

Endogenous Superallergen Protein Fv Interacts with the V_H3 Region of IgE to Induce Cytokine Secretion from Human Basophils

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Key Words

Basophils · FcεRI · Histamine · IL-4 · IL-13 · Protein Fv

Abstract

Background: Protein Fv is an endogenous protein, synthesized in liver and largely released in the digestive tract during acute and chronic viral hepatitis, that binds to immunoglobulin (Ig) from various mammalian and nonmammalian species. **Methods:** Basophils obtained from normal subjects were purified by a double Percoll gradient and elutriation. The secretion of histamine induced by protein Fv was assayed by a fluorometric technique, the extracellular protein levels of IL-4 and IL-13 were measured by ELISA, and IL-4 mRNA levels were evaluated by RT-PCR. **Results:** Protein Fv concentration-dependently induced histamine and IL-4 and IL-13 release from purified basophils. IL-4 mRNA, constitutively present in basophils, was increased after stimulation by protein Fv. Histamine and IL-4 secretion from basophils, but not histamine and IL-13 release, activated by protein Fv was significantly correlated ($r_s = 0.70$, $p < 0.001$). Basophils from which IgE had been dissociated by brief exposure to lactic acid no longer released IL-4 in response to protein Fv and anti-IgE. Two preparations of human V_H3⁺ monoclonal IgM inhibited protein Fv-induced secretion of IL-4 and histamine from basophils. In contrast, V_H6⁺ monoclonal IgM did not inhibit the release of

mediators caused by protein Fv. **Conclusions:** These results indicate that protein Fv, which acts as an endogenous superallergen, interacts with the V_H3 domain of IgE to induce the synthesis and release of IL-4, IL-13 and the secretion of histamine from basophils.

Introduction

The immunoglobulin (Ig)-binding protein Fv (pFv) is present in normal liver and is released in biological fluids of patients affected by acute and chronic viral hepatitis B (≈35%), C (≈43%), A and E [1]. pFv binds the variable (V) domain of heavy (H) chains (V_H), irrespective of Ig class, subclass, allotype and L chain type [2]. The binding of this molecule involves mainly the human V_H3 family [2] and since pFv binds outside the antigen-binding site of Ab, it belongs to the B cell superantigen family [2, 3].

Skin rashes, urticarial reactions involving skin mast cells, and cutaneous and systemic vasculitis occur in patients with viral hepatitis [3–5]. Human basophils and mast cells, through the release of vasoactive and proinflammatory mediators (histamine) [6] and the elaboration of various cytokines (IL-4, IL-13) [7–10], play a role in cutaneous and systemic vasculitis and stimulate the collagen synthesis characteristic of the fibrotic response in chronic liver diseases [10].

We previously found that pFv induces the release of proinflammatory mediators from human basophils and mast cells by interacting with the IgE bound to FcεRI [11]. Here we describe the effect of increasing concentrations of pFv purified from patients F.A.R. and T.E.R. on the release of preformed mediator (histamine) and de novo synthesized cytokines (IL-4 and IL-13) from human basophils.

Materials and Methods

Preparation of pFv and Human Monoclonal IgE and IgM

pFv was purified from 2 patients (F.A.R. and T.E.R.) affected by HCV hepatitis [1]. IgE myeloma proteins were purified from 2 myeloma patients (P.P. and A.D.Z.) [11]. Monoclonal IgM were purified from the sera of patients with Waldenström's macroglobulinemia, as described [2].

Purification of Peripheral Blood Basophils and Isolation of Cellular mRNA and RT-PCR

Basophils were purified from peripheral blood of normal subjects (mean age 31.5 ± 6.1 years), undergoing hemapheris [7]. RNA was isolated by harvesting the basophils from culture wells and centrifuging for 30 s at 10,000 *g*. After removing the supernatants, the cell pellet was extracted with RNazol B (Tel-test, Friendswood, Tex., USA), which is a modified guanidinium thiocyanate single-step procedure. An aliquot of total cellular mRNA was reverse-transcribed to cDNA and PCR expanded with the GeneAmp RNA PCR Core Kit (Perkin Elmer International, Nieuwerkerk, The Netherlands) according to the manufacturer's protocol. The primers for IL-4 and IFN- γ were synthesized by commercial source (Life Technologies Italia srl, Milan, Italy). An aliquot (10 μ l) of the reaction product was visualized on 2% agarose in buffer containing 0.5 μ g/ml of ethidium bromide. As a negative control, an aliquot of each RNA sample was subjected to PCR amplification without the reverse transcription step. In some experiments an aliquot of total cellular mRNA was reversed-transcribed to cDNA and PCR expanded for quantitative PCR using a Cytopress™ Detection Kit (BioSource International, Camarillo, Calif., USA) [12].

Histamine, IL-4 and IL-13 Release from Basophils

Histamine release from purified basophils was performed as described elsewhere [11]. Histamine was measured with an automated fluorometric technique [7]. The harvested supernatants were assayed for IL-4 and IL-13 with the Quantikine™ kit (R&D Systems, Minneapolis, Minn., USA) [12, 13].

Results and Discussion

pFv (0.1–1 ng/ml) concentration-dependently induced the release of histamine, IL-4 and IL-13 from basophils purified from 7 donors. Two preparations of pFv (obtained from patients F.A.R. and T.E.R.) absorbed with protein A-Sepharose coated with human polyclonal IgG, which were negative for Ig binding, did not cause cytokine release.

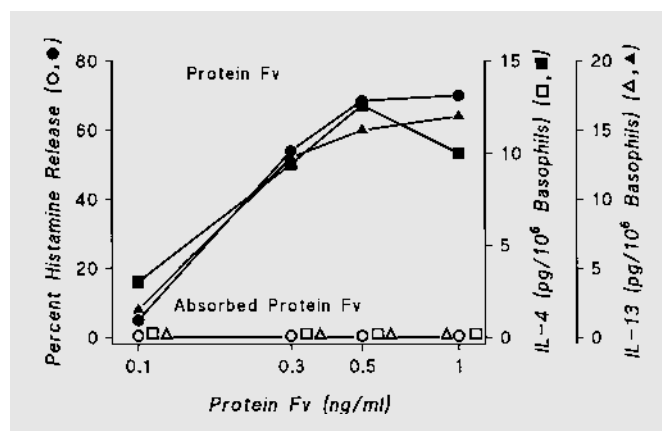


Fig. 1. Effects of increasing concentrations of pFv on histamine, IL-4 and IL-13 secretion from basophils obtained from a normal donor. pFv was absorbed with protein A-Sepharose coated with polyclonal IgG or nonabsorbed. Each symbol represents the mean mediator release from duplicate incubations or the mean of duplicate determinations from separate experiments.

Figure 1 shows a representative experiment in which pFv (0.1–1 ng/ml) concentration-dependently induced the release of histamine, IL-4 and IL-13 from purified (96%) basophils. The same preparations of pFv (F.A.R. and T.E.R.) absorbed with protein A-sepharose coated with normal IgG did not induce mediator release. We next evaluated series of experiments with IL-4 mRNA in resting and in pFv-stimulated basophils. Purified basophils ($\approx 99\%$) were cultured with rhIL-3 (10 ng/ml) for 16 h, washed, and challenged with anti-IgE (0.3 μ g/ml) or pFv (0.5 ng/ml) in the presence of rhIL-3 (10 ng/ml). IL-4 mRNA is constitutively expressed in human basophils, and pFv induced an apparent increase in IL-4 mRNA similar to the previously reported stimulation with anti-IgE [12, 13]. In these experiments, in which IL-4 mRNA was detected by RT-PCR, IFN- γ mRNA expression was not detected in control basophils or pFv- and anti-IgE-stimulated basophils. Therefore, IL-4 mRNA was extracted from purified preparations of basophils uncontaminated by IFN- γ -synthesizing leukocytes (e.g., lymphocytes and monocytes). A significant correlation was found between the percent histamine release and IL-4 secretion induced by pFv ($r_s = 0.70$; $p < 0.001$), but not between histamine and IL-13 release. Brief exposure to low pH removes most of the IgE bound on basophils, thus markedly reducing the activating properties of IgE-mediated stimuli [11]. In three experiments, brief exposure of basophils to lactic acid completely blocked the effect of pFv and of anti-IgE on the release of histamine, IL-4 and IL-13

from basophils. Preincubation of basophils with three preparations of monoclonal IgM (M3, M11, and LAN), which possess a V_H3 domain [2], inhibited the histamine-releasing activity of pFv in a dose-dependent fashion. In contrast, a monoclonal IgM (M14), which possesses a V_H6 domain, had a very low effect.

Our studies demonstrate that pFv acts as an endogenous superallergen. In fact, it causes the release of such important cytokines as IL-4 and IL-13 that exert immunoregulatory functions from human FcεRI⁺ cells. The mechanism of activation of FcεRI⁺ cells appears to be mediated by interaction pFv with the V_H3 region of membrane-bound IgE. Our data strongly suggest that an endogenous superallergen, pFv, which is released during viral infections, can induce the synthesis and secretion of cytokines IL-4 and IL-13 from basophils. pFv, released in patients with acute and chronic HCV or HBV infection, might contribute, through the release of IL-4 from FcεRI⁺, to the increased serum IgE levels found in these conditions [14]. We demonstrate that viruses

can induce in vivo the release of an endogenous superallergen (pFv) that acts on human FcεRI⁺ cells to release proinflammatory mediators and cytokines. Given the complex immunoregulatory role played by basophils, mast cells and their mediators [10], our data might be of interest not only to students of allergic diseases involving these cells [8, 11]. The mechanism of basophils/mast cell activation by pFv is a new model of a pathogenetic link between viral infections, FcεRI⁺ cell activation and cytokine release.

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