation in the eyes of such animals typically shows infiltration of the uveal tract and limbus by mononuclear cells and neutrophils. Eosinophils and basophils have not generally been recognized as a prominent feature of delayed-type reactions in the eye.

Recently, we showed that two distinct patterns of delayed hypersensitivity can be elicited in the guinea pig cornea following immunization with protein antigens. When guinea pigs are primed for DH by immunization with antigen in IFA and intracorneal challenge is carried out 5 to 7 days later, the limbal inflammatory reaction contains moderate numbers of basophils and eosinophils. In contrast, animals primed for DH with antigen in CFA and challenged 6 weeks later develop highly cellular reactions containing large numbers of mononuclear cells and neutrophils, with relatively few basophils and eosinophils.

Our current study employs similar methods of sensitization. However, challenge is made into the vitreous rather than the cornea. Again, a striking difference in the intensity of the ocular inflammatory response was observed. The DH reactions were much more severe clinically and more cellular histologically than the CBH reactions. The differential cell counts in the two forms of delayed reactivity also showed marked differences. Mononuclear cells were more numerous in DH reactions, where they accounted for 60% to 70% of the cells infiltrating the choroid, ciliary body, and limbus, whereas in CBH reactions, mononuclear cells made up only 40% to 50% of the infiltrate. Eosinophils, on the other hand, were more numerous in CBH reactions, accounting for 46% to 60% of the infiltrating cells, compared with 19% to 33% of the cells in DH reactions.

The iris appeared to be somewhat unusual in that the total number of infiltrating cells were fewer and mononuclear cells were by far the most numerous cells observed in animals primed for either DH or CBH. Basophils, although a constant feature of CBH skin reactions, were only seen rarely in the uveal tract or at the limbus when challenge was given intravitreally.

The ocular findings contrasted markedly to those in the skin. In CBH-primed animals, basophils comprised 51% of the inflammatory cells in skin and less than 1% in ocular tissues (p < 0.001). Eosinophils accounted for only 7% of inflammatory cells in skin and 47% and 58% in the choroid and ciliary body, respectively (p < 0.001).

The data presented here extend to the uveal tract the observation made several years ago in skin; namely, that two distinct
patterns of delayed-onset, cell-mediated hypersensitivity may be elicited to the same antigen, depending on the mode of immunization and the interval between immunization and challenge. They also supplement our finding that eosinophils are a prominent feature of delayed hypersensitivity reactions in the eye even though simultaneous reactions in the skin contain very few eosinophils and many basophils. We suggested previously that local tissue factors in some way influence the character of the inflammatory cell infiltrate. The nature and mode of action of these local factors is presently unknown. It has been postulated that the distribution and character of the microvasculature in different regions of the body may contribute significantly to differences in local inflammatory reactions. A similar situation where the cellular response is influenced by the site of challenge has been observed in the rejection of chemically induced tumor cells. In the skin, tumor rejection is associated with extensive infiltration of basophilic leukocytes, whereas macrophages and neutrophils were predominant when tumor cells were rejected in the peritoneal cavity. Even within the skin, variations in cellular infiltrates are encountered. CBH reactions may contain dense infiltrates of basophils in the papillary dermis, whereas an irregular and less dense infiltration of basophils occurs in the reticular dermis. In the guinea pig tuberculin reaction, mononuclear cells accumulate predominantly in the deeper dermis, and basophils may sometimes be found in the superficial dermis. Flax and Waksman have shown that DH skin reactions in the rat involve primarily the deeper dermis, largely sparing the superficial layers.

Although the abundance of eosinophils in the uveal tract and at the limbus following intravitreal challenge may reflect certain anatomical features, route of challenge, or local antigen concentration, the reason for their accumulation is not known. Eosinophil granules are known to contain enzymes, including arylsulfatase, histaminase, and phospholipase D, which can inactivate the products of basophil/mast cell secretion and presumably modulate the allergic inflammatory response. If this is the case in the ocular delayed reactions, perhaps mast cells rather than basophils are involved in this interaction.

Recently, Dvorak et al. showed that a systemic form of CBH is characterized by widespread inflammatory cell infiltration of tissues. Although the skin rash lesions contained many basophils, as expected in CBH, focal collections of eosinophils and a smaller number of basophils were found in the lung and spleen. This supports the concept that local factors play a determinative role in regulating the inflammatory response and demonstrates that an eosinophil response as seen in the eye may occur as an expression of delayed reactivity in other organs.

REFERENCES


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