Vol. 166, No. 1, 1990 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
January 15, 1990 Pages 187-192

NAP-1/IL-8 INDUCES UP-REGULATION OF CR1 RECEPTORS IN HUMAN NEUTROPHIL LEUKOCYTES

J.-P. Paccaud, J.A. Schifferli, and M. Baggiolini°

Division of Nephrology, Centre Médical Universitaire, Geneva, and °Theodor-Kocher Institut, University of Bern, Switzerland

Received November 14, 1989

The effect of the neutrophil-activating peptide NAP-1/IL-8 on the expression of complement receptor type 1 (CR1) in human neutrophils was studied. NAP-1/IL-8 enhanced CR1 expression at concentrations between 10^{-10} and 10^{-8} M. The maximum increase with respect to unstimulated control cells was on average 2.3 fold. The effect was rapid: Half-maximum enhancement was obtained in 4 min and the plateau was reached in 15 min. The chemotactic peptide fMLP, tested for comparison, was effective between 10^{-9} and 10^{-7} M, showed a similar time course and a somewhat higher maximum effect (2.8 fold increase). The effect of NAP-1/IL-8 was prevented by pretreatment of the cells with *B.pertussis* toxin and desensitization was observed following restimulation. Stimulus combination experiments suggested that NAP-1/IL-8 mobilizes the same or a similar intracellular pool of CR1 receptors as fMLP or C5a.

NAP-1/IL-8 is a recently discovered neutrophil-activating peptide produced by mononuclear phagocytes and a wide variety of tissue cells following stimulation with LPS, interleukin-1 or tumor necrosis factor (reviewed in reference 1). Like other chemotactic agonists, NAP-1/IL-8 induces a transient increase in the cytosolic free calcium concentration, shape change, chemotaxis, exocytosis and the respiratory burst. It acts via a selective receptor and appears to share, at least in part, the signal transduction system that operates for agonists like fMLP and C5a (1).

As a product of tissue cells, NAP-1/IL-8 is likely to mediate neutrophil invasion in a variety of inflammatory processes (1). Neutrophil activation is accompanied by the translocation of receptors for C3b/C4b (CR1) from their intracellular pool to the cell surface (2,3). These receptors are involved in the inactivation of C3b as well as in the binding and phagocytosis of C3b-coated particles (4,5) and are upregulated by stimulation of

0006-291X/90 $1.50

the neutrophils with chemoattractants like fMLP, C5a, LTB_4 and PAF (3,6,7), or cytokines like tumor necrosis factor (8).

We have now studied the effects of NAP-1/IL-8 on CR1 expression in human neutrophils and report here that the novel peptide upregulates CR1 in a similar way as the classical chemotaxins, fMLP and C5a.

METHODS

Buffers and Reagents. Bovine serum albumin (BSA), Ficoll 400 and Dextran T 500 were obtained from Pharmacia, and N-formyl-methionyl-leucyl-phenylalanine (fMLP) from Sigma. Purified human C5a was kindly provided by Dr. C. Dahinden, Institute of Clinical Immunology, University of Bern; and NAP-1/IL-8 was obtained from the Sandoz Research Institute, Vienna, Austria (9). fMLP was dissolved in dimethylsulfoxyde at 10^{-3} M and diluted with PBS, and the other stimuli were dissolved in PBS. Phosphate-buffered saline, pH 7.4, (Oxoid, Basingstoke, England) as such (PBS) or supplemented with 5 mg/ml BSA (PBSB) was used. When indicated, 1 mM $CaCl_2$ was added to PBSB.

Cell purification. Neutrophils were isolated at 4°C from fresh donor blood (10) and kept in PBSB at a concentration of 10^7 cells/ml on melting ice before use. Such preparations consisted of >96% neutrophils and >99% of the cells excluded trypan blue. All experiments were started within 30 min following cell preparation.

Stimulations and CR1 assays. Cell samples (150 µl) were prewarmed for 5 min at 37°C, exposed to a stimulus, and the response was stopped after different times by cooling on ice. CR1 expression was determined (11) using radiolabelled monoclonal antibody E11 kindly provided by Dr. N. Hogg, Imperial Cancer Research Fund, London (12). *B.pertussis* toxin treatment was performed by incubating the cell suspension with 1 µg/ml of the toxin (List Biochemical) for 60 min at 37°C. The treatment was terminated by centrifugation, and the cells were resuspended at the original concentration in PBSB.

RESULTS

The ability of NAP-1/IL-8 to increase the expression of CR1 was compared to that of fMLP. As shown in Fig. 1 the effects of both agonists were concentration dependent. The treshold concentration was 10^{-10} M for NAP-1/IL-8 and 10^{-9} M for fMLP. Maximum expression was obtained at 10^{-8} and 10^{-7} M, respectively, and no further increase was observed with 10^{-7} and 10^{-6} M NAP-1/IL-8 or 10^{-6} M fMLP. As clearly indicated by the crossing of the dose-response curves, NAP-1/IL-8 was superior to fMLP at low concentrations, but induced a somewhat lower maximum expression of CR1 (226 ± 20% versus 277 ± 25%). The time course of the response was similar for both agonists. In either case, CR1 expression reached a maximum 15 min after

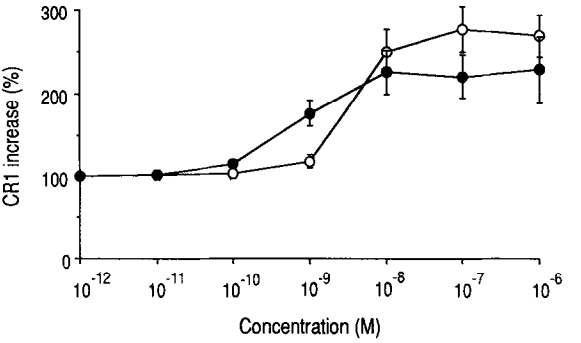

Figure 1. Effect of NAF and fMLP on CR1 expression in human PMN. The cells were exposed to increasing concentrations of NAP-1/IL-8 or fMLP, and CR1 expression was determined after 15 min. Relative increase with respect to unstimulated PMN, mean ± SEM of 6 experiments.

stimulation and half-maximum values were obtained within 4 min (Fig. 2). The experiments illustrated in Figs. 1 and 2 were done in PBSB without added Ca^{2+}. In two additional experiments performed in the presence of 1 mM Ca^{2+} no differences in the magnitude or in the time course of the response were observed.

fMLP and other chemotactic agonists are thought to mobilize the same intracellular CR1 storage compartment (8). Information on the compartment that is mobilized by NAP-1/IL-8 was obtained by repeated stimulation with combinations of NAP-1/IL-8, fMLP and C5a. The cells were first exposed for 15 min to NAP-1/IL-8 and then for another 15 min to the same agonist, fMLP or C5a. Restimulation with an

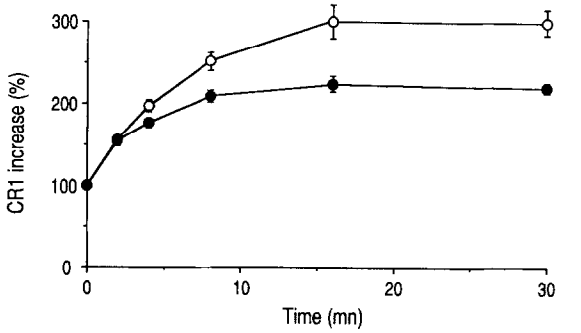

Figure 2. Time course of CR1 expression induced by NAP-1/IL-8 and fMLP in human PMN. The cells were exposed to 10^{-8} M NAP-1/IL-8 or 10^{-7} m fMLP and the relative increase in CR1 expression over time was determined (mean ± SEM of 6 experiments).

unrelated agonist resulted in a rise in CR1 expression over the level obtained with NAP-1/IL-8 alone, but no effect was obtained upon resti-mulation with NAP-1/IL-8. As shown in Fig. 3a, fMLP or C5a as the second stimulus induced a rise in CR1 density even after a maximum effective concentration of NAP-1/IL-8 (10^{-8} M). Upon repeated application, however, the effects of the two stimuli were not additive. The degree of CR1 expression obtained after stimulation with NAP-1/IL-8 plus fMLP or NAP-1/IL-8 plus C5a was about the same as after stimulation with maximum effective concentrations of fMLP or C5a alone, suggesting that all three agonists induce mobilization of the same intracellular pool of receptors. Similar experiments were done by exposing the neutrophils first to one of the three agonists and then to NAP-1/IL-8. As shown in Fig. 3b, no further enhancement of CR1 expression could be induced by NAP-1/IL-8, when maximum effective concentrations of fMLP or C5a were used initially. The desensitization of the neutrophils to repeated stimulation with NAP-1/IL-8 and the lack of cross-desensitization towards fMLP or C5a indicate that NAP-1/IL-8 acts via a selective receptor, a conclusion which is in accord with former observations concerning other neutrophils responses (1). In the present experiments, desensitization was also observed when the cells were stimulated twice with fMLP or C5a.

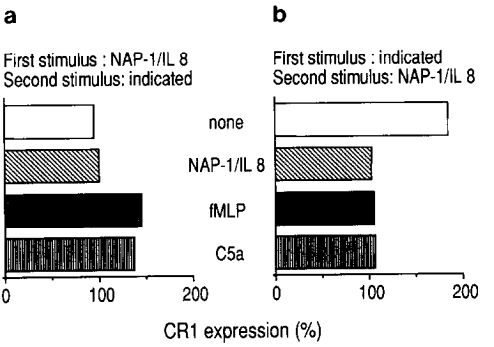

Figure 3. Effect of combined stimulations. (a) The PMN were stimulated with 10^{-8} M NAP-1/IL-8 and then exposed to PBSB (Control), 10^{-8} M NAP-1/IL-8, 10^{-7} M fMLP or 10^{-8} C5a. Relative changes in CR1 expression with respect to the value obtained with a single stimulation with 10^{-8} M NAP-1/IL-8 (100%) are shown. (b) The PMN were exposed to PBSB (control), 10^{-8} NAP-1/IL-8, 10^{-7} M fMLP or 10^{-8} M C5a, and stimulated with 10^{-8} M NAP-1/IL-8 after 15 min. Relative changes in CR1 expression with respect to the value obtained with the first treatment (100%) are shown. The results are representative for 3 separate experiments.

Further evidence that CR1 mobilization is a receptor-mediated event was obtained by pretreating the cells with *B.pertussis* toxin to block G-protein function. Under these conditions, no enhancement of CR1 expression was observed after stimulation with NAP-1/IL-8, fMLP or C5a while the upregulation induced by PMA remained unaffected (data not shown).

Double stimulation was also used to assess a possible priming effect of NAP-1/IL-8. Neutrophils were exposed for 15 min to treshold concentrations of NAP-1/IL-8 (10^{-10} M) and then to suboptimal concentrations of NAP-1/IL-8, fMLP or C5a. A response to the second stimulation was observed in all cases, but CR1 expression was not enhanced by pretreatment with NAP-1/IL-8, indicating that this peptide does not prime the neutrophils.

DISCUSSION

The present study shows that the novel chemotactic peptide NAP-1/IL-8 enhances the expression of CR1 on human neutrophils by a receptor-dependent and selective mechanism. As compared to fMLP, NAP-1/IL-8 had a lower threshold concentration, a similar time course of action and a somewhat lower maximum effect. The latter difference could be due to only partial mobilization of the intracellular pool of receptors or to a higher rate of re-internalization. Two lines of evidence argue in favor of the first possibility: (a) additional mobilization could be obtained with fMLP after maximum stimulation with NAP-1/IL-8, and (b) expression remained constant after reaching the maximum, indicating that re-uptake was minimal. NAP-1/IL-8, like other agonists, differed from PMA which enhances both expression and internalization (13). NAP-1/IL-8 did not enhance further CR1 expression in neutrophils stimulated with maximum effective amounts of either fMLP or C5a, while some enhancement was observed with the reversed order of stimulation. This suggests that all three agonists are effecting the same intracellular pool of receptors and that NAP-1/IL-8 is unable to mobilize this pool completely. The possibility remains open, however, that neutrophils contain more than one intracellular CR1 compartment, and that the action of NAP-1/IL-8 is more restricted than that of other agonists. Analysis of the internal pool(s) of CR1 would be necessary to clarify this issue. It is well known, on the other

hand, that the magnitude of neutrophil responses to unrelated agonists may differ considerably, as shown for instance by studies on the respiratory burst (14). The present observations could also reflect a difference in the duration of action or in part of the signal transduction system of NAP-1/IL-8 and the other peptides. It has been shown, however, that the mechanism of activation of neutrophils stimulated with NAP-1/IL-8 is qualitatively similar to that observed with other agonists (1).

The upregulation of CR1 demonstrated in this study indicates that neutrophils recruited into inflamed tissues by NAP-1/IL-8 generated locally not only release enzymes, oxygen-derived radicals and other phlogogenic products, but also acquire enhanced functional capabilities through receptor upregulation.

ACKNOWLEDGMENTS

We thank Miss Gertraud Steiger for excellent technical assistance and Sabine Imer for editorial assistance. This work was supported by the Swiss National Science Foundation, grants 32-25606.88 and 31-25700.88. J.A.S. is recipient of a Max Cloëtta Career Development Award.

REFERENCES

1. Baggiolini, M., Walz, A. and Kunkel, S.L. (1989) J. Clin. Invest. **84**, 1045-1049.
2. Fearon, D.T. (1980) J. Exp. Med. **152**, 20-30.
3. Fearon, D.T. and Collins L.A. (1983) J. Immunol. **130**, 370-375.
4. Wright S.D. and Silverstein S.C. (1983) J.Exp. Med. **158**, 2016-2023.
5. Medof, M.E., Iida, K., Mold, C. and Nussenzweig, V. (1982) J. Exp. Med. **156**, 1739-1754.
6. Berger, M., Birx, D.L., Wetzler, E.M., O'Shea, J.J., Brown, E.J. and Cross, A.S. (1985) J. Immunol. **135**, 1342-1348.
7. Shalit, M., Von Allmen, C., Atkins, P.C. and Zweiman, B. (1988) J. Leuk. Biol., **44**, 212-217.
8. Berger, M., Wetzler, E.M. and Wallis, R.S. (1988) Blood, 71, 151-158.
9. Lindley, I., Aschauer, H., Seifert, J.M., Lam, C., Brunowsky, W., Kownatzki, E., Thelen, M., Peveri, P., Dewald, B., von Tscharner, V., Walz, A., Baggiolini, M. (1988) Proc. Natl. Acad. Sci. USA **85**, 9199-9203.
10. Boyüm, A. (1976) Scan. J. Immunol. **5**, 9-15.
11. Ross, G.D., Yount, W.J., Walport, M.J., Winfield, J.B., Parker, C.J., Fuller, C., Taylor, R.P., Myones, B.L. and Lachmann, P.J. (1985) J. Immunol., **135**, 2005-2014.
12. Hogg, N., Ross, G.D., Jones, D.B., Slusarenko, M., Walport, M.J. and Lachmann, P.J. (1984) Eur. J. Immunol. **14**, 236-243.
13. Changelian, P.S., Jack, R.M., Collins, L.A. et al. (1985) J. Immunol. **134**, 1851-1858.
14. Wymann, M.P., von Tscharner, V., Deranleau, D.A., Baggiolini, M. (1987) J. Biol. Chem. **262**, 12048-12053.