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DIFFERENTIAL EFFECTS OF NEUTROPHIL-ACTIVATING PEPTIDE 1/IL-8 AND ITS HOMOLOGUES ON LEUKOCYTE ADHESION AND PHAGOCYTOSIS[1]

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Several structural homologues of the chemotactic peptide neutrophil-activating peptide 1/IL-8 (NAP-1/IL-8) were tested for their ability to influence the expression and function of adhesion-promoting receptors on human polymorphonuclear leukocytes (PMN). NAP-2, melanoma growth stimulatory activity, and two forms of NAP-1/IL-8 (ser-NAP-1/IL-8 and ala-NAP-1/IL-8, consisting of 72 and 77 amino acids, respectively), each caused an increase in the expression of CD11b/CD18 (CR3) and CR1, which was accompanied by a decrease in the expression of leukocyte adhesion molecule-1 (LAM-1, LECAM-1). The binding activity of CD11b/CD18 was also enhanced 3- to 10-fold by these peptides, but enhanced function was transient: binding of erythrocytes coated with C3bi reached a maximum by 30 min and declined thereafter. Ser-NAP-1/IL-8, ala-NAP-1/IL-8, NAP-2, and melanoma growth stimulatory activity also caused a two- to threefold enhancement of the phagocytosis of IgG-coated erythrocytes (EIgG) by PMN without causing a large increase in the expression of Fcγ receptors. Enhanced phagocytosis of EIgG appeared to be mediated through CD11b/CD18, because F(ab')$_2$ fragments of an antibody directed against CD18 inhibited NAP-1/IL-8-stimulated ingestion of EIgG. The four active peptides caused a rapid, transient increase in the amount of F-actin within PMN, indicating that they are capable of influencing the structure of the microfilamentous cytoskeleton, which participates in phagocytosis. Two other NAP-1/IL-8-related peptides, platelet factor 4 and connective tissue-activating peptide III, were without effect on expression of CD11b/CD18, CR1, and LAM-1, binding activity of CD11b/CD18, or Fc-mediated phagocytosis, and increased actin polymerization only slightly. Our observations indicate that several members of the NAP-1/IL-8 family of peptides were capable of promoting integrin-mediated adhesion and Fc-mediated phagocytosis, processes important in the recruitment of PMN to sites of inflammation and antimicrobial responses of PMN.

NAP-1/IL-8[3] is released in at least two different forms by monocytes (1) and endothelial cells (2) stimulated with bacterial LPS, IL-1, or TNF. The originally described form of NAP-1/IL-8 consists of 72 residues with a serine at the N-terminus (ser-NAP-1/IL-8), whereas the other common form has five additional N-terminal amino acids starting with an alanine (ala-NAP-1/IL-8). NAP-1/IL-8 belongs to a growing family of structurally related cytokines, which includes the human proteins IFN-γ-induced 10-kDa protein (3), *gro*/MGSA (4), PF-4 (5), CTAP-III (6), and its cleavage product NAP-2 (7), as well as the murine protein macrophage inflammatory protein 2 (8). Several of these proteins have stimulatory effects on human PMN, causing chemotaxis, cytosolic free calcium changes, and exocytosis.

We have previously shown that ser-NAP-1/IL-8 increases the expression of the adhesion-promoting receptor CD11b/CD18 (CR3) on the surface of PMN and also enhances the binding activity of this receptor for several ligands, including C3bi, LPS, fibrinogen, and a structure on endothelial cells (9). We have now compared the effects of the two major forms of NAP-1/IL-8 consisting of 72 and 77 amino acids (ser-NAP-1/IL-8, ala-NAP-1/IL-8), *gro*/MGSA, NAP-2, PF-4, and CTAP-III on the expression and function of several receptors involved in leukocyte adhesion and the interaction of PMN with opsonized particles. We report that both forms of NAP-1/IL-8, *gro*/MGSA and NAP-2 enhanced the expression of CD11b/CD18 and CR1 on PMN. Increased expression of CD11b/CD18 was accompanied by decreased expression of leukocyte adhesion molecule-1 (LAM-1, LECAM-1). The four active peptides also strongly stimulated the binding activity of CD11b/CD18, caused a two- to threefold enhancement of Fc-mediated phagocytosis, and induced rapid, transient polymerization of actin. In contrast, PF-4 and CTAP-III caused no change in receptor expression or function and stimulated actin polymerization only slightly. The ability of NAP-1/IL-8 and related peptides

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[3] Abbreviations used in this paper: NAP-1/IL-8, neutrophil-activating peptide 1/IL-8; NAP-2, neutrophil-activating peptide 2; *gro*/MGSA, melanoma growth stimulatory activity; LAM-1, leukocyte adhesion molecule 1; EC3bi, erythrocytes coated with C3bi; EIgG, erythrocytes coated with IgG; PF-4, platelet factor 4; CTAP-III, connective tissue-activating peptide III; PMN, polymorphonuclear leukocyte; fNLLP, formyl-norleucyl-leucyl-phenylalanine; PD, PBS without $CaCl_2$ or $MgCl_2$ but with 0.02% NaN_3; HSA, human serum albumin; HAP, PBS, 0.05% glucose, 0.5 mg/ml HSA and 0.3 U/ml aprotinin; AI, attachment index; PI, phagocytic index.

to stimulate the interaction of PMN with opsonized particles suggests that they may also enhance antimicrobial activities or clearance of debris at sites of infection or inflammation.

MATERIALS AND METHODS

Reagents and buffers. Ser-NAP-1/IL-8, ala-NAP-1/IL-8, and *gro*/MGSA were prepared by automated synthesis (4, 10), and NAP-2, PF-4, and CTAP-III were purified from thrombin-induced platelet release supernatants (7). All peptides were stored frozen at concentrations higher than 10^{-5} M. Aprotinin and fNLLP were purchased from Sigma Chemical Co., St. Louis, MO. Pyrogen-free HSA was supplied by Armour Pharmaceutical (Kankakee, IL) and Alpha Therapeutic Corp. (Los Angeles, CA). Buffers used were: pyrogen-free PBS (137 mM NaCl, 2.7 mM KCl, 0.9 mM $CaCl_2$, 0.5 mM $MgCl_2$, and 8 mM phosphate, pH 7.4) (Sigma); PD; and HAP.

Antibodies. mAb provided by colleagues were: OKM10, directed against CD11b (11), from Dr. Patricia Rao (Ortho Pharmaceuticals, Raritan, NJ); YZ, directed against CR1 (12), from Dr. Richard Jack (Harvard Medical School, Boston, MA); IV.3, directed against FcγRII (13), from Dr. Michael Fanger (Dartmouth Medical School, Hanover, NH). Anti-Leu 8 was purchased from Becton Dickinson Immunocytometry Systems (Mountain View, CA). 3G8, directed against FcγRIII (14), IB4, directed against CD18 (11), OKT3, directed against CD3 (15), and W6/32, directed against HLA-A,B,C (16), were as described. $F(ab')_2$ fragments of IB4 prepared by papain cleavage were provided by Dr. Samuel Wright (Rockefeller University, New York, NY).

Preparation of PMN and opsonized erythrocytes. Freshly drawn, heparinized blood from normal human donors was used for the isolation of PMN on neutrophil isolation medium (NIM, Los Alamos Diagnostics, Los Alamos, NM): 8 ml of blood were layered on 4 ml of NIM and centrifuged at ambient temperature for 30 min at 400 × *g*. The layer containing PMN was removed to a separate tube, and contaminating erythrocytes were lysed by brief exposure to hypotonic buffer. PMN were suspended in HAP buffer at a final concentration of 1×10^6 cells/ml for attachment and phagocytosis assays or 2×10^6 cells/ml for flow cytometry.

Sheep erythrocytes were coated with IgG or IgM as previously described (17). EC3bi were prepared by incubating EIgM in C5-deficient human serum (Sigma) as described (18).

Flow cytometry. The expression of cell surface Ag was measured after PMN were exposed to mAb (5 μg/ml in PD, 0.1% HSA) for 20 min at 0°C, washed, and incubated with fluoresceinated affinity-purified goat anti-mouse IgG $F(ab)'_2$ (7 μg/ml in PD, 0.1% HSA) (Tago, Burlingame, CA) for 45 min at 0°C. Fluorescence was measured on a Becton Dickinson FACScan cell analyzer. Forward and right angle light scatter were used to select PMN and eliminate the few contaminating erythrocytes. The mAb OKT3 was used as a negative control in all experiments, and the mean fluorescent channel derived from this antibody, which was the same as that obtained by omitting the primary antibody (mean fluorescent channel ≤4), was subtracted from those of experimental samples. The background staining remained very constant between experiments and exhibited no change upon stimulation of the PMN. Data are shown for representative experiments, along with the mean fold increase (±SEM) in mean fluorescent channel.

The fluorescent probe NBD-phallacidin (Molecular Probes, Junction City, OR), which binds specifically to filamentous actin (19), was used to measure the relative amount of filamentous actin in PMN as described previously (20). Briefly, PMN samples in microtiter plates were fixed in 3.7% formaldehyde and exposed to 0.33 μM NBD-phallacidin in PBS containing 100 μg/ml lysophosphatidylcholine (Sigma) overnight at 4°C. After washing, fluorescence was analyzed on a FACScan.

Assays for attachment and phagocytosis of ligand-coated erythrocytes. These were performed essentially as described previously (17). Briefly, opsonized erythrocytes were added to monolayers of PMN in Terasaki tissue culture plates and incubated for 30 min at 37°C. Unattached erythrocytes were removed by washing, and those remaining were quantitated by phase-contrast microscopy. An AI is expressed as the number of erythrocytes bound/100 PMN. Unopsonized erythrocytes are not bound (AI<1) by PMN (21). After removal of erythrocytes by hypotonic lysis, a PI was determined as the number of ingested erythrocytes/100 PMN. Data are shown for representative experiments, along with the mean fold increase (± SEM) in AI or PI.

RESULTS

Expression of CD11b/CD18, CR1, and LAM-1. Flow cytometry was used to measure the expression of receptors on the surface of PMN treated with serial dilutions of NAP-1/IL-8 and related peptides. Ser-NAP-1/IL-8, ala-NAP-1/IL-8, NAP-2, and *gro*/MGSA enhanced expression of CD11b/CD18 and CR1 in a concentration-dependent manner (Fig. 1). The threshold concentration was between 10^{-10} and 10^{-9} M, and maximum effects were obtained at 10^{-8} M with both forms of NAP-1/IL-8 and *gro*/MGSA, and at 10^{-7} M with NAP-2. Ser-NAP-1/IL-8 and ala-NAP-1/IL-8 caused a more than twofold increase in receptor expression, whereas lesser effects were observed with NAP-2 and *gro*/MGSA (Fig. 1). In contrast, PF-4 and CTAP-III were completely inactive within the concentration range tested (10^{-10} to 10^{-7} M) and did not alter the expression of either receptor (Fig. 1).

An up-regulation of CD11b/CD18 on murine PMN in response to a variety of other chemotactic peptides is accompanied by a decrease in the expression of the peripheral lymph node homing receptor, the murine homologue of LAM-1 (LECAM-1, Leu 8 Ag) (22). The change in expression of LAM-1 in response to NAP-1/IL-8 and related peptides was assessed by flow cytometry on PMN that had been stimulated with the near optimal concentration of 10^{-8} M. As shown in Figure 2, ser-NAP-1/IL-8, ala-NAP-1/IL-8, and *gro*/MGSA induced a decline in LAM-1 expression, whereas no effect was observed with this concentration of NAP-2 or with PF-4. For each of the

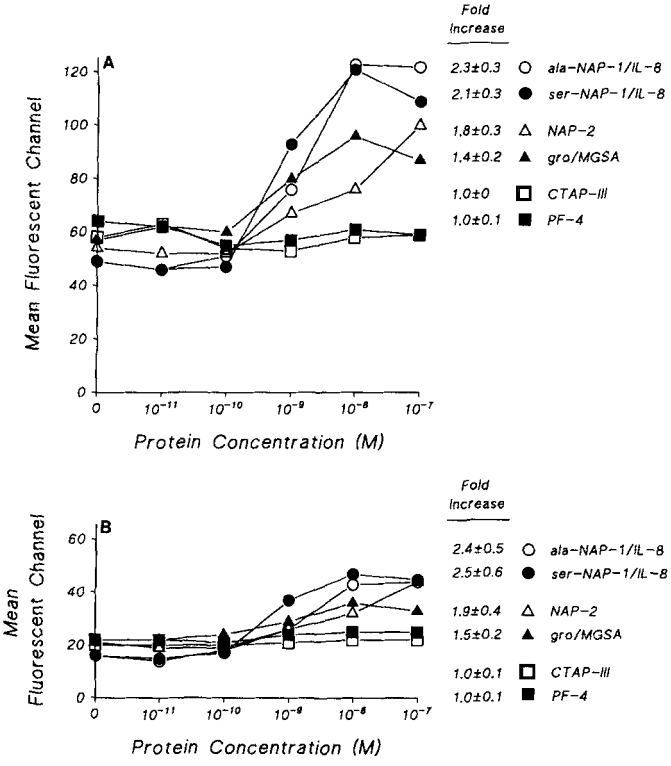

Figure 1. Expression of CD11b/CD18 and CR1 on the surface of PMN. PMN were exposed to increasing concentrations of ser-NAP-1/IL-8, ala-NAP-1/IL-8, *gro*/MGSA, NAP-2, PF-4, or CTAP-III for 30 min at 37°C. Control cells received no stimulus but were incubated for the same time at 37°C. Cells were stained for flow cytometry with mAb OKM10 directed against CD11b (*A*) or YZ directed against CR1 (*B*) as described in *Materials and Methods*. The mean fold increase (± SEM, $n = 3$) in mean fluorescent channel after stimulation with 10^{-7} M peptide is given.

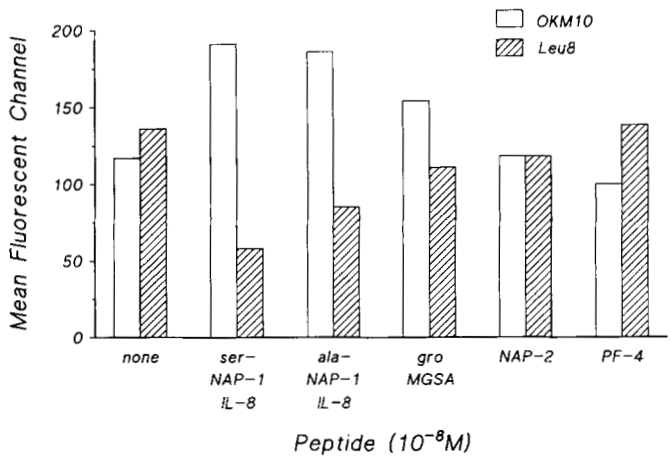

Figure 2. Expression of CD11b/CD18 and LAM-1 on the surface of PMN. PMN were treated with 10^{-8} M peptide for 30 min at 37°C, whereas control cells were incubated at 37°C without stimulus. Cells were stained for flow cytometry with mAb OKM10 (*open bars*) or anti-Leu 8 (*cross-hatched bars*) as described in *Materials and Methods*. These data are from one of three experiments with similar results.

active peptides, the extent of decline in LAM-1 was proportional to the enhancement in expression of CD11b/CD18.

Binding activity of CD11b/CD18 for C3bi. We have previously reported that ser-NAP-1/IL-8 enhances the binding activity of CD11b/CD18 for several ligands (9). Using the attachment of EC3bi to PMN treated with serial dilutions of both forms of NAP-1/IL-8 and related peptides to measure the binding activity of CD11b/CD18, we found that ser-NAP-1/IL-8, ala-NAP-1/IL-8, *gro*/MGSA, and NAP-2 all caused a concentration-dependent 3- to 10-fold increase in the AI (Fig. 3*A*). The threshold concentration for increased binding activity was about 10^{-9} M, and the maximum effect was observed at 10^{-7} M for all of the peptides. PF-4 and CTAP-III caused no enhancement of binding activity at concentrations up to 10^{-7} M (Fig. 3*A*).

To determine the time course for stimulation of the binding activity of CD11b/CD18, PMN were treated for different times with ser-NAP-1/IL-8, ala-NAP-1/IL-8, *gro*/MGSA, or NAP-2, washed free of stimulant, and exposed to EC3bi. In all cases attachment of EC3bi increased rapidly, peaked at about 30 min, and declined upon further incubation (Fig. 3*B*), indicating that the effect of NAP-1/IL-8 and the related peptides on the binding activity of CD11b/CD18 was transient. The loss of binding activity after 60 min appeared to be due to a failure of the receptors to respond further to the peptides rather than to degradation of the peptides themselves. Attachment of EC3bi remained low on PMN that were treated with ser-NAP-1/IL-8 for 75 min at 37°C, washed, and exposed to fresh ser-NAP-1/IL-8 or NAP-2 for an additional 20 min (Fig. 4). PMN treated with ser-NAP-1/IL-8 were, however, able to respond to fNLLP with increased binding of EC3bi. Cells treated with HAP buffer for 75 min at 37°C exhibited enhanced binding of EC3bi in response to ser-NAP-1/IL-8, NAP-2, and fNLLP.

Phagocytosis of EIgG and expression of FcγR. The chemotactic stimulus FMLP not only enhances the binding activity of CD11b/CD18 (9) but also stimulates Fc-mediated phagocytosis (21). When NAP-1/IL-8 and the related peptides were tested for their ability to modulate Fc-mediated phagocytosis, we found that ser-NAP-1/IL-

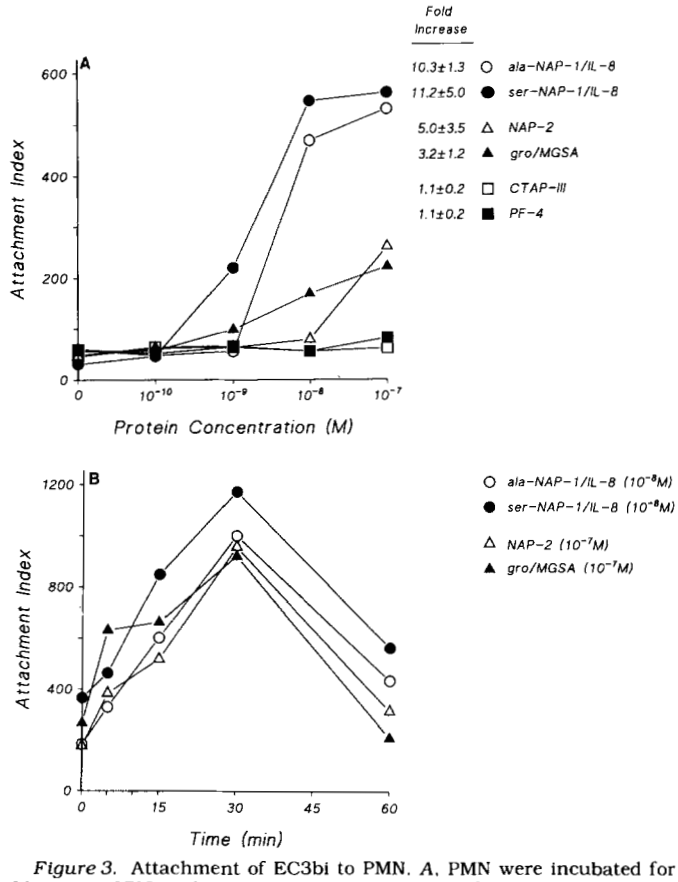

Figure 3. Attachment of EC3bi to PMN. *A*, PMN were incubated for 20 min at 37°C with increasing concentrations of ser-NAP-1/IL-8, ala-NAP-1/IL-8, *gro*/MGSA, NAP-2, PF-4, or CTAP-III. Control cells received no stimulus for 20 min at 37°C. After removal of the peptides by washing with PBS, attachment of EC3bi was measured as described in *Materials and Methods*. The mean fold increase (±SEM, $n = 4$) in AI after treatment with 10^{-7} M peptide is given. The increase in AI in response to 10^{-7} M NAP-2 shown in the graph is less than what was usually observed (see fold increase). *B*, PMN were incubated for 60 min at 37°C with peptides added for the indicated times. At the end of the incubation the cells were washed with PBS and binding of EC3bi was measured. 0-min cells received no stimulus but were incubated at 37°C. Three separate experiments were performed with similar results.

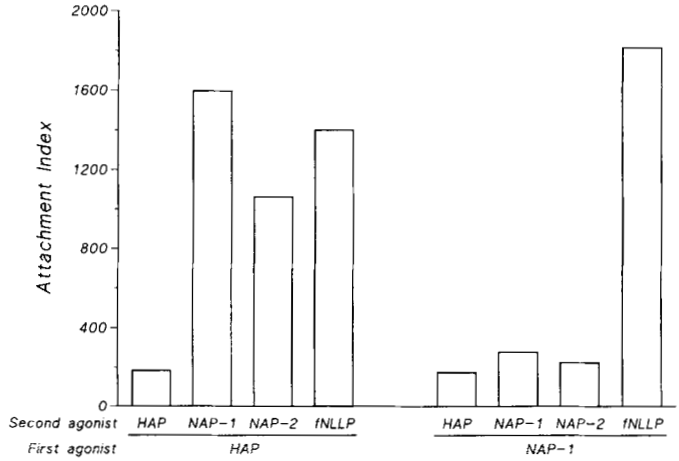

Figure 4. Attachment of EC3bi after readdition of NAP-1/IL-8. PMN were incubated for 75 min at 37°C with either 10^{-7} M ser-NAP-1/IL-8 or HAP buffer (first agonist). The cells were washed and fresh ser-NAP-1/IL-8 (10^{-7} M), NAP-2 (10^{-7} M), fNLLP (5×10^{-8} M), or HAP buffer was added for 20 min at 37°C (second agonist). After washing again EC3bi were added, and the AI was measured as in *Materials and Methods*. The data are representative of three separate experiments.

8, ala-NAP-1/IL-8, *gro*/MGSA, and NAP-2 all caused concentration-dependent increases in ingestion of EIgG (Fig. 5*A*). The threshold concentration for enhancement of phagocytosis was again between 10^{-10} and 10^{-9} M, and the magnitude of the effect was greater for both ser-NAP-1/IL-8 and ala-NAP-1/IL-8 than for *gro*/MGSA and NAP-2 at the highest concentration tested. Phagocytosis of EIgG was rapidly stimulated by the active peptides at the near optimal dose of 10^{-8} M, increasing by 5 min and reaching a plateau by 15 min (Fig. 5*B*). PF-4 and CTAP-III were inactive at stimulating Fc-mediated phagocytosis at all concentrations tested (range, 10^{-10} to 10^{-7} M) (Fig. 5*A*).

To test whether enhancement of Fc-mediated phagocytosis was associated with increased expression of FcγR, we used flow cytometry to measure the expression of FcγRII and FcγRIII on the surface of PMN after treatment for 30 min at 37°C with 10^{-8} M NAP-1/IL-8 and related peptides, a concentration that gave near maximal stimulation of phagocytosis. The expression of FcγRII, measured with mAb IV.3, did not change in response to any of the peptides tested (data not shown). Ser-NAP-1/IL-8 and ala-NAP-1/IL-8 both caused a 1.3-fold increase in the expression of FcγRIII, whereas *gro*/MGSA, NAP-2, and PF-4 did not change the amount of FcγRIII appreciably (data not shown). Phagocytosis of EIgG was thus strongly stimulated in the absence of a large increase in the expression of the receptors responsible for binding the opsonized particles and promoting phagocytosis, suggesting that another mechanism was responsible for the enhanced uptake of EIgG.

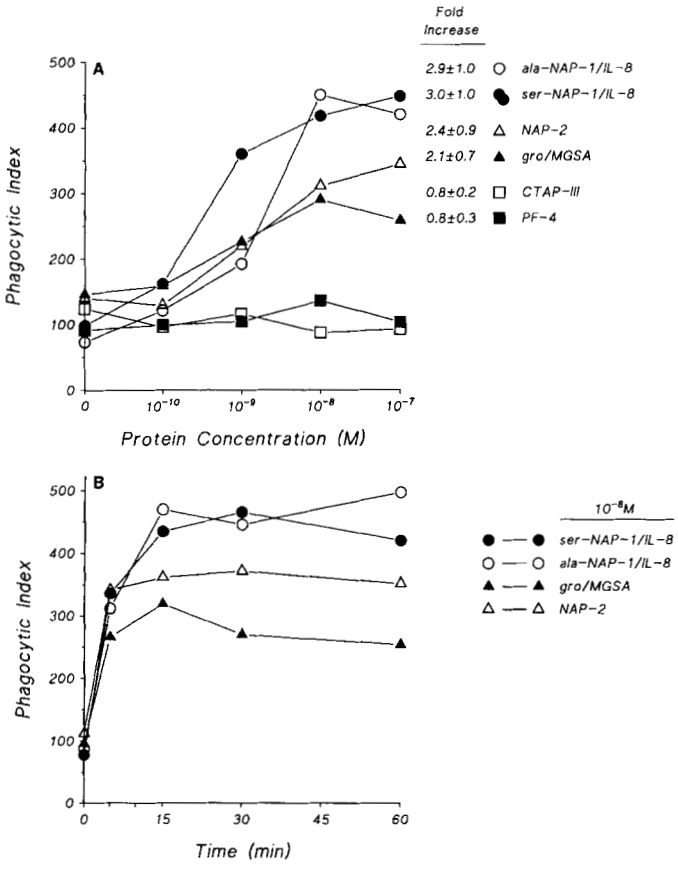

Figure 5. Phagocytosis of EIgG by PMN. *A*, PMN were incubated for 20 min at 37°C with increasing concentrations of peptides. Control cells received no stimulus during this incubation. After washing with PBS, EIgG were added and the PI was measured as described in *Materials and Methods*. The mean fold increase (±SEM, $n = 4$) in PI after treatment with 10^{-7} M peptide is given as fold increase. *B*, PMN were incubated for 60 min at 37°C with 10^{-8} M peptides added for the indicated times. At the end of the incubation the cells were washed with PBS, EIgG were added for an additional 30 min at 37°C, and the PI was measured. 0-min cells received no stimulus but were incubated and washed in the same way as the experimental ones. These data are representative of three experiments.

It has been previously reported that antibodies directed against CD18 inhibit Fc-mediated phagocytosis stimulated by FMLP (23), suggesting a role for CD11b/CD18 in the phagocytic process. We found that $F(ab')_2$ fragments of the anti-CD18 mAb IB4 inhibited NAP-1/IL-8-stimulated phagocytosis of EIgG by PMN. The PI for PMN incubated for 15 min at 37°C without agonist was 118 ± 45 ($n = 4$), whereas the PI increased to 309 ± 104 ($n = 4$) after 15 min at 37°C with 10^{-8} M NAP-1/IL-8. When 30 μg/ml $F(ab')_2$ fragments of IB4 was added for 15 min at 37°C after stimulation with NAP-1/IL-8, the PI was reduced to 125 ± 33 ($n = 4$). IB4 did not reduce phagocytosis of EIgG by unstimulated PMN below control levels, and, when used at similar concentrations, the control antibody W6/32 against HLA-A,B,C had no effect on phagocytosis of EIgG by either unstimulated or NAP-1/IL-8-stimulated PMN (data not shown). These observations suggest that augmentation of Fc-mediated phagocytosis by FMLP and NAP-1 involves a similar mechanism and may require the participation of CD11b/CD18.

Polymerization of actin. Participation of the microfilamentous cytoskeleton is required for the process of phagocytosis, and actin polymerization may occur at the site of formation of pseudopods (24). Another, perhaps related, burst of actin polymerization occurs upon stimulation of PMN with chemotactic formyl peptides (19). To test the ability of NAP-1/IL-8 and related peptides to initiate structural changes in the cytoskeleton of PMN, we used NBD-phallacidin to measure the relative F-actin content of cells treated with serial dilutions of peptides. Ser-NAP-1/IL-8, ala-NAP-1/IL-8, *gro*/MGSA, and NAP-2 each stimulated actin polymerization, as shown by a two- to threefold increase in the amount of fluorescence from NBD-phallacidin bound to cellular F-actin (Fig. 6*A*). The magnitude of the response to these peptides was comparable with that observed for the formyl peptide fNLLP. Actin polymerization in response to the neutrophil-activating peptides and *gro*/MGSA was rapid and transient, reaching a maximum by 1 min and declining nearly to resting levels by 5 min (Fig. 6*B*). Neither PF-4 nor CTAP-III were as active as the other peptides at stimulating actin polymerization (Fig. 6*A*).

DISCUSSION

The repertoire of receptors displayed on the surface of PMN is readily changed by exocytosis of granular components. Specific granules contain CD11b/CD18 as well as other members of the integrin family of receptors that promote adhesion of PMN to other cells and components of the extracellular matrix (25). NAP-1/IL-8 has been shown to cause an increase in the expression of CD11b/CD18 on PMN (9) and to induce the release of vitamin B_{12}-binding protein (26), indicating that NAP-1/IL-8 is capable of promoting the exocytosis of specific granules. NAP-1/IL-8 also promotes the release of a separate intracellular compartment that contains CR1 (9, 27). Here we report that the surface expression of CD11b/CD18 and

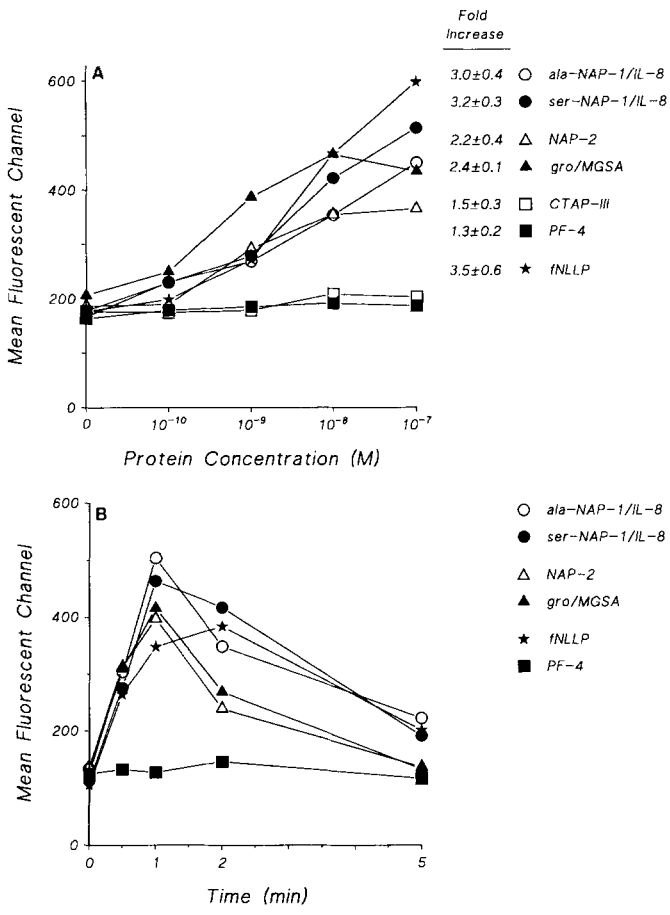

Figure 6. Polymerization of actin. *A*, PMN were held at ambient temperature for 15 min before addition of serial dilutions of peptides for 1 min. After fixation in formaldehyde the relative F-actin content was measured as mean fluorescent channel from NBD-phallacidin fluorescence as described in *Materials and Methods*. Control cells received no stimulus. The mean fold increase (±SEM, n = 4) in mean fluorescent channel after stimulation with 10^{-7} M peptide is given as fold increase. *B*, PMN were held at ambient temperature for 15 min before addition of 10^{-8} M peptides for the times indicated. 0-min samples received no stimulus. Relative F-actin content was measured as the mean fluorescent channel of NBD-phallacidin fluorescence as described in *Materials and Methods*. Similar results were obtained in three separate experiments.

CR1 on PMN increased significantly after treatment with either of the two common forms of NAP-1/IL-8 (ser-NAP-1/IL-8 and ala-NAP/1-IL-8), *gro*/MGSA, or NAP-2. These results indicate that all four of these related peptides are able to cause the exocytosis of specific granules and the CR1-containing vesicular compartment. Each of these peptides is therefore able to influence the deployment of integrins on the PMN surface and thereby change the adhesive interactions of leukocytes with their surroundings.

Remodeling of the PMN plasma membrane can also involve the loss of cell surface receptors. Treatment of human PMN with a variety of factors that augment the expression of CD11b/CD18, including granulocyte-macrophage CSF, TNF, FMLP, and leukotriene B_4, causes a decline in the cell surface expression of LAM-1 (LECAM-1) (28). A similar reciprocal behavior of CD11b/CD18 and the peripheral lymph node homing receptor, the murine homologue of LAM-1, was observed upon stimulation of murine PMN with chemotactic agents (22). We have observed that ser-NAP-1/IL-8 and ala-NAP-1/IL-8 also caused a decline in the expression of LAM-1 on PMN. This observation may provide an explanation for two apparently opposing effects of NAP-1/IL-8 on the adhesive interactions between PMN and endothelial cells. We and others have found that ser-NAP-1/IL-8 enhances the ability of PMN to adhere to unstimulated human umbilical vein endothelial cells in a CD18-dependent manner (9, 29). It has also been reported that both ser-NAP-1/IL-8 and ala-NAP-1/IL-8 inhibit the attachment of PMN to cytokine-stimulated endothelial cells, with ser-NAP-1/IL-8 being more potent in this regard (2). In addition, intravascular NAP-1/IL-8 inhibits the recruitment of PMN to sites of inflammation in rabbits (30). Because mAb directed against the peripheral lymph node homing receptor can block binding of PMN to lymph node endothelium in vitro and recruitment of PMN to inflammatory sites in vivo (31, 32), LAM-1 may contribute to the interaction between human PMN and endothelial cells. The loss of LAM-1 upon stimulation with NAP-1/IL-8 may thus lead to loss of adhesiveness under conditions that depend on CD18-independent adhesion.

Our previous results indicate that treatment of PMN with ser-NAP-1/IL-8 enhances the binding activity of CD11b/CD18 for several different ligands (9). We have confirmed and extended these results by showing that the binding activity of CD11b/CD18 for its ligand C3bi was strongly enhanced not only by ser-NAP-1/IL-8, but also by ala-NAP-1/IL-8, NAP-2, and *gro*/MGSA. The dose dependency for the effect was very similar to that observed for the increase in the expression of CD11b/CD18, but binding activity increased more (3- to 10-fold) than receptor expression (1.5- to 2.3-fold). Both of these effects were observed within the range of peptide concentrations that also elicit chemotaxis and transient increases in intracellular calcium (7).

Here we report that the enhancement of CD11b/CD18 binding activity caused by each of the four active peptides is transient, reaching a maximum at 30 min and declining thereafter. A decline in CD11b/CD18 activity was not detected in the shorter time course studies previously reported (9). Transient activation of CD11b/CD18 has been observed before for phorbol-stimulated binding of EC3bi (33) and TNF- and C5a-stimulated binding of PMN to endothelial cells (34). Because detachment must occur for an adherent cell to move across a surface, the transient binding activity observed in response to NAP-1/IL-8 may be important in the process of chemotaxis.

The decline in the ability of CD11b/CD18 to bind ligand after a 60- min incubation with NAP-1/IL-8 appears to be due to a desensitization of the response. Addition of fresh NAP-1/IL-8 to PMN already stimulated with NAP-1/IL-8 failed to elicit a new binding response. NAP-2 was also incapable of promoting binding activity in NAP-1/IL-8-stimulated cells, suggesting that these two peptides bind to the same receptor. When fNLLP was added to NAP-1/IL-8-treated PMN, however, the cells responded with an increase in CD11b/CD18 binding activity. Similarly, FMLP can elicit additional superoxide production from NAP-1/IL-8-stimulated PMN (26). Formyl peptides do not compete with NAP-1/IL-8 for binding to PMN (35), and therefore appear to bind to receptors distinct from those for NAP-1/IL-8.

Phagocytosis of opsonized particles represents an important mechanism of antimicrobial defense. Although neither NAP-1/IL-8 nor any of the related peptides promoted phagocytosis of EC3bi, ser-NAP-1/IL-8, ala-NAP-

1/IL-8, NAP-2, and *gro*/MGSA all enhanced the ingestion of EIgG, in the absence of a large increase in the expression of either FcγRII or FcγRIII. Other work has shown enhancement of Fc-mediated phagocytosis to accompany enhancement in CD11b/CD18 binding activity caused by FMLP (9, 21) and TNF (ref. 36 and Detmers, unpublished observations). Two lines of evidence suggest that activation of CD11b/CD18 is necessary for the enhancement of Fc-mediated phagocytosis. Fc-mediated phagocytosis stimulated by NAP-1/IL-8 was inhibited by $F(ab')_2$ fragments of an anti-CD18 mAb, and others have shown that FMLP-stimulated ingestion of EIgG is inhibited in a similar manner by an anti-CD18 antibody (23). In addition, PMN from patients deficient in all the members of the CD11/CD18 family (leukocyte adhesion deficiency) exhibit depressed ability to phagocytose EIgG in response to stimuli (37).

For phagocytosis of an opsonized particle to occur, pseudopods rich in filamentous actin must form and engulf the particle. Agents that block actin polymerization, such as cytochalasins, also block phagocytosis (38), suggesting that polymerization of actin may be required for the formation of pseudopods. Both forms of NAP-1/IL-8 (ser-NAP-1/IL-8 and ala-NAP-1/IL-8) as well as NAP-2 and *gro*/MGSA caused rapid, transient polymerization of actin in PMN, demonstrating that these four peptides are able to influence the structure of the microfilamentous cytoskeleton.

While the two common forms of NAP-1/IL-8 and the two homologous peptides NAP-2 and *gro*/MGSA induced marked changes in the expression and function of adhesion-promoting receptors, two other homologues, PF-4 and CTAP-III, lacked significant effects. Neither PF-4 nor CTAP-III enhanced expression of either CD11b/CD18 or CR1, binding activity of CD11b/CD18 for C3bi, or phagocytosis of EIgG at concentrations in the range 10^{-10} to 10^{-7} M. At the highest concentration tested PF-4 and CTAP-III caused a small amount of actin polymerization. These findings are consistent with a previous report that CTAP-III and PF-4 are inactive in assays of PMN chemotaxis and elastase release except at very high concentrations (7). Our results suggest that although peptides may show strong structural homology to NAP-1/IL-8, they may exhibit marked differences in their biologic properties.

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