Histamine and Prostacyclin

Primary and Secondary Release in Allergic Conjunctivitis

Laurent Helleboid, Mahin Khatami, Zhi-Gang Wei, and John H. Rockey

The relationship between the release of histamine, a major mast cell mediator of conjunctival type I reactions, and the production of a prostanoid, prostacyclin (prostaglandin I$_2$, PGI$_2$), was examined in a guinea pig model of allergic conjunctivitis. Guinea pigs were sensitized topically and challenged by repeated conjunctival instillation of fluoresceinyl ovalbumin. Histamine and 6-keto-PGF$_{1a}$, the stable product of the spontaneous degradation of PGI$_2$, were measured in tears by radioimmunoassays. Clinical type I reactions and tear histamine appeared by 8 days and increased up to 22 days during the initial sensitization, with notable variations between animals. The kinetics of histamine and 6-keto-PGF$_{1a}$ release in tears were examined over a 24-hr period after the antigen challenge. Histamine release was maximal during the first 10 min and returned to baseline values by 1 hr in all instances. The 6-keto-PGF$_{1a}$ release also peaked during the first 10 min but continued for an extended period. The ratio of tear 6-keto-PGF$_{1a}$ to histamine increased more than 16-fold over the 2 hr after antigen challenge. Late-phase reactions with second peaks of histamine or 6-keto-PGF$_{1a}$ in the tears were observed in two different guinea pigs 4–8 hr after antigen challenge. Histamine applied to the eyes of naive guinea pigs also induced the release of 6-keto-PGF$_{1a}$ in tears. Histamine appeared to act as a primary mediator, stimulating the secondary production and release of PGI$_2$ by constitutive (eg, vascular) and possibly infiltrating inflammatory cells during an allergic conjunctival reaction. Invest Ophthalmol Vis Sci 32:2281-2289, 1991

Human allergic ocular diseases share common features (immunoglobulin [Ig] E antibodies, mast cell degranulation, and eosinophil activation) but differ substantially in clinical and histopathologic expression and response to treatment. The basis for these varied ocular allergic reactions is not fully understood, but it may be due to the addition and complex interactions of other humoral and cellular immunopathologic mechanisms in some of the disease processes. For example, although type I allergic reactions alone may be sufficient to produce recurrent hay fever-type allergic conjunctivitis, the addition of other immunopathologic processes may be necessary to produce vernal keratoconjunctivitis.$^{1,2}$

To study the roles of distinct combinations of humoral and cellular mechanisms in causing, maintaining, or suppressing allergic ocular diseases, we established animal models of hay fever-type and vernal conjunctivitis in guinea pigs, passively sensitized with IgE antibody, or actively immunized by chronic topical (conjunctival) challenge with antigen.$^{1-4}$ Not only may more than a single immunopathologic mechanism occur at one time in vivo, but also interactions between the different mechanisms may alter the response and effect of an individual mechanism substantially. Studies in animal models of integrated immune mechanisms offer a more complete picture of such interactions (eg, inclusive of factors contributed by the circulation) not fully duplicated by studies in cell, tissue, or organ culture.$^{1,3}$ We examined the interactions between a major mast cell mediator, histamine, released by a type I reaction in topically immunized and challenged guinea pigs, and the production of a prostanoid, prostacyclin (prostaglandin I$_2$, PGI$_2$).

Materials and Methods

Sensitization and Challenge Schedule

All studies were done in accordance with the ARVO Resolution on the Use of Animals in Research. Eight female Hartley guinea pigs were sensi-
tized topically (conjunctivally) unilaterally (right eye) by repeated ocular instillation of one drop of 10 mg/ml fluoresceinyl-ovalbumin (FL-OA) three times a day from days 1–6, once a day from days 7–11, and then once every 3–4 days from days 12–22. They subsequently were challenged in the same eye with one drop of FL-OA on days 52 and 95.

Tear Sampling

To study the evolution of histamine release in the tears during the initial development of the conjunctival hypersensitivity, tears were sampled from the right eye before and 15 min after instillation of antigen from days 1–22. They also were collected from the unchallenged left eye, after the topical challenge of the right eye, on days 1, 11, and 18. To evaluate the kinetics of the release of histamine and 6-keto-PGF$_{1a}$ (the stable spontaneous degradation product of PGI$_2$) during a type I reaction in guinea pigs presenting various degrees of clinical responsiveness to the antigen (graded 1–4.5+, vide infra), tear samples were collected before antigen challenge; 10, 20, and 30 min after; and 1, 2, 4, 6, 8, 10, and 24 hr after challenge with 1 drop of FL-OA in the right eye. In some instances, tear samples also were taken at 2 and 7 min or 5 min. Samples were collected four times (on days 15, 22, 52, and 95), permitting the study of eight reactions for histamine and ten reactions for 6-keto-PGF$_{1a}$.

The tears were sampled by washing the conjunctival surface twice with 20 µl of saline, and the fluids (which will be called “tears”) were collected with a micropipette held close to the internal canthus. When collecting tears for 6-keto-PGF$_{1a}$, the pipette was rinsed with 20 µl of assay buffer, and this was added to the sample. The total volume of the tears collected was recorded. The tears were frozen within 5 min and stored at −20°C until assayed. The clinical reactions were graded (always by the same observer) on a 0–5+ scale, weighing edema 40%, vasodilation 40%, and tearing 20%.

 Conjunctival Stimulation by Histamine

To understand the role of histamine in the release of PGI$_2$ from constitutive conjunctival cells, two naive guinea pigs received one drop of 0.1 M histamine on both eyes, and tears were collected for 6-keto-PGF$_{1a}$ assay at 15, 30, and 60 min.

Assay Methods

Histamine was measured routinely in duplicate by radioimmunoassay. Tear samples, immediately after thawing, were brought to 100 µl by dilution with saline. The histamine in the samples and standards was acetylated first with succinylglycinamide-N-hydroxysuccinimide (SGA) and then mixed in competition with $^{125}$I-labeled SGA-histamine in polypropylene tubes coated with a monoclonal anti-SGA-histamine antibody (Amac, Westbrook, ME). Cross reactivities were 0.8% for PGE$_1$, 0.6% for PGF$_{2a}$, and 0.4% for thromboxane B$_2$, PGE$_2$, and PGI$_2$. After an 18-hr incubation, the antibody–antigen complexes were precipitated with polyethylene glycol and centrifuged. The supernatants were discarded, and the tubes containing the precipitated complexes were counted as described. A standard curve was constructed each time the assay was done. The limit of sensitivity of the radioimmunoassay was 2 pg of histamine. After appropriate corrections for assay dilution, the results were expressed as picograms of histamine recovered from the eye surface.

Tear samples were collected for histamine and 6-keto-PGF$_{1a}$ assays during the initial acute type I reaction after short intervals to determine more accurately the peak time of mediator release. Thereafter, tear samples were collected after longer intervals to maximize the recovery of histamine and 6-keto-PGF$_{1a}$ (above the limits of the radioimmunoassays) measurable in the accumulated tears. When mediator release in tears over different intervals were to be compared, the average rate of accumulation over a given interval was determined as: mediator recovered versus interval between time of prior sampling and present sampling (min).

Histology

Normal female Hartley guinea pig eyes were removed with the lids attached and cut in half vertically.
One half was fixed either in 0.6% formaldehyde, 0.5% acetic acid, pH 2.9 (IFAA), or Carnoy’s solution, at 4°C for 24 hr. The other half was fixed in 10% buffered formalin for 24 hr. Hartley guinea pigs, sensitized as described, were killed (without antigen instillation on the same day) on days 5, 8, 11, 15, and 22. The eyes were removed with the lids attached and fixed in IFAA at 4°C for 24 hr. All tissues were processed, embedded in paraffin, and sectioned at 5 μm. Histologic sections were stained with 0.5% toluidine blue at pH 4.0 or pH 0.5. The densities of granulated and degranulated mast cells per 40x field were counted using a Zeiss Universal microscope (Zeiss, Oberkochen, West Germany). Mast cells were counted in 20 fields per section (excluding the limbus and lid margin). Mast cells without a nucleus appearing in the same section were not included in the cell count.

Results

Histamine release in tears, measured by radioimmunoassay, and clinical responses of individual animals during the sensitization period are summarized in Figure 1. The first appearance of a clinical response and of histamine in the tears showed marked differences between animals. The quantity of histamine released also increased to different levels among the animals. During the sensitization period, histamine assayed by the radioenzymatic method was 2.2 and 6.4 pg/μl in reactions graded 1 and 3+, respectively. Although no clinical reaction was noted in the unchallenged left eyes, small amounts of histamine occasionally were recovered from the surface of the left eye after antigen instillation in the right eyes. For one animal, 12.6 pg of histamine was recovered on day 11 (right eye, clinical reaction graded 2+; histamine recovered, 206 pg) and 7.2 pg on day 18 (right eye, clinical reaction graded 4+; histamine recovered, 1035 pg). For another animal, 14.3 pg of histamine was recovered on day 18 (right eye, clinical reaction graded 3+; histamine recovered, 376.3 pg). Histamine recovery from the unchallenged left eye was not related to either the intensity of the clinical reaction, or the histamine output in the challenged right eye.

The clinical reactions in sensitized animals appeared rapidly after the conjunctival instillation of FL-OA (2–5 min) and reached a maximum at 7–10 min in all cases except one. In that instance, a clinical reaction graded 1+, consisting of vasodilation, but no edema or tearing, appeared at 2 hr, subsided to 0.5+ at 4 hr, and disappeared by 6 hr. Individual guinea pigs rechallenged at different dates had similar reactions from one occasion to another. The reactions of individual animals, however, varied significantly in intensity between animals. Small reactions, peaking at 0.5–1.5+, consisted mainly of vasodilation. All had returned to normal within 2 hr except one, which lasted up to 4 hr. Intense reactions, peaking at 3–4.5+, were associated with marked tearing, vasodilation, and edema. The tearing usually disappeared by 2 hr, but in some cases persisted for as long as 6 hr. The edema also cleared by 2–6 hr, depending on the intensity of the initial chemosis. The vasodilation was the longest lasting phenomenon, taking sometimes more than 10 hr to disappear (Fig. 2, Table 1). No second peak of edema, vasodilation, or tearing was observed after the initial peak had begun to subside. The clinical reaction always had completely disappeared by 24 hr after antigen challenge.

The time course of the recovery of histamine in tears, and of the clinical reaction, after antigen challenge in a sensitized guinea pig with a typical type I reaction is illustrated in Figure 2A. Histamine was always undetectable in the tears before antigen challenge. It remained undetectable in the animals showing little or no clinical response after antigen challenge (maximal clinical score, ≤0.5+). When a clinical reaction was present, histamine recovery peaked early, between 2 and 10 min (Fig. 2, Table 1). The peak levels varied widely among the animals (range, 31.6–535.5 pg). The average rate of accumulation of histamine was maximal between 0 and 10 min (Table 1). Histamine recovery then decreased rapidly, reaching undetectable levels at 1 hr in all cases. Pearson’s
Histamine (pg)  
Clinical reaction (x100)

Fig. 2. Recovery of histamine in tears after antigen challenge in two representative unilaterally (OD) topically sensitized animals. Typical reaction from right eye of one guinea pig (A) and occurrence of a late-phase histamine release from right eye of a second animal (B). (Sample measurements that were below the sensitivity of the assay were assigned a value of 0.)

correlation coefficient between the scores of the clinical reactions and the corresponding histamine recoveries was 72.4%.

Histamine usually remained undetectable from 1-24 hr. However, in one animal (Fig. 2B), after a peak of 236.5 pg at 10 min and a return to baseline at 1 hr, the histamine recovery increased again from 2-8 hr (peak, 78.8 pg at 4 hr). The average rates of accumulation of histamine on the eye surface over each time interval were: < 0.32 pg/min between 30 min and 1 hr (below the limit of sensitivity of the assay); 0.76 pg/min from 1-2 hr; and 0.66 pg/min from 2-4 hr. No second peak of tearing, edema, or vasodilation was observed in this animal.

The time course of the recovery of 6-keto-PGF\(_{1\alpha}\) after antigen challenge in sensitized guinea pigs is illustrated in Figure 3 and Table 1. No 6-keto-PGF\(_{1\alpha}\) was detectable in tears before antigen challenge. Tear fluid 6-keto-PGF\(_{1\alpha}\) increased after antigen challenge when the clinical reaction scored 1.5+ or more. When the clinical reaction was mild (two cases of reactions scoring 1.5+), low levels of 6-keto-PGF\(_{1\alpha}\) were found at only one time (11.3 and 7 pg at 5 and 2 min, respectively). In animals with more pronounced reactions, the level of 6-keto-PGF\(_{1\alpha}\) recovered in the tears started to increase at 2-10 min, reaching a maximum between 10 min and 1 hr, and then slowly decreased, becoming undetectable at 4-10 hr. A typical reaction is illustrated in Figure 3A. The average rate of accu-

Table 1. Histamine and 6-keto-PGF\(_{1\alpha}\) recovery, average rate of accumulation in tears, and clinical reactions, after antigen challenge

<table>
<thead>
<tr>
<th>Time</th>
<th>Histamine (n = 8)</th>
<th>6-keto-PGF(_{1\alpha}) (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Recovery (pg)</td>
<td>Rate (pg/min)</td>
</tr>
<tr>
<td>BC</td>
<td>225.6 ± 393.8</td>
<td>22.6 ± 39.4</td>
</tr>
<tr>
<td>10 min</td>
<td>(0-1093)</td>
<td>(0-109.3)</td>
</tr>
<tr>
<td>20 min</td>
<td>27.1 ± 36.4</td>
<td>2.7 ± 3.6</td>
</tr>
<tr>
<td></td>
<td>(0-80.9)</td>
<td>(0-81)</td>
</tr>
<tr>
<td>30 min</td>
<td>20.7 ± 28.4</td>
<td>2.1 ± 2.8</td>
</tr>
<tr>
<td></td>
<td>(0-72)</td>
<td>(0-7.2)</td>
</tr>
<tr>
<td>1 h</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2 h</td>
<td>7.9 ± 17.1</td>
<td>0.1 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>(0-45.8)</td>
<td>(0-0.8)</td>
</tr>
<tr>
<td>4 h</td>
<td>11.2 ± 29.8</td>
<td>0.1 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>(0-78.7)</td>
<td>(0-0.7)</td>
</tr>
<tr>
<td>6 h</td>
<td>5.7 ± 15.0</td>
<td>0.05 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>(0-39.8)</td>
<td>(0-0.3)</td>
</tr>
<tr>
<td>8 h</td>
<td>5.1 ± 13.5</td>
<td>0.04 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>(0-35.9)</td>
<td>(0-0.3)</td>
</tr>
<tr>
<td>10 h</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

All values presented as mean ± SD (min-max).
Sample measurements that were below the sensitivity of the assay were assigned a value of 0.
BC = before challenge.

Recovery is the total quantity recovered from the eye surface at the time point indicated.
Rate is the average rates of accumulation on the eye surface during the interval ending with the sample recovery.
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Table 2A. Tear histamine and 6-keto-PGF<sub>1α</sub> release, and ratio of 6-keto-PGF<sub>1α</sub>/histamine, in a guinea pig with a strong Type I reaction

<table>
<thead>
<tr>
<th></th>
<th>10 min</th>
<th>20 min</th>
<th>30 min</th>
<th>1 h</th>
<th>2 h</th>
<th>4 h</th>
<th>6 h</th>
<th>8 h</th>
<th>10 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Histamine</td>
<td>1093</td>
<td>80.9</td>
<td>35.7</td>
<td>&lt;9</td>
<td>&lt;9</td>
<td>&lt;9</td>
<td>&lt;9</td>
<td>&lt;9</td>
<td>&lt;9</td>
</tr>
<tr>
<td>(pg recovered)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6-Keto-PGF&lt;sub&gt;1α&lt;/sub&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(pg recovered)</td>
<td>13.5</td>
<td>12.6</td>
<td>10.5</td>
<td>9.9</td>
<td>18.5</td>
<td>16.5</td>
<td>11.5</td>
<td>7.1</td>
<td>&lt;6</td>
</tr>
<tr>
<td>6-Keto-PGF&lt;sub&gt;1α&lt;/sub&gt;/Histamine</td>
<td>0.012</td>
<td>0.16</td>
<td>0.29</td>
<td>&gt;1.1</td>
<td>&gt;2.1</td>
<td>&gt;1.8</td>
<td>&gt;1.3</td>
<td>&gt;0.8</td>
<td></td>
</tr>
</tbody>
</table>

* Clinical grading 3.5+ at 10 min after conjunctival challenge with FL-OA.
eye by circulating homocytotropic (eg, IgE and IgG₁) antibody³ and systemic passage of the antigen after topical challenge.⁴ Sensitized animals, receiving a topical instillation of antigen as long as 43 days after the last challenge, had clinical and tear histamine responses comparable with those observed after the previous challenge. The persistence of the sensitization indicated either that the mast cells had been sensitized with a homocytotropic antibody with a long tissue life (eg, IgE), or that a prolonged production of the antibody (eg, IgG₁) continued even in the absence of an intervening antigenic stimulus.³⁴¹¹

We may question how well variations in tear levels of histamine reflect those in the conjunctival substantia propria (not readily measurable directly in vivo). The quantity of histamine appearing on the ocular surface depends on the concentration in the underlying tissue and the leakage rate. The permeability of the conjunctival vessels increases under the influence of several mast cell mediators (eg, histamine, bradykinin, PGD₂, and leukotriene C₄) released early during the reaction. This leakage causes conjunctival edema and ultimately results in a flow of tissue fluids and serum proteins across the epithelium to the ocular surface, evidenced by fluorescent microscopy of labeled serum proteins, and sometimes by coagulation of the tears.¹¹ Histamine may diffuse independently or be carried by this flow to the ocular surface. The liquid collected from the eye surface at this stage is a mixture of “leaking” serum and reflex tearing from the lacrimal glands. The proportions of these elements may differ substantially, causing variations in the concentration of histamine in the tears. Examining the total quantity of histamine recovered after washing the ocular surface and collecting all of the resulting liquid, therefore, seems to be a more reliable method to approach the actual histamine output in the conjunctival tissue.

The metabolism of histamine by histaminase (a diamine oxidase) to imidazoleacetic acid, or by methyltransferase to methylhistamine, in the conjunctival tissue or on the ocular surface, also may influence the histamine measurements. In our study, the tear samples were frozen immediately to minimize possible enzymatic alteration of histamine after collection. Enhancement of vascular permeability during the inflammatory reaction caused proteins (among them, histaminase) to leak from the blood into the conjunctival tissue. Histaminase also is one of the lysosomal enzymes of eosinophils. Its release by guinea pig eosinophils is triggered by various inflammatory mediators, in particular leukotriene B₄.¹² After the early peak of histamine due to the initial degranulation of mast cells, the release of histaminase may have an effect on the levels of histamine in the tissue and on the ocular surface (in particular during the interval between tear collections).

Histamine release typically showed an early peak (2–10 min) and a return to baseline by 1 hr. In one instance, the kinetics of histamine release showed a biphasic early and late reaction. Calculating the rate of accumulation of histamine on the eye surface showed that the second peak was not created artificially by the differences of time intervals between successive tear collections. Late-phase reactions were described first in asthma and refer to the biphasic recurrence of the same phenomenon (eg, bronchoconstriction and histamine or leukotriene release).¹³ The late-phase reaction could be a result of the restimulation of the mast cell histamine release by a biphasic penetration of antigen, by anaphylatoxins C₃α or C₅α produced by an IgG₂-dependent type III mechanism,² or by other degranulating agents such as the cytokines histamine releasing factors,¹⁴ or neuropeptides (eg,

### Table 2B. Mean tear histamine and 6-keto-PGF₁α release, and ratio of 6-keto-PGF₁α/histamine, in guinea pigs with moderate and strong Type I reactions

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Histamine* (pg recovered)</th>
<th>6-Keto-PGF₁α† (pg recovered)</th>
<th>6-Keto-PGF₁α/Histamine</th>
<th>Clinical Reaction (mean)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 min</td>
<td>135.1</td>
<td>18.3</td>
<td>0.14</td>
<td></td>
</tr>
<tr>
<td>20 min</td>
<td>33.4</td>
<td>15.1</td>
<td>0.45</td>
<td></td>
</tr>
<tr>
<td>30 min</td>
<td>27.3</td>
<td>19.8</td>
<td>0.73</td>
<td></td>
</tr>
<tr>
<td>1 h</td>
<td>&lt;9</td>
<td>19.7</td>
<td>&gt;2.2</td>
<td>&lt;4.45 ± 0.5</td>
</tr>
<tr>
<td>2 h</td>
<td>&lt;8.8</td>
<td>20.2</td>
<td>&gt;2.3</td>
<td>116.3 ± 15.0</td>
</tr>
<tr>
<td>4 h</td>
<td>&lt;9.4</td>
<td>13</td>
<td>&gt;1.4</td>
<td>30.9 ± 14.9</td>
</tr>
<tr>
<td>6 h</td>
<td>&lt;9.0</td>
<td>8.7</td>
<td>&gt;1</td>
<td>41.1 ± 5.6</td>
</tr>
<tr>
<td>8 h</td>
<td>&lt;8.9</td>
<td>9</td>
<td>&gt;1</td>
<td>19.7 ± 14.9</td>
</tr>
</tbody>
</table>

* Five Guinea pigs.
† Six Guinea pigs.
vasoactive intestinal peptide, substance P, somatostatin, neurotensin, or calcitonin gene-related peptide). We observed that guinea pig conjunctival mast cells may be induced to release histamine by stimulation with the neuropeptides, somatostatin, bradykinin, and substance P (unpublished observations). The washout of histamine to the ocular surface also could occur in two waves, the first from histamine itself and the second from another late-appearing vasoactive mediator promoting recurrent vascular leakage (eg, leukotrienes C₄ and D₄, PGD₂, -E₂, or -I₂).

The compound PGI₂ also was produced by the topically sensitized conjunctival tissue after antigen challenge, as shown by assaying its stable metabolite, 6-keto-PGF₁α. It is a vasodilator and potentiates vascular leakage induced by histamine and bradykinin. It also disaggregates platelets and inhibits platelet aggregation. The production of eicosanoids (prostanoids, prostaglandins, leukotrienes, and lipoxins) and of reactive oxidants and free radicals during an inflammatory reaction may be linked intimately, and PGI₂ may function as an antioxidant and free-radical scavenger. It is converted rapidly to 6-keto-PGF₁α in vivo (half-life, 3 min), and its function may be predominately local as an autocrine or paracrine. After antigenic stimulation, PGI₂ appeared rapidly on the eye surface (in one case a significant level was found at 2 min). The peak recovery of 6-keto-PGF₁α occurred during the first hour. The metabolite 6-keto-PGF₁α was accumulated at significantly elevated rates for a much longer period than histamine. The ratio of 6-keto-PGF₁α to histamine increased on average by a factor of 16 between 10 min and 2 hr. This changing ratio may reflect the fact that histamine is released immediately by the conjunctival mast cells after stimulation by antigen; PGI₂ may be synthesized and released over a longer period of time. Also, 6-keto-PGF₁α may be released more slowly because of its lipophilic nature. Tears were not collected with a lipid-solubilizing agent because this would have damaged the ocular surface and the tear film, altering the reaction that was being studied. It was not possible to assay both histamine and 6-keto-PGF₁α in the same samples without sacrificing the assay sensitivities. However, measurements were made in the same group of responsive, sensitized animals, on the same dates, alternating which assay was done on a given animal from one occasion to the next. Even though there were large differences between the animals, probably because of varying degrees of sensitization, each individual animal's clinical reactions were similar in character and stayed within a limited range on the challenge dates. Once sensitized, each animal achieved similar clinical scores for all the challenges.

An important question is which cells synthesize the PGI₂ released during the conjunctival type I reaction. It must be emphasized that normal, constitutive conjunctival cells are capable of producing it. Our results show that the application of histamine on naive guinea pig eyes elicited a clinical response and a rapid increase of tear 6-keto-PGF₁α at levels comparable with those found in sensitized animals stimulated by antigen.

Mast cells produce arachidonic acid metabolites, mainly PGD₂ and leukotrienes B₄ and C₄. However, they do not appear to synthesize PGI₂. High-performance liquid chromatographic analyses of the arachidonate metabolites released by antigenic stimulation of sensitized purified mast cells showed high levels of PGD₂ and leukotriene C₄, but no 6-keto-PGF₁α. Possible sources of PGI₂ before infiltrating inflammatory cells are present include vascular cells (endothelial cells), smooth muscle cells, and fibroblasts and adipocytes. Rabbit corneal epithelial, stromal, and endothelial cells also are capable of producing PGI₂. Histamine stimulates the production of PGI₂ in vitro by human, rat, and murine vascular endothelial cells and bovine corneal endothelial cells through an H₁ receptor. Bradyskinin also stimulates PGI₂ production by adipocytes and vascular endothelial cells in vitro. We may therefore hypothesize that immediately after antigenic stimulation in sensitized eyes, mast cells release histamine and possibly other mediators, which then secondarily stimulate other types of constitutive cells (vascular, fibroblasts, and adipocytes) to produce and release PGI₂.

During later stages of the reaction, infiltrating cells such as macrophages and eosinophils also may release PGI₂. This late source of PGI₂ could be responsible for the prolonged release of 6-keto-PGF₁α and for the late peak of 6-keto-PGF₁α that was found in one instance (other mechanisms also may explain this late-phase reaction, particularly a second peak of histamine release or stimulation by another late-appearing mediator, vide supra).

The argument that 6-keto-PGF₁α may originate from the blood is difficult to verify, given the unreliability of 6-keto-PGF₁α blood levels. However, it seems unlikely that passive leakage from the blood could result in the high tear levels that were found because measurements of 6-keto-PGF₁α in the blood have shown levels ranging from 0.1 pg to a few picograms per microliter, most of which may result from the local reaction after the venous puncture. In our study, tears diluted more than ten times had similar levels.

Our findings illustrate the importance of interacting systems in ocular allergic reactions in vivo. Mast cell degranulation during a type I reaction may be the...
first step in a cascade of events involving complex intercellular interactions. Histamine, and possibly other mast cell mediators, may act as primary mediators stimulating the production of secondary mediators by other cell types, including both constitutive and infiltrating inflammatory cells. An understanding of the basis for the differences between diseases such as recurrent hay fever and vernal allergic conjunctivitis, and for effective therapy, may require a detailed knowledge of the interacting immunopathologic mechanisms operating in the living animal in each disease entity.

Key words: histamine, prostacyclin, allergic conjunctivitis, type I hypersensitivity, tears

Acknowledgments

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