Vol. 179, No. 1, 1991
August 30, 1991

BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
Pages 628-633

INTERLEUKIN 8 - INHIBITOR AND INDUCER OF HISTAMINE AND LEUKOTRIENE RELEASE IN HUMAN BASOPHILS

Stephan C. Bischoff+, Marco Baggiolini*,
Alain L. de Weck+, and Clemens A. Dahinden+

+ Institute of Clinical Immunology, Inselspital, and * Theodor-Kocher Institute, University of Bern, CH-3010-Bern, Switzerland

Received July 18, 1991

We have shown previously that interleukin 8 (IL-8) induces histamine and leukotriene release in human basophils exposed to interleukin 3 (IL-3). We now found that pretreatment with low concentrations of IL-8 selectively inhibits this response. Inhibition was significant at 0.01 nM IL-8 and virtually complete at 1 nM, which is about 100-fold lower than the concentration required for induction of mediator release. IL-8 dependent responses were also inhibited, albeit to a lesser extent, by preincubation with neutrophil-activating peptide 2 (NAP-2), but not with connective tissue-activating peptide III (CTAP-III) or platelet factor 4 (PF4). Release induced by C5a, fMet-Leu-Phe, or anti-IgE antibody, by contrast, was not affected.

Human basophils release inflammatory mediators such as histamine and sulfidoleukotrienes in response to chemotactic receptor agonists like C5a or fMet-Leu-Phe (1). Similar effects are ascribed to the so-called histamine-releasing factors (HRF) that are produced by different cells including lymphocytes, monocytes, neutrophils and platelets (2-4). Because of the potential involvement of basophils in late-phase reactions (5) and the increasing interest in IgE-independent mediator release, the study of cell-derived HRF has gained considerable importance (6-9). Several cytokines have been studied, but no clear evidence for a direct mediator-releasing activity on basophils was found so far in healthy donors (9-12). We have recently shown that the neutrophil-activating cytokine, IL-8 (13), induces the release of histamine and leukotrienes from basophils pretreated with the hematopoietic growth factor IL-3 (14).

Factors derived from blood mononuclear cells, that induce and inhibit histamine release were recently reported (15). Since IL-8 is a major product of these cells and has some activity on basophils (14), we studied its ability to inhibit histamine and leukotriene release. We found that preincubation of basophils with IL-8 strongly inhibits the release reaction induced by the subsequent treatment with IL-3 and IL-8. Inhibition

0006-291X/91 $1.50

was observed at IL-8 concentrations that were about 100 times lower than those required for basophil stimulation.

METHODS

Reagents. Dextran and Ficoll Hypaque were obtained from Pharmacia (Uppsala, Sweden); Hepes from Callbiochem-Behring Corp. (La Jolla, CA, USA); bovine serum albumin (BSA) from Boehringer Inc. (Mannheim, FRG).

Basophil preparation. The cells were prepared essentially as described previously (11,12). Freshly-drawn blood from unselected donors was anticoagulated with 10 mM EDTA and sedimented for 90 min at room temperature after addition of 0.25 volumes of 6 % dextran. After washing in HA buffer (20 mM Hepes, 125 mM NaCl, 5 mM KCl, 0.5 mM glucose, 0.025% BSA, adjusted to pH 7.4), leukocytes were fractionated by Ficoll Hypaque density centrifugation (400g, 40 min, 20°C). The basophil-rich MNC layer was harvested, washed three times in HA buffer (400g, 10min, 4°C), and finally suspended in HACM buffer (HA buffer, 1 mM $CaCl_2$, 1 mM $MgCl_2$) at a density of 3-6x10^6 cells/ml. Cell preparations contained 1-8% basophils (mean: 3%) as determined by differential counting of May-Grünwald-stained cytocentrifuge slides.

Stimuli. Human recombinant IL-8 (16) was obtained from Dr. E. Liehl, Sandoz Research Institute Vienna, Austria. NAP-2, CTAP-III, and PF4 purified from platelet release supernatants (17) were kindly supplied by Dr. A. Walz, Theodor-Kocher Institute, Bern, Switzerland. The sources and use of human C5a, fMet-Leu-Phe, human recombinant IL-3 and mouse monoclonal anti-IgE antibody (Ab) LE 27 were described previously (11,12).

Mediator release. The experiments were performed at 37°C in a shaking water bath. After a warming-up for 10 min, the cells were preincubated with control buffer (HACM buffer) or different concentrations of IL-8. IL-3 (20 ng/ml) was added after 5 min and IL-8 (100 nM) 10 min later. The reaction was stopped after 30 min by placing the cells on ice. After centrifugation (400g, 10 min, 4°C) histamine and leukotriene release were determined in the supernatant by fluorometry and radioimmunoassy, respectively (11,12,14).

Presentation of data. Histamine release was expressed in percent of the total histamine content per sample (determined after cell lysis). Leukotriene generation was expressed as pg LTC4/D4/E4 per ng total histamine. Percentage inhibition of mediator release was calculated by the formula: [(b-a) - (d-c)] x 100/(b-a) according to Alam et al. (15), where a = buffer control, b = IL-3 + IL-8, c = IL-8 preincubation alone, d = IL-8 preincubation followed by stimulation with IL-3 and IL-8.

RESULTS

In agreement with our previous observations (14), histamine release was induced by IL-8 only when the basophils were pretreated with IL-3. With preparations from a total of 12 different donors, histamine release was significant after stimulation with 10 nM IL-8, and reached 10-25 % at the near-maximum effective IL-8 concentration of 100 nM. This level corresponds to about a third of the maximum release obtained in IL-3 pretreated cells challenged with C5a or anti-IgE Ab (11,12). These results confirm that IL-8 has HRF properties. Therefore, we investigated whether the same peptide is also able to inhibit basophil responses, and thus to act as HRIF. As

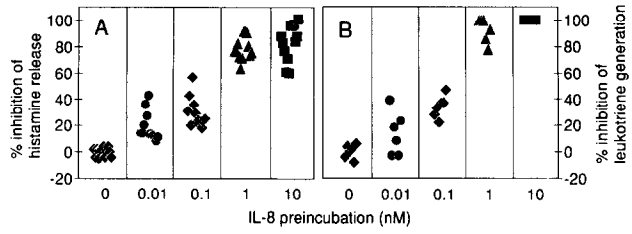

Fig.1. Inhibition of histamine (A) and sulfidoleukotriene (B) release from basophils by IL-8. Cells from four donors were preincubated with IL-8 (0-10 nM) for 5 min and then exposed to 20 ng/ml IL-3 and, 5 min later, 100 nM IL-8. After 30 min, the tubes were cooled on ice and centrifuged. In the absence of inhibition, specific histamine release (minus spontaneous release) was 10-25% and leukotriene release 0-22 pg/ng histamine. Cells from 2 of 4 donors did not release leukotrienes. Single values from experiments in triplicate are shown.

shown in Fig. 1, when basophils were pretreated with IL-8, the release of histamine and leukotrienes induced by sequential stimulation with IL-3 and IL-8 was inhibited. We have shown previously that leukotriene release does not always parallel the release of histamine (14), and in the present experiments only two of the four donors examined exhibited both responses. The inhibitory effect of IL-8 was already apparent at 0.01 nM and inhibition approached 100% at 1-10 nM. The concentration dependence was similar for both, histamine and leukotriene release, and the IC_{50} for IL-8 was approximately 0.3 nM.

We also assessed the ability of IL-8 to inhibit histamine release induced by other stimuli. As shown in Fig. 2, preincubation of basophils with 10 nM IL-8, a concentration that nearly abolishes IL-8 dependent basophil activation, did not influence histamine release in response to C5a, fMet-Leu-Phe or anti-IgE Ab, either given alone or after pretreatment with IL-3. In analogy, IL-8 did not affect leukotriene release induced under the same experimental conditions by the other stimuli (data not shown).

IL-8 is structurally related to the platelet granule constituents, platelet basic protein, CTAP-III, and PF4, and to the neutrophil-activating amino-terminal truncation product of CTAP-III, NAP-2 (17). Therefore, all these peptides were compared with IL-8 as potential inhibitors of IL-8 induced histamine release. The results of a representative experiment are shown in Fig. 3. NAP-2 exhibited weak, but significant inhibition of IL-8 induced histamine release, while CTAP-III and PF4 were completely ineffective.

In view of its selectivity, inhibition by IL-8 could result from receptor desensitization. We have, therefore, assessed the effects of pretreatment with IL-8 or C5a on basophil responses induced by maximally effective concentrations of the same agonists. As shown in Fig. 4, IL-8 and C5a induced histamine and leukotriene release

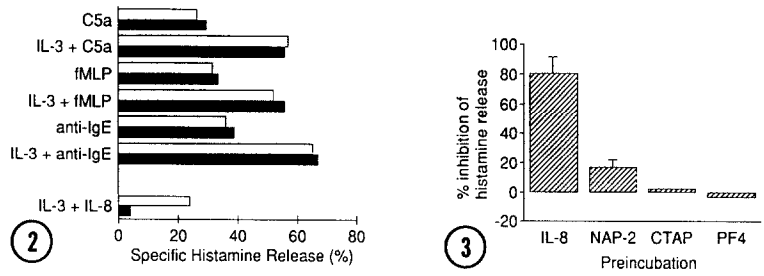

Fig. 2. Effect of preincubation with IL-8 on histamine release from basophils triggered by different stimuli. Cells were preincubated with (solid bars) or without (open bars) 10 nM IL-8 and then challenged with 10 nM C5a, 2.5 μM fMet-Leu-Phe, 100 ng/ml anti-IgE Ab or 100 nM IL-8. Where indicated, IL-3 was added 5 min prior to challenge. Experimental protocol as in Fig. 1. Mean values from 3 experiments with SD values below 10%.

Fig. 3. Effect of preincubation with IL-8 and homologues on histamine release from basophils. The cells were preincubated with the peptides at 10 nM and then challenged with IL-3 and IL-8 as described in Fig. 1. Mean values ± SD from 3 determinations.

in IL-3 pretreated cells. In confirmation of former observations (12,14), C5a was considerably more potent than IL-8 (ED_{50} values: 0.1-0.2 and 20 nM, respectively). Pretreatment of the cells with IL-8 or C5a resulted in a concentration-dependent

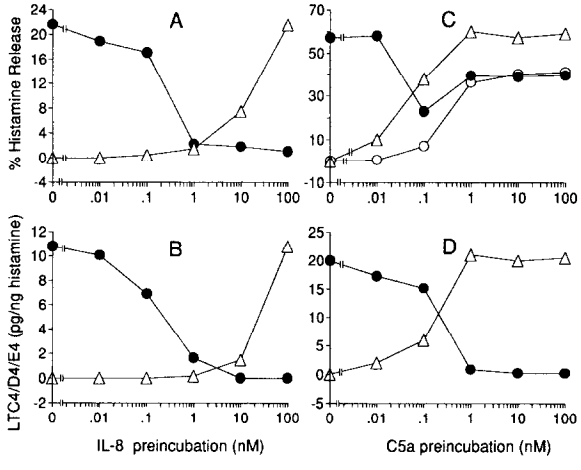

Fig.4. Inhibition of histamine and sulfidoleukotriene release from basophils by preincubation with IL-8 or C5a. Solid circles: Preincubation of the cells with increasing concentrations of IL-8 (A,B) or C5a (C,D) for 5 min, followed by exposure to 20 ng/ml IL-3 for 5 min, and then challenge with 100 nM IL-8 (A,B) or 1 nM C5a (C,D). Open triangles: Preincubation of the cells with medium alone for 5 min, followed by exposure to 20 ng/ml IL-3 for 5 min, and then challenge with increasing concentrations of IL-8 (A,B) or C5a (C,D). Open circles, panel C: Cells challenged with increasing concentrations of C5a only. Absence of open circles in panel A, B and D indicates that no mediator release was observed without exposure to IL-3. Means of triplicate values from one out of 3 experiments.

inhibition of the subsequent response to the same agonist. A comparison of the two sets of curves (A,B, and C,D) shows that IL-8 is inhibitory at concentrations that are about 100 times lower that those required for the induction of release (0.01-10 nM versus 1-1000 nM). In analogy to IL-8 (Fig. 2), pretreatment of the cells with C5a did not affect the release responses induced by unrelated stimuli such as anti-IgE Ab or fMet-Leu-Phe (data not shown).

DISCUSSION

This study shows that pretreatment with IL-8 selectively attenuates or abolishes the response of basophils to IL-8 dependent stimulation. A similar, but less potent inhibition was obtained with the IL-8 homologue, NAP-2, but not with CTAP-III or PF4. A comparable type of selective inhibition of homologous stimulation was also observed with C5a. With IL-8, however, inhibition was obtained at concentrations that were about 100-fold lower than those inducing release, while C5a showed inhibitory and agonistic effects in the same concentration range.

The present demonstration that IL-8 can act as inhibitor or inducer of basophil mediator release may be viewed in the context of reports on the generation of histamine-releasing and histamine release-inhibiting factors (HRF and HRIF, respectively) by blood mononuclear cells (2,4,8,9,15,18). Of particular interest is the HRIF recently described by Alam et al. (15), which shares with IL-8 both the the cell of origin and the apparent molecular weight of 8-10 kD. In addition, like IL-8, which was considerably more potent as inhibitor than as inducer of release, HRIF was reported to act at lower concentrations than the corresponding HRF. A potential role for IL-8 in the regulation of histamine release is also suggested by a report attributing agonistic properties to CTAP-III related peptides (18). CTAP-III itself, however, is unlikely to be active. In our hands, it consistently failed to induce histamine release, in contrast to IL-8 and NAP-2, even at concentrations as high as 1 μM, and upon pretreatment of the cells with IL-3. In addition, as shown in the present study, CTAP-III was ineffective (in contrast to IL-8 and NAP-2) as inhibitor of IL-8 dependent basophil activation.

The observed inhibition by IL-8 and C5a could result from desensitization, a phenomenon frequently observed upon repeated stimulation with the same agonist and known to occur in basophils (19). This presumably applies for C5a which showed inhibiting and activating effects in the same concentration range. IL-8, by contrast, was already highly inhibitory at concentrations that were 10 to 100-fold lower than those required for a release response, which is unusual for homologous desensitization (19,20). Rather, IL-8 appears to act on basophils like an antagonist with some intrinsic

agonistic activity. In view of the difference in the potency of the described effects, IL-8 is more likely to act physiologically as inhibitor rather than activator of basophils. Its activity, however, could be modified by the presence of other cytokines, as shown here for IL-3, which renders basophils responsive to IL-8 and can thus condition its action as inducer of release.

Acknowledgments: We thank Drs. M. Schreier and E. Liehl (Sandoz Ltd.) for providing us with cytokines, A. Walz (Theodor-Kocher Institute, Bern) for IL-8 homologues, and J. Zingg, P. Winkler and A. Sohn for excellent technical assistance. This study was supported by the Swiss National Science Foundation, grant 31-27980.89. S.C.B. is supported by a grant from the Deutsche Forschungsgemeinschaft.

REFERENCES

1. Grant, J. A., Lett-Brown, M. A., Warner, J. A., Plaut, M., Lichtenstein, L. M., Haak-Frendscho, M., and Kaplan, A. P. (1986) Fed.Proc. 45, 2653-2658.
2. Thueson, D. O., Speck, L. S., Lett-Brown, M. A., and Grant, J. A. (1979) J. Immunol. 123, 626-632.
3. Kaplan, A. P., Haak-Frendscho, M., Fauci, A., Dinarello C., and Halbert, E. (1985) J. Immunol. 135, 2027-2032.
4. Alam, R., Forsythe, P. A., Lett-Brown, M. A., and Grant, J. A. (1989) J. Immunol. 142, 3951-3956.
5. Naclerio, R. M., Proud D., Togias, A. G., Adkinson, N. F., Meyers, D. A., Kagey-Sobotka, A., Plaut, M., Norman, P. S., and Lichtenstein, L. M. (1985) N. Engl. J. Med. 313, 65-70.
6. Haak-Frendscho, M., Arai, N., Arai, K., Baeza, M. L., Finn, A., and Kaplan, A. P. (1988) J.Clin.Invest. 82, 17-20.
7. MacDonald, S. M., Schleimer, R. P., Kagey-Sobotka, A., Gillis, S., and Lichtenstein, L. M. (1989) J.Immunol. 142, 3527-3532.
8. Baeza, M. L., Reddigari, S., Haak-Frendscho, M., and Kaplan, A. P. (1989) J. Clin. Invest. 83,1204-1210.
9. Alam, R., Welter, J. B., Forsythe, P. A., Lett-Brown, M. A., and Grant, J. A. (1989) J.Immunol. 142, 3431-3435.
10. Hirai, K., Morita, Y., Misaki, Y., Ohta, K., Takaishi, T., Suzuki, S., Motoyoshi, K., and Miyamoto, T. (1988) J. Immunol. 141, 3958-3964.
11. Bischoff, S. C., Brunner, T., De Weck, A. L., and Dahinden, C. A. (1990) J. Exp. Med. 172, 1577-1582.
12. Kurimoto, Y., De Weck, A.L., and Dahinden, C.A. (1991) Eur. J. Immunol. 21, 361-368.
13. Baggiolini, M., Walz, A., and Kunkel, S. L. (1989) J. Clin. Invest. 84, 1045-1049.
14. Dahinden, C. A., Kurimoto, Y., De Weck, A. L., Lindlay, I., Dewald, B., and Baggiolini, M. (1989) J. Exp. Med. 170, 1787-1792.
15. Alam, R., Grant, J. A., and Lett-Brown, M. A. (1988) J. Clin. Invest. 82, 2056-2062.
16. Lindley, I., Aschauer, H., Seifert, J. M., Lam, C., Brunowsky, W., Kownatzki, E., Thelen, M., Peveri, P., Dewald, B., von Tscharner, V., Walz, A., and Baggiolini, M. (1988) Proc. Natl. Acad. Sci. USA. 85, 9199-9203.
17. Walz, A., Dewald, B., von Tscharner, V., and Baggiolini, M. (1989) J. Exp. Med. 170, 1745-1750.
18. Baeza, M. L., Reddigari, S. R., Kornfeld, D., Ramani, N., Smith, E. M., Hossler, P. A., Fischer, T., Castor, C. W., Gorevic, P. G., and Kaplan, A. P. (1990) J. Clin. Invest. 85, 1516-1521.
19. Siraganian, R., and Hook, W. A. (1977) J. Immunol. 119, 2078-2083.
20. Henson, P. M., Zanolari, B., Schwartzman, N. A., and Hong, S. R. (1978) J. Immunol. 121, 851-855.