

THE NEUTROPHIL-ACTIVATING PEPTIDE NAF/NAP-1  
INDUCES HISTAMINE AND LEUKOTRIENE RELEASE  
BY INTERLEUKIN 3-PRIMED BASOPHILS

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Basophils are an infrequent type of leukocyte that bear high affinity IgE receptors, contain important amounts of histamine in their granules, and are capable of generating high levels of sulfidoleukotrienes. They are activated after IgE receptor cross-linking, and their products lead to the well-known signs of immediate-type hypersensitivity (1). Basophils are also activated by IgE-independent mechanisms, and may thus contribute to nonallergic inflammatory responses. So far two well-defined chemotactic peptides, the anaphylatoxin C5a and the bacterial product analogue FMLP, have been shown to induce basophil degranulation (1, 2). In addition, several cell-derived factors have been repeatedly implicated in basophil activation. However, up to now no defined and pure cytokine was found to have a potent and consistent histamine-releasing activity for basophils incubated in physiological buffers (3, 4).

NAF/NAP-1 is a novel neutrophil-activating peptide that was originally isolated from the culture fluids of stimulated human monocytes (5-7), and was subsequently shown to be produced by a variety of different cells (reviewed in reference 8). Like C5a and FMLP, NAF/NAP-1 activates human neutrophils, inducing chemotaxis, shape change, granule release, and the respiratory burst (8). In this paper we show that NAF/NAP-1 induces the release of histamine and leukotriene C<sub>4</sub> (LTC<sub>4</sub>) from human basophils that have been exposed to IL-3. Our results indicate that in the presence of IL-3, NAF/NAP-1 fulfills the role of a "histamine-releasing factor" and may thus mediate IgE-independent hypersensitivity reactions.

### Materials and Methods

*Mediator Release from Human Basophils.* Blood collection, cell isolation, and the mediator release assays were performed exactly as described (9). The mononuclear cell fractions containing the basophils were suspended at a cell density of  $2.5 \times 10^6$  cells in 20 mM Hepes, 125 mM NaCl, 5 mM KCl, 1 mM CaCl<sub>2</sub>, 1 mM MgCl<sub>2</sub>, 0.5 mM glucose, 0.025 human serum albumin (Calbiochem-Behring Corp., La Jolla, CA), pH 7.4. The release experiments were performed in a shaking water bath at 37°C and the reaction was stopped by placing the tubes in an ice bath, except for kinetic experiments where the cells were rapidly cooled by adding

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1 vol of ice cold buffer. Histamine release is expressed as percent of the total cellular histamine content minus the release in the buffer control sample. The total histamine content in the different cell preparations ranged from 41 to 168 ng histamine per  $2.5 \times 10^6$  cells. Sulfidoleukotriene generation is expressed as picograms sulfidoleukotriene per nanogram total cellular histamine. Each experiment was performed at least three times in triplicates.

**Granule Release from Human Neutrophils.** The release of primary and secondary granules in response to NAF/NAP-1 was assessed as described in detail (10). The release experiments were performed with  $4 \times 10^6$  neutrophils per milliliter preincubated with  $5 \mu\text{g/ml}$  cytochalasin B (Sigma Chemical Co., St. Louis, MO) for 5 min, and the reaction was stopped in an ice bath 15 min after the addition of the stimulus. Granule marker release is expressed as percent of the cellular content (determined from Triton X-100 lysed cells), minus the release in buffer controls.

**Stimuli.** Human rIL-3 (bioactivity of  $10^6$  U/1.5 mg) was a generous gift of Dr. M. Schreier, Sandoz, Basel, Switzerland. 1 U/ml is defined as the concentration of IL-3 giving a 50% of maximal [ $^3\text{H}$ ]TdR incorporation in cultures of leukocytes from chronic myeloid leukemia patients. Human recombinant NAF/NAP-1 from *Escherichia coli* had the same neutrophil-activating potency as the natural product isolated from LPS-stimulated mononuclear cells (11). The peptides were stored at  $-70^\circ\text{C}$  and added to the cells in a 1:100 volume ratio.

### Results and Discussion

Histamine release from basophils in response to NAF/NAP-1 was studied in blood mononuclear cells with and without pretreatment with IL-3, a pluripotent myeloid cell growth factor (12, 13) that was recently found to enhance mediator release by mature basophils in response to anti-IgE, FMLP, and C5a (9, 14). As shown in Fig. 1, stimulation with 100 nM NAF/NAP-1 alone did not result in histamine release, and in further experiments NAF/NAP-1 was equally ineffective in a concentration range of 1 to 1,000 nM. Thus NAF/NAP-1 differs from the chemotactic peptides C5a and FMLP, which induce basophil degranulation in a concentration-dependent manner (1, 2, 9). IL-3 alone also failed to induce histamine release at 20 ng/ml (Fig. 1) or at concentrations up to 1,000 ng/ml, which is consistent with former observations (9, 14). In contrast, when the cells were exposed to IL-3 (20 ng/ml) and then stimulated with NAF/NAP-1 (100 nM), a marked release of histamine corresponding to  $\sim 30\%$  of the cellular content was regularly observed. The situation was similar when production of  $\text{LTC}_4$  was determined. Basophils pretreated with 20 ng/ml IL-3 released on average  $2.3 \pm 0.47$  pg (SEM;  $n = 24$ )  $\text{LTC}_4$  per nanogram of cellular histamine upon stimulation with 100 nM NAF/NAP-1, whereas no sulfidoleukotriene production was detected after addition of either peptide alone, even at 10-fold higher

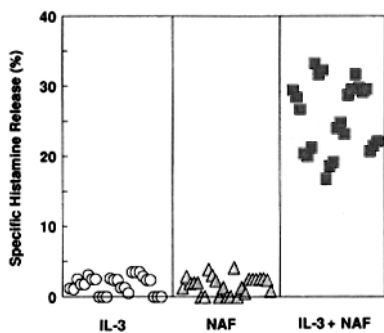


FIGURE 1. Histamine release by human blood basophils. The cells were treated with 20 ng/ml IL-3 for 25 min (IL-3, circles); 100 nM NAF/NAP-1 for 20 min (NAF, triangles); or 20 ng/ml IL-3 for 5 min followed by 100 nM NAF/NAP-1 for 20 min (IL-3 + NAF, squares). 24 determinations with cells from 8 different donors are shown.

concentrations. Although significant, the amount of LTC<sub>4</sub> released upon stimulation with NAF/NAP-1 was only 5–10% of that obtained after optimal IgE receptor crosslinking (1) or sequential treatment of basophils with IL-3 and C5a (9). It is known, however, that LTC<sub>4</sub> is ~1,000 times more potent than histamine on most target tissues (15), suggesting that the amounts of leukotrienes released by NAF/NAP-1-stimulated cells are sufficient to exert biological effects.

The induction of NAF/NAP-1 responsiveness by IL-3 was a rapid process. Some histamine release was apparent when IL-3 and NAF/NAP-1 were added together, but the maximal responsiveness to NAF/NAP-1 was observed when the cells were pretreated with IL-3 for 5 min (data not shown). Thus, the time required for IL-3-induced NAF/NAP-1 responsiveness was identical to that shown to be optimal for an enhanced degranulation response of basophils stimulated with C5a (9).

The effect of IL-3 was concentration dependent with a threshold between 0.1 and 1 ng/ml (Fig. 2). The response of IL-3-pretreated basophils to different concentrations of NAF/NAP-1 is illustrated in Fig. 3a. Histamine and sulfidoleukotriene re-

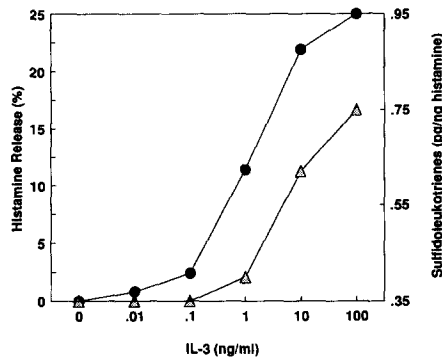


FIGURE 2. Effect of the IL-3 concentration on the NAF/NAP-1-induced basophil activation. The cells were exposed to IL-3 for 5 min and then stimulated with 100 nM NAF/NAP-1 for 20 min. Histamine release (*circles*) and sulfidoleukotriene generation (*triangles*). The lowest value of the right hand ordinate indicates the detection limit for sulfidoleukotrienes. Mean values of triplicates from a representative experiment.

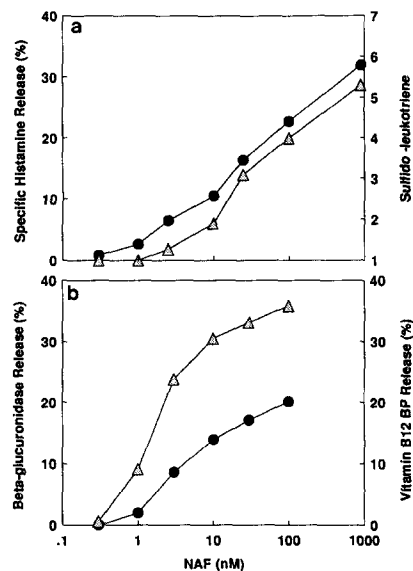


FIGURE 3. Activation of basophils (*a*) and neutrophils (*b*) by NAF/NAP-1. Dependence on the NAF/NAP-1 concentration. (*a*) Histamine release (*circles*) and generation of sulfidoleukotrienes (*triangles*) by basophils treated for 5 min with 20 ng/ml IL-3 and then stimulated with the indicated concentrations of NAF/NAP-1. (*b*) Release of  $\beta$ -glucuronidase from primary granules (*circles*) and vitamin B12-binding protein from secondary granules (*triangles*) induced by NAF/NAP-1 in human neutrophils. Percent of total cellular content released minus the values in unstimulated controls. Mean values of triplicate determinations.

lease increased progressively with the concentration of NAF/NAP-1 between 1 and 1,000 nM. For comparison, the well-established effects of NAP/NAP-1 on the release of neutrophil granule constituents were examined in parallel experiments (Fig. 3 *b*). These results show that IL-3-pretreated basophils are activated by NAF/NAP-1 in a similar concentration range as neutrophils. In additional experiments it was found that agonist-induced exocytosis of azurophil and specific granules in human neutrophils was not influenced by IL-3 (not shown). The time course of the release response of basophils induced by NAF/NAP-1 is shown in Fig. 4. Histamine and sulfidoleukotrienes were released rapidly, the maximum being reached within 5–10 min of stimulus addition.

NAF/NAP-1 is known to activate and attract neutrophils *in vitro* and to induce a neutrophilic cellular infiltration *in vivo* (5–8). Except for the reported chemotactic activity towards T lymphocytes (16), NAF/NAP-1 appeared to be a selective stimulus for neutrophils, since in contrast to other neutrophil agonists like C5a and FMLP, it does not activate monocytes or eosinophils (8, 17). We now show that upon treatment with IL-3, NAF/NAP-1 also triggers basophils to release histamine and sulfidoleukotrienes, mediators that can promote vascular leakage, smooth muscle contraction and mucus formation. Thus, in the presence of IL-3, NAF/NAP-1 functions like a “histamine-releasing factor” and may provoke symptoms of hypersensitivity reactions and induce edema.

IL-3 has been recently shown to enhance degranulation of basophils stimulated with anti-IgE, FMLP, and C5a (9, 14) and to change the mediator profile of basophils triggered by C5a (9). The present study demonstrates that IL-3 also has the capacity to render basophils responsive to NAF/NAP-1. It is well established that IL-3 is a product of T lymphocytes (12). Furthermore, IL-3 and NAF/NAP-1 are likely to be co-produced at sites of inflammation, since IL-3 (in addition to other cytokines) induces NAF/NAP-1 formation in monocytes (our unpublished observation). Thus,

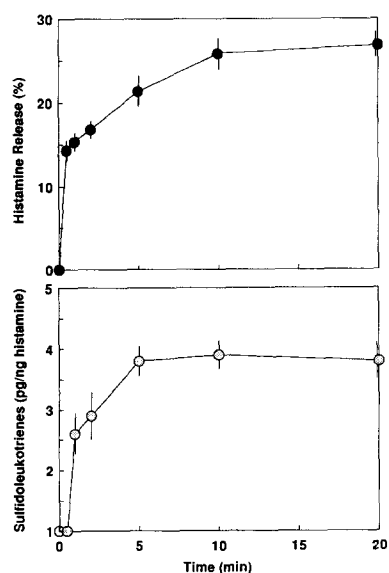


FIGURE 4. Time-course of product release by NAF/NAP-1-stimulated basophils. Cells were pretreated with 20 ng/ml IL-3 for 5 min and then stimulated with 100 nM NAP-1. Histamine and sulfidoleukotriene release was determined at different times after NAF/NAP-1 addition. Mean values  $\pm$  SEM from nine separate assays.

NAF/NAP-1-dependent basophil degranulation may occur in pathological conditions associated with T cell activation.

On the other hand, it has been shown recently that cultured murine mast cells release IL-3 after IgE receptor crosslinking (18, 19), suggesting that basophil activation by IL-3 and NAF/NAP-1 may also ensue from IgE-dependent events. It is interesting that immediately after antigen challenge of allergic individuals, the mast cell mediators histamine, proteases, sulfidoleukotrienes, and prostaglandin D<sub>2</sub> are detected in nasal washes. In so called late-phase reactions, which occur several hours after allergen challenge, a second peak of the same mediators was measured, but prostaglandin D<sub>2</sub> was lacking (20). From these observations it has been suggested that basophils are a major effector cell type in allergic late-phase reactions, since basophils are incapable of producing prostaglandin D<sub>2</sub> and since basophils are present in cellular infiltrates of late-phase reactions (20). Thus, basophil activation as described here could also be involved in the pathogenesis of allergic late-phase reactions, which are thought to be responsible for the major symptoms in clinical allergy.

### Summary

IgE-independent mediator release from basophils is considered an important event in inflammation, particularly in nonallergic immediate hypersensitivity and in allergic late-phase reactions. This study demonstrates that after exposure to IL-3, basophils release histamine and leukotrienes in response to the neutrophil-activating peptide NAF/NAP-1. Thus, the sequential action of two pure cytokines can promote basophils mediator release. In the presence of IL-3, NAF/NAP-1 functions like a "histamine-releasing factor" and may therefore not only induce cellular infiltration but also provoke symptoms of hypersensitivity reactions.

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