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# Inhibition of Allergen-Induced Histamine Release from Human Basophils by Cyclosporine A and FK-506

## Abstract

A number of structurally different allergens trigger the release of mediators from basophils by cross-linking of IgE receptors. In this study, we analyzed the effects of cyclosporine A (CSA) and FK-506 on allergen-induced histamine release in human blood basophils obtained from birch- or grass-pollen-allergic donors (n=12). Preincubation of basophils with CSA (0.003–3 µg/ml) or FK-506 (0.003–3 µg/ml) led to inhibition of histamine release induced by purified recombinant tree pollen allergens (r Bet v 1, r Bet v 2) and timothy grass pollen allergens (r Phl p 1, r Phl p 2, r Phl p 5). The effects of CSA and FK-506 were dose dependent, with IC<sub>50</sub> values ranging between 0.03 and 0.3 µg/ml for both CSA and FK-506. Cyclosporine H, an inactive CSA analog, did not show any effect on allergen-induced histamine secretion. IgE dependency of the reaction was demonstrated in passive transfer experiments using highly enriched human basophils (>95% pure) and specific IgE from a patient allergic to Bet v 2. In summary, our data show that CSA and FK-506 inhibit recombinant-allergen-induced histamine release from peripheral blood basophils in allergic donors.

## Key Words

Basophils  
Histamine release  
Recombinant allergens

## Introduction

Type I allergy caused by plant allergens (pollen- and plant-derived food stuff) represents a major health problem in industrialized countries. Clinical symptoms include allergic rhinitis, conjunctivitis and bronchial asthma. Such symptoms are primarily caused by vasoactive mediators produced in human basophils and/or mast cells. Allergens derived from plants can induce the release of these mediators by binding to membrane-bound IgE and consecutive cross-linking IgE receptors on mast cells and basophils [1, 2]. Both the natural as well as the recombinant birch pollen allergens [3] and the grass pollen allergens [4] can induce release of histamine from peripheral blood basophils of allergic donors [3–5].

Cyclosporine A (CSA) and FK-506 are two potent immunosuppressive drugs used in transplantation medicine and inflammatory disease states [6–11]. Both drugs exert anti-inflammatory effects on a variety of immune cells including T cells, mast cells and basophils [10]. Inhibition of IgE-dependent histamine release in mast cells and basophils by CSA and FK-506 has also been demonstrated [12–17]. However, so far the effects of these drugs on allergen-dependent histamine release have not been characterized in detail. The aims of the present study were to analyze systematically the effects of CSA and FK-506 on mediator release induced by 5 defined recombinant plant allergens.

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## Materials and Methods

### Patients

Birch-pollen- and grass-pollen-allergic patients were selected according to case history, positive RAST (birch pollen and/or grass pollen) and skin testing as described [3, 18, 19]. In addition, sera were tested in vitro (ELISA, IgE immunoblot) for IgE reactivity to recombinant birch pollen allergens (r Bet v 1 [20], r Bet v 2 [21]) and recombinant timothy grass pollen allergens (r Phl p 1 [22], r Phl p 2 [23], r Phl p 5 [4]) as described [24, 25].

### Expression and Purification of Recombinant Plant Allergens

Recombinant birch pollen (r Bet v 1, r Bet v 2) and timothy grass pollen allergens (r Phl p 1, r Phl p 2, r Phl p 5) were expressed as non-fusion proteins in lysogenic *Escherichia coli* BL21 (DE3) using pET expression plasmids (Novagen, Madison, Wisc., USA) in liquid culture after induction with isopropyl- $\beta$ -thiogalactopyranoside. r Bet v 2 was purified by poly-L-proline affinity chromatography as described [26, 27]. The purification of r Bet v 1 is described by Ferreira et al. [28], and recombinant timothy grass pollen allergens were purified by conventional chromatography [25].

### Isolation of Basophils

Peripheral blood was obtained from allergic patients (n=12) by venipuncture, after informed consent had been given. Granulocytes were recovered from whole blood by dextran sedimentation according to published techniques [29]. In brief, blood cells were fractionated by incubation in 1.1% dextran 70 and 8  $\mu$ mol/l ethylenediamine tetra-acetic acid for 90 min at room temperature. Thereafter, cells were washed 3 times in phosphate-buffered saline and then activation experiments were carried out.

### Purification of Human Blood Basophils to Homogeneity

Pure populations of peripheral blood basophils were obtained by a multi-step separation protocol essentially as described previously [30]. In brief, 500 ml of venous blood were collected (from healthy volunteers, n = 3) and the mononuclear blood cells isolated by use of Ficoll. Then, basophil-rich fractions were separated from lymphocytes by counterflow centrifugation, by loading  $3-8 \times 10^6$  mononuclear blood cells at 6 ml/min into a Beckman elutriator (Beckman Instruments, Palo Alto, Calif., USA), equipped with a JE-6B rotor (2,030 rpm at room temperature). Fractions were recovered at increasing flow rates and a selection was performed based on the content of basophils. In a second step, elutriated basophil-rich mononuclear blood cells were incubated with a monoclonal antibody against lactocyclamide, CDw17 (monoclonal antibody T5A7, obtained from the International Workshop and Conference on Leucocyte Differentiation Antigens, Oxford, 1987), and sorted for CDw17++ cells (basophils). After sorting, >95% of the cells were basophils as assessed by Giemsa staining. Cell viability was >80% in all experiments.

### Histamine Release Assay

The histamine release assay was performed on enriched peripheral blood basophils according to published techniques [29]. Basophils were preincubated with CSA or FK-506 (0.00003–3  $\mu$ g/ml) or control medium (Ca-containing PIPES buffer) for 15 min. Thereafter, cells were incubated with r Bet v 1, r Bet v 2, r Phl p 1, r Phl p 2 or r Phl p 5 (0.01–10  $\mu$ g/ml) for 30 min (37°C; 5% CO<sub>2</sub>). As positive control, the cells were also exposed to the anti-IgE monoclonal antibody E-124-2-8 (De2; Immunotech, Marseille, France; 0.1–1  $\mu$ g/ml). Cells were in-

cubated in 96-well microtiter plates (Costar, Cambridge, Mass., USA). After incubation, the cells were centrifuged at 4°C and the cell-free supernatants recovered and analyzed for the presence of histamine.

Histamine was measured in cell lysates or supernatants by a commercial radioimmunoassay (Immunotech) as described [29]. Total histamine was determined in cell lysates after freeze-thawing. Histamine release was calculated as percent of total histamine.

### Passive Sensitization Experiments

Highly enriched normal peripheral blood basophils were cultured for 24 h in RPMI 1640 medium supplemented with 10% FCS, glutamine, antibiotics and recombinant human IL-3, 1 U/ml (Sandoz, Vienna, Austria). Thereafter, the cells were incubated with serum recovered from a patient allergic to Bet v 2 or with IgE (myeloma derived; Chemicon AG, Temecula, Calif., USA) at 4°C for 3 h. Then, cells were washed 3 times and the IgE-preincubated cells exposed to anti-IgE (0.1–1  $\mu$ g/ml). The cells that had been preincubated with serum (from a Bet-v-2-allergic donor) were washed, exposed to CSA (3  $\mu$ g/ml), FK-506 (3  $\mu$ g/ml) or control medium for 15 min and thereafter challenged with the specific allergen (Bet v 2: 0.1–10  $\mu$ g/ml) for 30 min. Histamine was measured in cell-free supernatants as described above.

### Statistical Evaluation of Data

Differences in mediator content and secretion were analyzed by standard tests including the paired Student's t test. Results were considered significantly different when  $p < 0.05$ .

## Results

### Characterization of Patients

Peripheral blood basophils were obtained from 12 allergic patients aged 23–50 years (4 females, 8 males). All patients had a history of allergic rhinitis or conjunctivitis following exposure to allergens; in 5 patients allergen-induced asthma was known. Allergen-specific IgE against at least one of the tested allergens could be detected by immunoblotting. The histamine release provoked by allergens correlated well with the immunoblot data (table 1). The allergens did not induce secretion of histamine from basophils of patients without specific IgE (i.e. negative immunoblot).

### Allergen-Induced Histamine Release

In a first set of experiments, the effects of r Bet v 1, r Bet v 2, r Phl p 1, r Phl p 2 and r Phl p 5 on mediator secretion by human basophils from allergic donors were analyzed. For this purpose, cells were incubated with increasing concentrations of the respective allergen. Incubation of basophils with the allergens induced specific histamine release. Nine patients allergic to Bet v 1 were tested. The nonspecific release ranged between 1.3 and 11.5% (mean  $\pm$  SD:  $4.6 \pm 3.2\%$ ). The release mediated by r Bet v 1 (1  $\mu$ g/ml) ranged from 17.1 to 75.4% (mean  $\pm$  SD:  $32.5 \pm 17.4\%$ ) of total histamine ( $p < 0.005$ ). In 3 patients allergic to Bet v 2, the specif-

**Table 1.** Patient's characteristics

| Donor          | Sex | Age<br>years | Symptoms | Bet v 1 |      | Bet v 2 |      | Phl p 1 |      | Phl p 2 |      | Phl p 5 |      |
|----------------|-----|--------------|----------|---------|------|---------|------|---------|------|---------|------|---------|------|
|                |     |              |          | blot    | h.r. | blot    | h.r. | blot    | h.r. | blot    | h.r. | blot    | h.r. |
| 1              | M   | 30           | r, c, a  | +       | +    | —       | n.t. | —       | n.t. | —       | n.t. | —       | n.t. |
| 2              | F   | 27           | r, c, a  | +       | +    | —       | n.t. | +       | +    | —       | n.t. | +       | n.t. |
| 3 <sup>1</sup> | M   | 46           | r, c     | +       | +    | —       | n.t. | +       | n.t. | —       | n.t. | +       | n.t. |
| 4              | M   | 29           | r, c, a  | +       | +    | —       | n.t. | +       | n.t. | +       | +    | +       | n.t. |
| 5              | F   | 42           | r, c     | +       | +    | —       | n.t. | +       | +    | +       | +    | —       | n.t. |
| 6              | F   | 23           | r, c, a  | +       | +    | —       | n.t. | —       | n.t. | —       | n.t. | —       | n.t. |
| 7              | M   | 28           | r, c     | +       | +    | —       | —    | —       | —    | —       | n.t. | —       | n.t. |
| 8              | M   | 34           | r, c     | —       | —    | +       | +    | +       | +    | +       | +    | +       | +    |
| 9 <sup>1</sup> | M   | 36           | r, c, a  | +       | +    | +       | +    | +       | n.t. | +       | n.t. | +       | +    |
| 10             | M   | 34           | r, c     | —       | n.t. | —       | n.t. | +       | +    | +       | n.t. | +       | n.t. |
| 11             | M   | 32           | r, c     | +       | n.t. | —       | n.t. | +       | n.t. | +       | n.t. | +       | n.t. |
| 12             | F   | 50           | r, c     | +       | +    | +       | +    | +       | n.t. | n.t.    | n.t. | +       | n.t. |

r = Rhinitis; c = conjunctivitis; a = asthma; h.r. = histamine release; n.t. = not tested.

<sup>1</sup> Patients with prior hyposensitization.

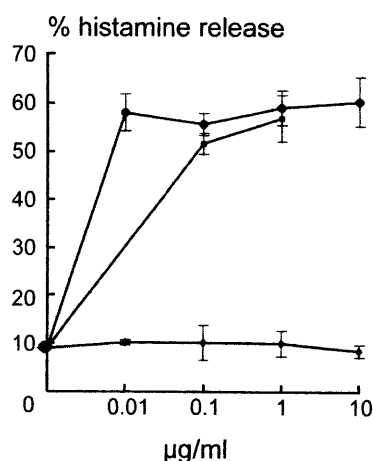


**Fig. 1.** Histamine release from human basophils of a donor (No. 8) allergic to Bet v 2. Peripheral blood basophils from a donor with a positive immunoblot for Bet v 2, but a negative blot for Bet v 1, were exposed to various concentrations (as indicated) of anti-IgE (■), r Bet v 1 (◆) or r Bet v 2 (●) for 30 min (37°C). After centrifugation, cell-free supernatants were analyzed for the presence of released histamine. Histamine release is expressed as percent of total histamine. The values represent the mean  $\pm$  SD.

ic release ranged between 27.1 and 71.3% (mean  $\pm$  SD: 49.6  $\pm$  16.5%) versus the control 0.8–17.7% (mean  $\pm$  SD: 6.2  $\pm$  6.0%). r Phl p 1 (1  $\mu$ g/ml) induced a histamine release of 9.7–81.9% (mean  $\pm$  SD: 43.8  $\pm$  26.7%) versus a nonspecific release of 0.6–15.6% (mean  $\pm$  SD: 4.6  $\pm$  5.1%) in 8 donors ( $p < 0.005$ ). Three patients allergic to Phl p 2 [nonspecific release, mean  $\pm$  SD: 7.4  $\pm$  5.7%, vs. r Phl p 2 (1  $\mu$ g/ml), mean

$\pm$  SD: 62.9  $\pm$  7.4%) and 2 patients allergic to Phl p 5 [control: 0.83 and 12.19%, mean: 6.8, vs. r Phl p 5 (1  $\mu$ g/ml): 60.7 and 68.7%, mean: 68.8] were tested. All allergens induced release of histamine from peripheral blood basophils in a dose-dependent way. In figure 1, the effect of r Bet v 2 on histamine release in one allergic donor (No. 8) is shown. Dose-dependent results were also obtained with r Bet v 2, r Phl p 1, r Phl p 2 and r Phl p 5 (not shown). No cross-reactivities between the different recombinant allergens were observed (fig. 1).

#### Effects of CSA and FK-506 on Allergen-Induced Histamine Release

The effects of CSA and FK-506 on allergen-induced histamine release of basophils were analyzed and compared. In typical experiments, cells were preincubated with CSA (3  $\mu$ g/ml) or FK-506 (3  $\mu$ g/ml) for 15 min. Thereafter, cells were incubated with allergens (0.1–10  $\mu$ g/ml) or (as control) with anti-IgE (0.1–1  $\mu$ g/ml). Preincubation with CSA or FK-506 resulted in inhibition of allergen-induced as well as anti-IgE-induced histamine release in basophils of allergic donors (fig. 2).

To assess whether the CSA-induced inhibition of histamine release was dose dependent, cells were preincubated with various concentrations of CSA (0.00003–3  $\mu$ g/ml) for 15 min before being stimulated with allergens (1  $\mu$ g/ml). CSA was found to downregulate allergen-dependent cell activation in a dose-dependent way, with an  $IC_{50}$  ranging between 0.03 and 0.3  $\mu$ g/ml. FK-506 also inhibited the allergen-dependent histamine release in a dose-dependent manner with  $IC_{50}$  values ranging between 0.03 and 0.3  $\mu$ g/

**Table 2.** Effects of CSA and FK-506 on allergen-induced histamine release (%) from human blood basophils of allergic donors

|               | r Bet v 1<br>(n = 5) | r Bet v 2<br>(n = 3) | r Phl p 1<br>(n = 7) | r Phl p 2<br>(n = 2) | r Phl p 5<br>(n = 1) | Mean      |
|---------------|----------------------|----------------------|----------------------|----------------------|----------------------|-----------|
| CSA, µg/ml    |                      |                      |                      |                      |                      |           |
| 0.00003       | 62.1±23.2            | 72.3±7.6             | 55.7±25.9            | 61.2                 | 69.0                 | 64.0±5.9  |
| 0.0003        | 70.2±21.2            | 59.6±16.8            | 38.1±20.4            | 54.7                 | 66.5                 | 57.3±11.2 |
| 0.003         | 49.4±21.3            | 62.0±16.3            | 48.5±19.4            | 50.3                 | 63.5                 | 54.7±6.5  |
| 0.03          | 34.3±19.1            | 65.4±2.3             | 47.2±14.5            | 42.2                 | 63.2                 | 50.5±12.0 |
| 0.3           | 11.9±8.4             | 42.5±1.7             | 27.9±9.6             | 36.4                 | 43.7                 | 32.5±11.7 |
| 3             | 16.1±15.5            | 39.3±7.9             | 29.9±9.6             | 44.6                 | 29.1                 | 31.6±9.8  |
| FK-506, µg/ml |                      |                      |                      |                      |                      |           |
| 0.00003       | 57.2±9.5             | 53.7±24.5            | 65.1±18.3            | 50.0                 | 69.0                 | 55.2±7.8  |
| 0.0003        | 64.2±18.2            | 53.5±19.1            | 44.5±15.1            | 52.2                 | 69.2                 | 56.8±8.9  |
| 0.003         | 46.9±9.8             | 54.6±13.0            | 48.5±11.4            | 54.9                 | 60.1                 | 49.6±10.3 |
| 0.03          | 41.8±11.1            | 54.8±18.6            | 44.2±17.1            | 46.1                 | 64.3                 | 50.2±8.3  |
| 0.3           | 20.1±19.2            | 42.5±18.4            | 34.0±18.2            | 39.6                 | 48.6                 | 39.1±11.5 |
| 3             | 11.4±10.3            | 34.9±2.7             | 26.1±17.1            | 39.9                 | 43.4                 | 31.2±11.4 |

The percentage of inhibition of histamine release was calculated by comparing histamine secretion in basophils preincubated with CSA (0.00003–3 µg/ml) or FK-506 (0.00003–3 µg/ml) with preincubation in control medium. The results for r Bet v 1, r Bet v 2 and r Phl p 1 represent the mean ± SD, the results of r Phl p 2 represent the mean of 2 donors.

ml. Table 2 gives a summary of the results obtained with CSA and FK-506.

The results of these experiments show that both CSA and FK-506 are potent inhibitors of allergen-induced histamine release in human basophils, with similar  $IC_{50}$  values for FK-506 and CSA. Cyclosporine H, an inactive CSA analog (lacking high affinity for cyclophylins), did not inhibit allergen-induced histamine release (not shown).

#### Passive Sensitization

The effects of CSA and FK-506 were also tested in a passive sensitization model. Normal basophils (nonallergic donors) were enriched to a purity of >95% by counterflow centrifugation and sorting. The highly enriched human basophils were preincubated with purified myeloma IgE or serum (specific IgE) of a patient allergic to Bet v 2.

When the purified basophils loaded with myeloma IgE were activated by anti-IgE, a dose-dependent secretion of histamine could be observed after 30 min [control: 3.6–15.5% vs. anti-IgE (10 µg/ml): 62.4–84.9%]. When cells were incubated with serum containing specific IgE, histamine release could be induced by the allergen. Cells were also incubated with CSA (3 µg/ml), FK-506 (3 µg/ml) or control medium, before being stimulated with r Bet v 2. Both CSA and FK-506 were able to downregulate the histamine release induced by Bet v 2 (Bet v 2: 13.7%; Bet v 2 + CSA: 5.4%; Bet v 2 + FK-506: 5.5%).

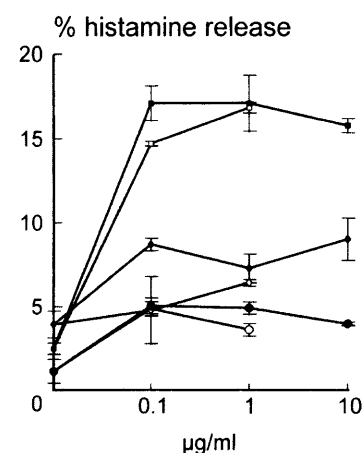


**Fig. 2.** Effect of r Bet v 1 or anti-IgE on basophils after preincubation with CSA, FK-506, or control medium. Peripheral blood basophils of a donor (No. 1) allergic to Bet v 1 (immunoblot Bet v 1 reactive) were preincubated with CSA (3 µg/ml), FK-506 (3 µg/ml) or control medium for 15 min. Cells were then challenged with r Bet v 1 (0.1–10 µg/ml), anti-IgE (0.1–1 µg/ml) or control medium. The filled symbols represent the exposure to r Bet v 1 (■), r Bet v 1 + CSA (◆) and r Bet v 1 + FK-506 (●). The open symbols represent the activation by anti-IgE (□), anti-IgE + CSA (◇) and anti-IgE + FK-506 (○). Cell-free supernatants (after centrifugation) were analyzed for the presence of released histamine. Histamine release is expressed as percent of total histamine. Values represent the mean ± SD of triplicate determinations.

## Discussion

CSA and FK-506 are potent immunosuppressive drugs with multifunctional anti-inflammatory activities. Both drugs have been described to inhibit immune cell activation and proliferation. Recently, CSA has been considered for use in patients with cortison-resistant asthma [31]. This consideration was based on the anti-inflammatory effects of CSA and FK-506 and on the *in vitro* inhibitory action of the drugs on lymphocyte [6, 8, 32–36], basophil and mast cell activation [12–17]. However, concerning basophils (and mast cells) such experiments had mostly been performed using experimental secretagogues but not a broader spectrum of environmental allergens. In this study, 5 different recombinant plant allergens were tested against the inhibitory effects of CSA and FK-506. Incubation of basophils from allergic patients with the defined recombinant allergens resulted in secretion of histamine. CSA and FK-506 were found to inhibit basophil histamine release induced by either anti-IgE, r Bet v 1, r Bet v 2 or recombinant grass pollen allergens of *Phleum pratense* (r Phl p 1, r Phl p 2 or r Phl p 5).

Allergic reactions in basophils are mediated via specific membrane-bound IgE and the IgE receptor [1]. So far, it has been shown that histamine release induced by cross-linking of the IgE receptor with either anti-IgE or the major mite allergen Der p 1 [12] can be downregulated by CSA and FK-506. In this study, IgE dependency of plant-allergen-induced cell activation could be demonstrated in passive sensitization experiments. In particular, highly enriched basophils from a nonallergic donor were incubated with serum (IgE) of a patient sensitized to birch profilin. Thereafter, the cells could be activated by the corresponding antigen (e.g. Bet v 2). In this model, the allergen-induced histamine release could again be downregulated by both CSA and FK-506, confirming the results obtained with basophils

from birch- or grass-pollen-allergic donors. Since 95% pure cells were used, our results suggest that CSA and FK-506 directly exert an inhibitory effect on human basophils.

In the last few years, a great effort has been undertaken to elucidate the mechanism of action of CSA and FK-506 [10, 37–41]. Both drugs apparently act on their target cells via a family of specific receptors (cyclophyllins) exhibiting rotamase activity. The existence of cyclophyllins has recently also been confirmed for human basophils [12, 15, 16]. Therefore, the involvement of such binding sites in CSA- and FK-506-induced inhibition seems likely. In line, the cyclophyllin-inactive CSA analog cyclosporine H showed no effect on allergen-dependent histamine release in this study.

The concentrations of CSA and FK-506 used in our experiments include those reached *in vivo* [7, 8, 31]. However, a full inhibition of allergen-induced basophil activation could only be achieved if high concentrations of CSA or FK-506 (1 µg/ml) were used. This fact and the well-known side effects of CSA and FK-506 argue against a wider use (over a long time) of such drugs in allergic disease. On the other hand, our data as well as the clinical observation by Alexander et al. [31] should encourage the search for basophil-specific, less toxic derivatives of CSA/FK-506.

In conclusion, our study provides evidence that CSA and FK-506 inhibit allergen-dependent activation and mediator secretion of human peripheral blood basophils in patients allergic to various plant allergens.

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