

Short communication

Tear tryptase levels and allergic conjunctivitis

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We measured tryptase, a neutral protease stored in the secretory granules of mast cells, by solid-phase radioimmunoassay in tears of 12 subjects with vernal keratoconjunctivitis (VKC) during remission phases, nine subjects with seasonal or perennial allergic conjunctivitis, and eight healthy controls. Mean values of tear tryptase levels were significantly ($P < 0.02$) increased in VKC patients ($14.5 \pm 13 \mu\text{g/l}$) when compared to those measured in patients with seasonal or perennial allergic conjunctivitis ($0.6 \pm 0.1 \mu\text{g/l}$) and in controls ($3.3 \pm 3.2 \mu\text{g/l}$). In subjects with allergic conjunctivitis, the levels of tryptase, almost undetectable before allergen conjunctival challenge, showed a significant increase in the challenged eye 20 min – but not 6 h – after provocation in 5/9 cases. Our results indicate that VKC, a severe ocular disease characterized by an increased number and abnormal distribution of mast cells in the conjunctiva, also shows elevated levels of tryptase in tears even during remission phases. Evidence of mast-cell activation, as revealed by a significant increase of tryptase levels in tears, is documented during the early-phase reaction, but not during the late-phase reaction, of allergic conjunctivitis patients challenged topically by specific allergen.

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Tryptase is a neutral protease stored in the secretory granules of mast cells. The enzyme is released after cross-linkage of high-affinity IgE Fc receptors by allergen or by the effect of other degranulating agents (1). The active form of tryptase is a tetramer, each subunit having one active site and common antigenic determinants; the tetrameric form is stabilized by binding to heparin and chondroitin-sulfate E (2–4). The large molecular size of the tryptase-heparin-proteoglycan complex is possibly responsible for the persistence of the active form of the enzyme in the site of mast-cell mediator release for a longer period than histamine (2, 5, 6). Moreover, while other mast-cell mediators are released by several inflammatory cells too, tryptase is specific for both tryptase- and chymase-tryptase-positive mast cells (in fact, basophils contain the enzyme in negligible amounts only). Finally, tryptase can be measured by a simple and reproducible immunoassay in both secretions and plasma or serum (7), while the measurement of histamine is often troublesome and its plasma levels can be

influenced by blood coagulation. Therefore, in view of its kinetics, specificity, and detectability, tryptase has been considered a useful marker in biologic fluids of the mast-cell activation and degranulation process (8, 9).

Evidence of the pathophysiologic and clinical usefulness of tryptase measurement has been reported in serum after systemic respiratory reactions and, more recently, in sudden death (10, 11). The enzyme has been detected also in nasal washings (12), bronchoalveolar lavage (13), and skin-chamber fluid (14) after allergen challenge.

In the eye, Fukagawa et al. (15) reported that tryptase was increased in the conjunctival epithelial cell suspension of six children with vernal keratoconjunctivitis (VKC). Butrus et al. (16) confirmed these findings by measuring tryptase levels in the tears of patients with various clinical pictures and phases of the disease or after allergen provocation.

Our recent research has focused on the pathophysiology of ocular allergy – in particular, vernal conjunctivitis – as a model for studying allergic

inflammation (17). The diagnosis of VKC is often difficult during asymptomatic phases since the typical clinical picture is not evident and IgE tests either are negative or are not specific for the disease. Butrus et al. (16) studied five patients with VKC. They all had increased tryptase tear levels but were symptomatic at the time of tear collection. Therefore, the first aim of this study was to investigate in both serum and tears whether tryptase levels are increased in vernal conjunctivitis during the asymptomatic phase, thus representing a useful clinical marker of ongoing inflammation of the eye. We also investigated whether tryptase values represent a distinct feature of different forms of "allergic conjunctivitis", such as RAST-positive and RAST-negative VKC or VKC and allergic conjunctivitis (seasonal or perennial).

In addition, a second problem is here addressed. Using the conjunctival provocation test with specific allergen in patients with allergic conjunctivitis, we were able to show that the ocular allergic response to allergen provocation is not confined to the typical early-phase reaction, but that long-standing inflammatory changes are also detectable 6–24 h after provocation (18). This late-phase reaction (LPR) in the eye is largely dependent on the dose of allergen used for provocation; the higher the dose of allergen, the more likely the ocular LPR to occur (19). The study of Butrus et al. (16) showed that tear tryptase levels increased 10 min after allergen provocation, returning to baseline values 40 min after challenge. However, the measurement of tryptase was confined to up to 4 h after provocation, and the minimal dose of allergen eliciting an early-phase reaction was employed. Therefore, the second aim of this paper was to investigate whether conjunctival provocation with high allergen doses and a more prolonged observation time allows detection of mast-cell involvement, as shown by tryptase release, during the late-phase ocular response.

Material and methods

Patients

We studied 21 subjects with allergic conjunctivitis (13 males and eight females aged 4–45 years; mean age 16 years) diagnosed as having vernal conjunctivitis (12 subjects) and seasonal or perennial allergic conjunctivitis (nine subjects). The diagnosis was based on clinical data, ocular examination and conjunctival scrapings. 12 VKC patients, six were skin-test- and/or RAST-positive (four to ryegrass, one to *Parietaria*, and one to more than one inhalant allergen). The nine patients with allergic conjunctivitis and the six with seasonal conjunctivitis

were shown to be sensitive to grass by skin test and RAST, while the three with perennial conjunctivitis were skin-test- and RAST-positive to *Dermatophagoides pteronyssinus* (two cases) or *Parietaria officinalis* (one case). All patients were studied during the asymptomatic phase and outside the pollen season. No treatment was given at least 15 days before the study.

Eight matched subjects (three males and five females aged 8–16 years; mean age 12 years) with no inflammatory eye symptoms or signs and negative skin test and RAST to common allergens were also studied.

Conjunctival provocation test (CPT)

The nine subjects affected by seasonal or perennial allergic conjunctivitis underwent ocular challenge with specific allergen. Challenges were performed at the lower conjunctival fornix of one eye, the other eye acting as control, with 100 000 SQ-U/ml in albumin (Pharmacia, ALK, Horsholm DK) of house-dust mite, *P. officinalis*, or ryegrass allergens. Each patient was clinically evaluated and tears were collected at the following time periods: before allergen challenge and 20 min and 6 h after allergen challenge. The criteria for positive conjunctival provocation test were redness, conjunctival edema, and itching, as well as inflammatory cell accumulation in tears, as previously described (19).

Tear collection

At least 10 µl of tear fluid was collected by placing a microcapillary tube in the inferior tarsal conjunctiva of each eye. The tears were stored at –20°C before the assay.

Tryptase determination

Tryptase levels were measured in both serum and tears of all subjects by a sandwich solid-phase radioimmunoassay (Tryptase RIACT, Pharmacia, Uppsala, Sweden) (7) based on the reaction between a monoclonal antibody linked to the solid phase of a test tube with the tryptase present in samples. The reaction is revealed by a second monoclonal antibody marked with radioactive ¹²⁵I. Tear fluid was diluted 1:5. The lower limit of sensitivity of the test with 50 µl of sample was shown to be 0.5 µg/l.

Statistical analysis

In view of the non-Gaussian distribution of individual values in each group, tear tryptase levels in all groups studied were expressed as median

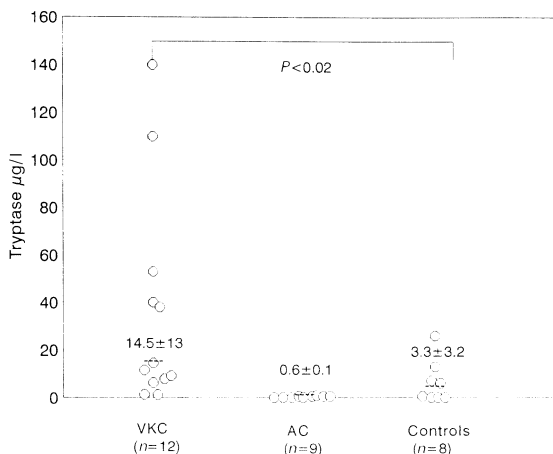


Fig. 1. Tryptase levels in tears were significantly higher ($P < 0.02$) in vernal keratoconjunctivitis (VKC) during remission phases than in seasonal and perennial allergic conjunctivitis (AC) and in controls.

values $\pm$ SE (and range), and statistical analysis was performed by the Mann-Whitney U test.

Results

Tear levels of tryptase in patients with VKC were significantly higher (14.5 ± 13 $\mu\text{g/l}$; range 1.2–140 $\mu\text{g/l}$) than those measured in patients with seasonal or perennial allergic conjunctivitis (0.6 ± 0.1 $\mu\text{g/l}$; range 0–0.9 $\mu\text{g/l}$) and in the eight control subjects (3.3 ± 3.2 $\mu\text{g/l}$; range 0–26 $\mu\text{g/l}$) ($P < 0.02$) (Fig. 1). All patients with VKC, but only 5/9 patients with seasonal or perennial allergic conjunctivitis and 4/8 controls, showed detectable (>0.5 $\mu\text{g/l}$) amounts of tryptase in tears. Patients with a positive skin test and/or RAST apparently had higher tear tryptase levels than those with negative skin tests and/or RAST (46.5 ± 22.8 in comparison with 10.3 ± 5.1 $\mu\text{g/l}$). However, and also in view of the low number of cases and high variability of values in the two groups, this difference did not reach statistical significance ($P = 0.086$).

Tryptase, almost undetectable before allergen provocation in both eyes of the nine patients with seasonal or perennial conjunctivitis, was shown to be present in significant amounts 20 min after challenge in the challenged eye of four grass-sensitive patients and one house-dust-mite-sensitive patient (values at least three times higher than basal values

and two times higher than those of the control nonchallenged eye). Six hours after challenge, the levels of tryptase returned to baseline values in both eyes.

In all serum samples tested from both patient and control groups, tryptase levels were below the sensitivity of the test used.

Discussion

Our data indicate that VKC patients, even during remission phases of the disease, have significantly increased levels of tryptase in tears.

VKC is a chronic bilateral, often severe inflammation of the eye, mainly affecting young males, with exacerbation during spring (20). VKC has been attributed to a type I hypersensitivity reaction because of the frequent positive history of atopy as well as of the increased IgE levels in both serum and tears (20). In fact, although IgE triggering is not always demonstrable, mast cells represent a constant and typical feature of VKC, as demonstrated by several immunohistochemical (21, 22) and cytologic studies (23). For instance, Irani et al. (21) showed that mast cells are increased in the substantia propria of the VKC conjunctiva when compared to the normal conjunctiva, and also populate the epithelium, which does not contain mast cells in normal subjects. Moreover, mast cells present in the substantia propria show extensive

apparent degranulation, as indicated by tissue deposits of tryptase and chymase along the outline of the mast-cell cytoplasmic membrane (21). These findings have been recently confirmed by Fukagawa et al. (15), who demonstrated significantly higher levels of tryptase in conjunctival epithelial cell suspensions of VKC and allergic conjunctivitis patients than in controls, thus indicating that mast cell are present in the conjunctival epithelium of these patients and, consequently, are directly involved in the local allergic inflammatory process. Morgan et al. confirmed these data and hypothesized that this "mast cell hyperplasia" could be controlled by T-cell factors, particularly interleukin-3 (22). The presence of measurable tryptase in tears is further functional evidence of the role of mast cells in causing hypersensitivity, inflammation, and symptoms in VKC patients, as previously suggested by Butrus et al. on the basis of preliminary observations (16).

Moreover, our data represent the first evidence that tryptase is present in tears also during asymptomatic phases of the disease. This would suggest an ongoing subclinical degranulation process in VKC patients, possibly requiring treatment also during remission of symptoms. In fact, mast-cell stabilizers are indicated in VKC (23). However, they may be unsuccessful during the active inflammatory phase, when only anti-inflammatory agents such as steroids are effective, with possibly severe side-effects (24). Therefore, we can speculate that the use of mast-cell stabilizers during asymptomatic phases might be useful to block the ongoing degranulation process and might prevent exacerbation of the disease.

In addition to the above reported considerations, our data in VKC patients may also have diagnostic relevance. In fact, the diagnosis of VKC is difficult during asymptomatic phases, when the typical clinical picture is not evident. Moreover, IgE tests are positive only in approximately 50% of patients (25). This study indicates that detectable amounts of tryptase are present in all asymptomatic VKC patients, both in RAST-positive- and in RAST-negative-forms, but not in hay fever conjunctivitis patients, thus representing a useful complementary, possibly diagnostic marker of the disease. Similar findings were also reported by Fukagawa et al. epithelial cell suspensions from patients with VKC and allergic conjunctivitis (15). However, for routine diagnostic purposes, tears represent a sample more easily collectable and standardizable than conjunctival epithelial cell suspensions.

On the other hand, tryptase was never detectable in the serum of both patients and controls. In fact, tryptase is released from mast cells which are only present at tissue level, and it is conceivable that a

degranulation process in a small confined tissue does not necessarily reflect increased levels of released mediators in the circulation.

With reference to the second aim of our paper, our data confirm the observations of Butrus et al. (16) that tear tryptase is increased during the early phase after allergen challenge. These data agree with those obtained in bronchial (13), nasal (12), and skin (14) provocation studies, which all underline the essential role of mast-cell activation during the early phase of allergic reaction.

In addition, we also measured tryptase levels 6 h after challenge with high allergen doses. In spite of a clinical and cytologic reaction, tryptase was not detectable in tears in all cases studied. The lack of this mast-cell-specific product during the LPR (when other mast cell/basophil products such as histamine are present) supports the theory that basophils are relevant during the LPR (26) while mast cells play a major role during the early phase. In fact, Naclerio et al. (27), after nasal challenge, measured increased levels of several mast-cell/basophil mediators during both the early and the late phases of allergic reaction, while prostaglandin D_2 , which is synthesized and released only by mast cells after allergen challenge, is present only during the early phase of allergic reaction.

In conclusion, this study provides additional information for a better understanding of the role of mast cells in allergic eye diseases and suggests that the measurement of tryptase in tears may have clinical relevance for the diagnosis and monitoring of these common pathologic conditions.

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