DEFECTIVE RELEASE OF EOSINOPHIL CHEMOTACTIC FACTOR FROM PERIPHERAL LEUKOCYTES IN PATIENTS WITH CHRONIC URTICARIA

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Release of eosinophil chemotactic factor (ECF) and histamine was studied in peripheral leukocytes of 16 normal and 12 ragweed allergic volunteers and compared to 15 patients with chronic urticaria. Cells were exposed to varying concentrations of anti-IgE or to ragweed antigen E in the case of allergic donors. Twelve of 15 patients with chronic urticaria showed defective release of ECF as well as histamine, while almost all normal and all allergic donors were able to release larger quantities of both mediators. Since the eosinophil is thought to have a modulating effect at sites of inflammation induced by anaphylactic mechanisms, the defective release of a factor attracting these cells may explain the persistence of symptoms in chronic urticaria.

Immunologic reactions of the immediate type are associated with the release of mediators from mast cells and basophils. Urticaria is a cutaneous reaction induced with a variety of allergens and by physicochemical means. For chronic urticaria, defined as a recurrence of lesions at frequent intervals over more than 6 weeks, a cause can be found only in a small percentage of cases [1].

While studying the release of mediators from basophils in these patients, Greaves et al [2] noted that they release considerably less of the histamine stored in their basophils by methods of reverse anaphylaxis (i.e., anti-IgE) in contrast to normal individuals. These findings were confirmed in our laboratory [3], but the significance and mechanisms underlying this observation have not been clarified. In the present study, an attempt was made to further define this problem by examining whether the release of another mediator from basophils, the eosinophil chemotactic factor (ECF) [4], is also defective in patients with chronic urticaria.

ECF-A is a low-molecular-weight peptide whose structure has recently been determined [5]. It can be released from tissue mast cells by reverse anaphylaxis [6]. A similar or identical factor (ECF) is released from normal human basophils by the same method [4] or by the calcium ionophore A23187 [7] and from human neutrophils by the ionophore [8] or by phagocytosis [9].

MATERIALS AND METHODS

Fifteen patients with chronic urticaria of unknown cause which had persisted for more than 6 weeks were studied together with 16 normal volunteers and 12 ragweed allergic individuals. None of the subjects received any medication for at least 3 days prior to testing.

The method for mediator release has been described in detail [4]. Briefly, venous blood was drawn into ethylenediaminetetraacetic acid (EDTA) and leukocytes were allowed to sediment in 6% dextran for 2 to 3 hr. The supernatant containing leukocytes and platelets in plasma was then aspirated, centrifuged gently to remove platelets, and the leukocytes were washed twice in Tris buffer (pH 7.4) containing human albumin (0.03%). For release of mediators, aliquots of these cells were incubated in Tris-albumin buffer with added calcium (0.6 mM) and magnesium (1.0 mM) and were exposed to varying concentrations of highly purified anti-human IgE, prepared as described previously [10]. Ragweed antigen E (a gift of Dr. T. P. King) [11] was used for release from leukocytes of allergic donors. After 30 min of incubation at 37°C, cells were separated from the supernatants by centrifugation at 400 g for 5 min, and the supernatants were tested for histamine and ECF activity.

Histamine determinations were performed in duplicate by a spectrophotofluorometric technique [12]. Total cellular histamine content was obtained by lysis or sonication of cells, and the amount of antigen-induced release was expressed as percentage of the total. For ECF determinations, supernatants were placed in the lower part of a modified Boyden chamber, and 2.5 x 10⁶ purified human or guinea-pig eosinophils were placed above a nitrocellulose filter (Sartorius Membran Filter GMBH, Göttingen, Germany). After 3 hr of incubation at 37°C in 100% humidity, the filters were stained, dried, and cleared in m-Xylene (Eastman Kodak, Rochester, NY). Eosinophils that had migrated completely through the filters were counted under high-dry magnification. All samples were tested at least in duplicate. Details of...
this method have been published elsewhere [4,8]. Peripheral leukocyte counts were performed by examining fresh smears stained with Wright’s stain and by counting 100 cells with a high-dry objective under the microscope.

RESULTS

The Figure shows the typical release curves for histamine and ECF at increasing anti-IgE concentration in a healthy control and a patient with urticaria. As previously described [4], histamine and ECR release from basophils of normal or allergic individuals follow parallel dose–response curves with depression of release in the region of antigen excess. The patient with chronic urticaria, in this case, however, releases less than 5% of the total histamine content from his peripheral basophils and, under the same conditions, very little ECF release was observed. It was not possible to express the ECF activity as percent of total cellular content because, unlike histamine, very little or no ECF is released from basophils on sonication [13], and inhibitors of ECF are liberated from other cells present in the buffy coat [14]. ECF release was therefore expressed in terms of a threefold increase at optimal stimulation over that induced by the least effective stimulus. The difference between control values and a threefold increase of ECF activity had a p value of <0.001 in all subjects with normal histamine release curves.

The Table summarizes all the results obtained. The number of subjects showing a greater than threefold increase in ECF release and a more than 25% of total cellular histamine release is compared in the three groups studied. All allergic patients were able to release significant amounts of mediators on antigen E stimulation, and almost all normal subjects could equally be induced to release the mediators after anti-IgE challenge of their peripheral leukocytes. In contrast, the cells of only 3 of 15 patients with chronic urticaria behaved like normal cells after anti-IgE stimulation, and 12 released little or no histamine of ECF. The release curves for these two mediators at different anti-IgE concentrations were therefore flat in the latter individuals.

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Releasing agent</th>
<th>No. of patients</th>
<th>25% HR</th>
<th>3 \times ECF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>Anti-IgE</td>
<td>16</td>
<td>14</td>
<td>15</td>
</tr>
<tr>
<td>Allergic</td>
<td>Antigen E</td>
<td>12</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>Urticaria</td>
<td>Anti-IgE</td>
<td>15</td>
<td>3</td>
<td>3</td>
</tr>
</tbody>
</table>

The total histamine content of basophils was in the range of normal for patients and control subjects. Total ECF content could not be measured for the reasons outlined above [4,8,13]. Differential counts of peripheral leukocytes showed no eosinophilia (>4%) in any of the chronic urticaria patients.

DISCUSSION

The decreased histamine release from basophils of chronic urticaria patients by reverse anaphylaxis is an intriguing finding, the underlying defect for which is not apparent. After extensive studies in this laboratory, a “desensitization” of the cell has been suggested as the most likely cause [3]. The finding that mediators other than histamine are also not released during anti-IgE challenge suggests that the secretory mechanism of the cell, at least as it relates to the release of the mediators of anaphylaxis, is generally suppressed. Our previous studies demonstrated that the number of circulating basophils in the peripheral blood of patients is normal [3]. Also, several investigators reported normal [15] or only slightly elevated [2,3] serum IgE levels, and the cyclic AMP levels of peripheral leukocytes did not differ from those of controls [3].

Many chemical inhibitors of mediator release from basophils are known [16]. Histamine, when it reaches a certain concentration, inhibits further histamine release by elevating cyclic AMP in the cell [17], and elevated serum histamine levels have been demonstrated in patients with urticaria [18]. Beta-adrenergic agents such as epineprine also inhibit histamine formation so that it is possible that the decreased response is due to circulating hormones [16]. On the other hand, patients with chronic urticaria may be thought of as being intermittently exposed to stimuli which activate the cellular release mechanism and lead to a refractory or desensitized state.

Eosinophils are prominent in many allergic reactions and have been observed to be elevated in urticarial reactions associated with parasitism, serum sickness, and drug reactions. They are thought to have a modulating effect on allergic reactions, and they are reported to contain an inhibitor of histamine release [19] and an inactivator of another mediator, the slow-reacting sub-
stance of anaphylaxis [20]. The significance of a defective release of a factor (ECF) attracting these cells to sites of inflammation can only be speculative, but may partly explain the persistence and chronicity of symptoms in patients with chronic urticaria. It is of interest, finally, that the defect in release in these patients is concordant with respect to both a preformed mediator, histamine, and ECF which is generated after the immunologic stimuli [13].

REFERENCES