Inhibition of Degranulation of Polymorphonuclear Leukocytes by Angiogenin and Its Tryptic Fragment*

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A degranulation inhibiting protein was purified to apparent homogeneity from plasma ultrafiltrates of patients with uremia using gel-permeation chromatography, ion-exchange chromatography, affinity chromatography on blue Sepharose, and ion-exchange chromatography on a Mono S HR 5/5 fast protein liquid chromatography column. The identity of the isolated degranulation inhibiting protein with angiogenin was demonstrated by amino acid sequence determination, an inhibitory effect of the isolated protein upon PMNL. The identity of this protein was confirmed by the payment of page charges. This article must therefore be solely to indicate this fact.

At concentrations in the nanomolar range, the protein inhibited spontaneous degranulation of polymorphonuclear leukocytes (PMNL) to 40%. The protein discharge of cells, which were preincubated with nanomolar concentrations of angiogenin and then stimulated with the cationic peptide formyl-norleucyl-leucyl-phenylalanyl-norleucyl-tyrosyl-leucine (FNLPNTL), was inhibited by 70%. Cellular functions such as chemotaxis, phagocytosis, and the oxidative respiratory burst were not obviously affected by angiogenin. A polyclonal antibody to human recombinant angiogenin abolished the inhibitory effect of the isolated protein upon PMNL.

The same but reduced effect was induced by the disulfide C23-C69 containing tryptic angiogenin fragment L-H-G-G-S-P-W-P-C58-S-Y-R-G-L-T-S-P-C65-K, indicating a new, so far unknown biologically active site of angiogenin which is different from the sites responsible for angiogenic or ribonucleolytic activity. Two synthetic peptides containing residues 83-95, one with C69 instead of C69, revealed the same inhibitory effect on the protein degranulation of PMNL as the entire tryptic fragment.

Patients with renal failure suffer from multimorbidity and show an increased susceptibility to infections. Among other reasons this has been attributed to abnormalities in granulocyte function (1, 2). Uremia, as well as damage of PMNL,

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† The abbreviations used are: PMNL, polymorphonuclear leukocytes; DIP, degranulation inhibiting protein; FPLC, fast protein liquid chromatography; HPLC, high performance liquid chromatography; NaCWP, phosphate-buffered saline; FNLPNTL, formyl-norleucyl-leucyl-phenylalanyl-norleucyl-tyrosyl-leucine; CB, cytochalasin B; ABTS, 2,2'-azinobis(3-ethylbenzthiazoline)sulfonic acid; MeO-Suc-Ala-Ala-Pro-Val-pNA, methoxysuccinyl-alanyl-alanyl-prolyl-valyl-paranitroanilide; PAGE, polyacrylamide gel electrophoresis.

caused by a hemodialysis procedure, could contribute to a dysfunction that becomes obvious in an impaired glycolytic enzyme activity (3), diminished intracellular ATP levels (4), reduced oxidative metabolism, and a restricted chemotactic activity (5). These observations led us to assume the presence of one or more factors in uremic serum that influence PMNL to respond to signal molecules. Such factors may be released from microbial organisms, plasma proteins, host cells, or the extracellular matrix, necessary to protect the body from infections.

Hör et al. (6) reported recently on the physicochemical characterization of a granulocyte inhibiting polypeptide present in plasma ultrafiltrates of patients with uremia. This protein was shown to inhibit the uptake of 3H-glucose, reduce the oxidative metabolism, diminish intracellular lysis of bacteria, and confine chemotaxis of PMNL.

In our report we describe another protein isolated from plasma ultrafiltrates of patients with uremia, which inhibits degranulation of PMNL at concentrations in the nanomolar range but does not block chemotaxis, phagocytosis, or the oxidative respiratory burst at comparable concentrations. The isolated factor is shown to be identical with angiogenin. Angiogenin, a 14.1-kDa protein, was first isolated and characterized from an HT 29 human adenocarcinoma cell line and has been shown to have an angiogenic (7) as well as a ribonucleolytic activity (8).

EXPERIMENTAL PROCEDURES

Materials

Ultratrol Aca54 and Q-Sepharose Fast Flow were purchased from Pharmacia (Upsala, Sweden). Blue Sepharose was prepared according to Pharmacia's prescription by coupling reactive Blue 5 (Sigma, München, Germany) to Sepharose CL6B. Resins and protected amino acid-active esters were purchased from Nova Biochem (Sandhausen, Germany). Cytochalasin B (CB), FNLPNTL, Angiogenin C-terminal peptide Glu18-Pro121, enzyme substrates (ABTS, MeO-Suc-Ala-Ala-Pro-Val-pNA, anti-human lactoferrin, Methocel and Histopaque 1977 were supplied by Sigma (Steinheim, Germany). Oposonized particles were prepared from zymosan (Sigma) as described (9). Culture dishes and Immunoplates-Maxisorb F96 were obtained from Nunc GmbH (Roskilde, Denmark). Ultravist 370 was a kind gift from Schering (Berlin, Germany). Fresh blood was supplied by Prof. Riesewiek, Herz-Zentrum Bad Oeynhausen (Bad Oeynhausen, Germany). Recombinant angiogenin was a gift from Dr. Bert L. Vallee, Harvard University (Boston, MA) and from Dr. Paques, Behringwerke (Marburg, Germany).

All other materials were of the purest grade available.

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Methods

Purification of Angiogenin from Plasma Ultrafiltrates—Plasma ultrafiltrates were prepared from patients with chronic renal failure in the Dialysis Unit of the Department of Medicine of the University of...
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Preparation of Antibodies to Angiogenin—Polyclonal antibodies to human recombinant angiogenin were prepared as described by Bläser et al. (19).

Preparation of Polymorphonuclear Leukocytes (PMNL)—PMNL were isolated from the fresh, heparinized venous blood of healthy human donors by modification of a method described by Metcalf et al. (24). PMNL were resuspended in a modified Dulbecco’s saline solution (140 mM NaCl, 5 mM KCl, 10 mM HEPES, pH 7.4, 1% glucose, 0.1% bovine serum albumin) and prewarmed to 37 °C. PMNL were exposed to PMNL-degranulation inhibitory factor (PMNL-DIF) for 40 min in suspension. PMNL-DIF was used at a concentration of 10 μg/ml.

Tests—The oxidative respiratory burst potential of PMNL was measured as described by Metcalf et al. (24) based on the cytochrome c absorption test and also tested by quantifying the activity of lactate dehydrogenase (EC 1.1.1.27) in the cell culture medium (21). Viability was found to be >90% with both test systems.

Preparation of Protein and Peptide Samples for the PMNL Degranulation Test—Concentrated protein fractions acquired after each chromatographic step were dialyzed against NaCVP, peptide samples and antibodies to angiogenin were biphosphated and dissolved in NaCVP.

PMNL Degranulation Test—PMNL were resuspended in NaCVP, + 0.1% glucose immediately after purification and prewarmed to 37 °C for 20 min in suspension. Protein and peptide samples were conveyed to culture dishes, filled up with NaCVP, plus 0.1% glucose to an end volume of 0.4 ml for each dish, and also adjusted to 37 °C for 20 min.

0.1 ml portions of the cell suspension containing approximately 1 x 10^6 PMNL were plated out into the prepared culture dishes where the cells were allowed to adhere. In order to test the effect of the samples on spontaneous degranulation, protein release (lactoferrin; leukocyte collagenase, gelatinase, and lactoferrin) was measured as described by Metcalf et al. (24) based on the cytochrome c absorption test and also tested by quantifying the activity of lactate dehydrogenase (EC 1.1.1.27) in the cell culture medium (21). Viability was found to be >90% with both test systems.

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The influence of samples on the protein release from stimulated PMNL after additional incubation with the micro1/2almitin (PMNL-DIF; CB) was examined by treating the cells with CB (5 μg/ml) for 20 min before stimulation with 10 μM FNLPLT for 20 min at 37 °C.

Supernatants were treated as described above before being tested for protein release (lactoferrin and leukocyte elastase (EC 3.4.24.27)). Enzyme-linked Immunoassay Assay Procedure for Collagenase, Gelatinase, and Lactoferrin—A sandwich-type enzyme-linked immunosorbent assay was used to quantify secretion of lactoferrin, collagenase, and gelatinase. Antibodies and enzyme standards were prepared according to Bergmann et al. (22). Microtiter plates were coated with the specific antibody (2 μg/ml in sodium carbonate buffer, pH 9.6). After overnight incubation, aliquots of PMNL supernatant were treated with 10 μl ml diisopropyl fluorophosphate (serine protease inhibiting agent; 100 mM in n-propanol) were added and incubated overnight (4 °C).

As an additional washing step was performed, and the second antibody (biotinylated peroxidase-conjugated) was added. After incubation for 2 h at room temperature and the washing steps, the resulting double-layer complex was visualized with ABTS as a substrate (0.5 mg/ml citrate buffer, pH 4.2 + 0.13 μl of 30% H₂O₂/ml) at 405 nm.

Photometric Assay of Elastase Activity—Elastase activity was monitored by hydrolysis of MeO-Suc-Ala-Ala-Pro-Val-pNA as a synthetic substrate at 25 °C using an Eppendorf photometer (605 nm). The test solution consisted of 0.2 μl of 10 μg/ml diisopropyl fluorophosphate (serine protease inhibiting agent; 100 mM in n-propanol) were added and incubated overnight (4 °C).

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Angiogenin was shown not to bind to the matrix of the cation-exchanger as (lane 5), blue Sepharose (lane 6) and FPLC on a Mono S cation-exchanger as final step. Protein concentrations for each sample on the SDS-PAGE were chosen to obtain a clearly visible band for the present constituents.

After each purification step, fractions corresponding to each elution peak were combined, concentrated, and screened for affinity chromatography on blue Sepharose, and ion-exchange chromatography on a Mono S HR 5/5 FPLC column was used to obtain homogeneous angiogenin.

The extent to which granule discharge could be enhanced by angiogenin concentration as low as 70 nM was used for testing peripheral human PMNL freshly prepared from human blood of healthy volunteers. This concentration was sufficient to induce maximum effects on the cells in the test suspension. The experiments were carried out in serum-free phosphate-buffered saline medium containing 0.1% (w/v) glucose with 2 x 10^5 cells/ml test solution.

The extent to which granule discharge could be enhanced by FNLPNTL at 10^-7 M alone served as reference for all other measurements and was arbitrarily set to 100% (Fig. 3D).

The measured values of secreted proteins after preincubation with angiogenin and stimulation with FNLPNTL fell be-
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Fig. 2. Demonstration of the identity of DIP with human recombinant angiogenin. Recognition of DIP in concentrated plasma ultrafiltrates from patients with chronic renal failure using a polyclonal antibody to human recombinant angiogenin on Western blotting. Figure shows concentrated plasma ultrafiltrate (lane 1), human recombinant angiogenin (lane 2), and molecular weight markers (lane 3).

Fig. 3. Influence of angiogenin and peptides on granule discharge of PMNL. Portions of a PMNL suspension adjusted to 37°C were transferred to prewarmed culture dishes containing buffer and samples of angiogenin (~70 nM), peptides (~200 nM) or solely buffer. After incubation (5 min) cells were stimulated with FNLPNTL (10 min) or left untreated for 15 min at 37°C. Protein release of cells stimulated solely with FNLPNTL, which were not treated with test samples, was set to 100%. A, cells treated with angiogenin alone; B, untreated cells; C, cells pretreated with angiogenin and stimulated with FNLPNTL; D, cells treated solely with FNLPNTL. E, cells pretreated with the tryptic fragment 12 and stimulated with FNLPNTL; F, cells pretreated with the synthetic peptide L-H-G-G-S-P-W-P-P-S-Q-Y-R and stimulated with FNLPNTL. Standard incubation conditions: 37°C, 5% CO₂.

low the levels of totally untreated cells to 29 ± 5% (n = 22) for lactoferrin, 35 ± 3% (n = 5) for collagenase, and 38 ± 3% (n = 5) for gelatinase (Fig. 3C). As mentioned above, the protein discharge of cells, which were solely stimulated with FNLPNTL, was set to 100%.

A mixture of angiogenin and FNLPNTL, which had been adjusted to 37°C for 10 min, caused a decrease in lactoferrin discharge to only 64 ± 16% (n = 5) (data not shown) instead of 29% (see above). In this case, the deviation in measured values was much higher than in those for the other experiments.

Biological activity of angiogenin was demonstrated to be a function of concentration. As shown in Figs. 4, 5, and 6, the dose-response curves for the inhibition of spontaneous degranulation (Fig. 4), as well as for the stimulating effect of FNLPNTL (Figs. 5 and 6), displayed a similar shape (n = 4). In both cases angiogenin at approximately 7 nM was required to achieve a minimum inhibitory effect. The extent of inhibition increased with rising concentrations of angiogenin until a maximum reduction in granule discharge was reached at a concentration of approximately 70 nM.

Whether the microfilament system could be involved in the inhibition of degranulation by angiogenin was investigated with cells previously treated with cytochalasin B, an agent that destroys their microfilament system. The cells were incubated with cytochalasin B (5 µg/ml) before preincubation with angiogenin and then stimulated with FNLPNTL at 10⁻² M. Cells supernatants were tested for secretion of elastase and lactoferrin. In both cases, the protein release was not suppressed by angiogenin (70 nM) compared with cells not preincubated with angiogenin.

The granulocyte functions phagocytosis, oxidative respiratory burst, and chemotaxis were tested as described under "Experimental Procedures." These cellular functions were not blocked by angiogenin concentrations in the range of 14–700 nM (data not shown).

The same cellular effects were induced in PMNL when re-
Angiogenin—the effect of monospecific, polyclonal antibodies against human angiogenin on the inhibitory activity of angiogenin residues Leu$^{3}$-Arg$^{45}$, containing Ser$^{31}$ instead of a cysteine, on lactoferrin discharge of PMNL. Portions of a PMNL suspension adjusted to 37°C were transferred to prewarmed culture dishes containing buffer and samples of angiogenin or solely buffer. After incubation (5 min) cells were stimulated with FNLPNTL (10 min) or left untreated for 15 min at 37°C. The cell release of cells stimulated solely with FNLPNTL, which were not treated with test samples, was set to 100%. Fragment amino acid sequences: I. Gln$^{1}$-Asp-Ser-Arg$^{2}$, 2. Ser$^{3}$-Ile-Lys$^{3}$ + Ile$^{31}$-Ser-Lys$^{31}$ + Arg$^{32}$; Pro: 3. Ile$^{36}$-His-Gly-Asn-Lys$^{36}$; 4. Asn$^{36}$-Gly-Asn-Pro-His-Arg$^{36}$; 5. Gln$^{4}$-Asn-Leu-Arg$^{29}$; 6. Leu$^{4}$-Thr-Gln-His-Tyr-Asp-Ala-Lys$^{4}$; 7. Ala$^{4}$-Thr-Ala-Gly-Phe-Arg$^{31}$; 8. Tyr$^{2}$-Thr-His-Phe$^{2}$; 9. Asp$^{4}$-Ile-Aas-Thr-Phe$^{4}$; 10. Tyr$^{2}$-Cys-Glu-Ser-Ile-Met-Arg$^{3}$ + Gln$^{37}$-Val-Thr-Thr-Cys-Lys$^{37}$; 11. Tyr$^{2}$-Cys-Glu-Ser-Ile-Met-Arg$^{3}$ + Ser$^{31}$-Ser-Phe-Glu-Gln-Val-Thr-Thr-Cys-Lys$^{31}$; 12. Gln$^{38}$-Leu-Thr-Ser-Pro-Cys-Lys$^{38}$ + Leu$^{38}$-His-Gly-Gly-Ser-Pro-Trp-Pro-Cys-Gln-Tyr-Arg$^{38}$; 13. Ala$^{35}$-Ile-Cys-Glu-Aas-Lys$^{35}$ + Asp$^{35}$-Val-Val-Ala-Cys-Glu-Aas-Ash-Leu-Asp-Gln-Ser-Ile-Met-Arg$^{35}$.

The C-terminal angiogenin fragment Glu$^{106}$-Pro$^{13}$ was reported to inhibit both the ribonucleolytic and the angiogenic activity (27). To investigate if this was the case for the inhibitory activity of angiogenin presented in this report, PMNL were treated with angiogenin and, in addition, with various concentrations of this angiogenin fragment. Then the cells were stimulated with FNLPNTL as described above. The C-terminal fragment Glu$^{106}$-Pro$^{13}$ had only little influence on the lactoferrin secretion since values between 91 and 106% were determined compared to only with FNLPNTL-treated cells using peptide concentrations of 20 nM up to 6 µM. The peptide was not able to inhibit the activity of angiogenin on the PMNL degradation. Even at the highest concentration, the C-terminal fragment caused no significant change. Cells treated with mixtures of angiogenin (280 nM) and the peptide (6 µM) showed a lactoferrin secretion of 39 ± 8%, and PMNL treated with angiogenin (280 nM) showed a value of 34 ± 6%, as compared to treatment with only FNLPNTL-stimulated cells (190%).

Activity of Tryptic Fragments of Angiogenin—Angiogenin was subjected to tryptic digestion, and the resulting fragments were separated by reversed phase chromatography on an RP-18 column. Thirteen fragments (see Table I) were isolated and tested for their inhibitory activity in the granule discharge test. Only fragment 12 exhibited degranulation inhibitory activity, which was, however, reduced compared to intact angiogenin. At a concentration of -200 nM, fragment 12 suppressed the lactoferrin and the collagenase secretion of PMNL to 81 ± 8% (n = 10) and 83 ± 5% (n = 5) (Fig. 3E), when cells were stimulated with FNLPNTL. The covalent structure of fragment 12 was elucidated by amino acid composition and amino acid sequence determination and revealed L-H-G-G-S-P-W-P-P-S-Q-Y-R G-L-T-S-P-C-K, as the tryptic fragment containing the disulfide bridge C$^{28}$-C$^{31}$ of angiogenin.

Activity of Synthesized Peptides—The corresponding peptides G-L-T-S-P-C-K and L-H-G-G-S-P-W-P-P-C-Q-Y-R, as well as their mixed disulfide and the homologous peptides containing serine in place of cysteine, were synthesized. The homologous short pentapeptides had no inhibitory effect on the degranulation of PMNL (data not shown). The other two homologous 13-residue peptides, corresponding to the angiogenin residues Leu$^{3}$-Arg$^{45}$, showed inhibitory activity in the same order of magnitude as fragment 12. Exposure of granulocytes to the peptides L-H-G-G-S-P-W-P-P-C-Q-Y-R and L-H-G-G-S-P-W-P-P-S-Q-Y-R caused a maximum inhibition of the lactoferrin discharge after stimulation with FNLPNTL to 80 ± 7% (n = 5) (data not shown) and 79 ± 7% (n = 22) (Fig. 3F) respectively, compared to cells solely treated with FNLPNTL. Besides the secretion of lactoferrin, the release of collagenase from cells pretreated with the serine-containing large peptide was also determined. The single-chain serine peptide caused a reduction in collagenase release of 23 ± 4% (n = 4) (Fig. 3F).

The inhibitory activity of the peptide L-H-G-G-S-P-W-P-P-S-Q-Y-R on the degranulation of PMNL was dose-dependent with a concentration profile similar to that determined for intact angiogenin. The dose-response curve for the inhibition of the lactoferrin secretion induced by the peptide as compared to angiogenin is shown in Fig. 5.

The N-terminal truncated peptide S-P-W-P-P-C-Q-Y-R, lacking the four residues L-H-G-G, however, exhibited no detectable inhibiting effect on the protein discharge of PMNL.

**DISCUSSION**

Following the observation that plasma ultrafiltrates of patients with chronic renal failure inhibited degranulation of PMNL, angiogenin was isolated from this source as a protein causing such an effect. Angiogenin, initially identified as a tumor-derived protein, was also found in human blood plasma (28) and has been reported to induce vascular growth. The protein belongs to the protein superfamily of ribonucleases, as it shows a characteristic ribonucleolytic activity and was demonstrated to have 35% identity with the pancreatic ribonuclease I containing the catalytic residues His$^{31}$, Lys$^{31}$, and His$^{41}$ of RNase A (9). The ribonucleolytic activity was reported to cause a cytotoxic effect in Xenopus oocytes cells (29) and the ability to inhibit protein synthesis in vitro (30). Angiogenin was shown to
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It was reported by Rybak et al. (27) that synthetic peptides corresponding to the C-terminal region of angiogenin inhibit the enzymatic and biological activities of the protein. Therefore, peptide concentrations in the micromolar range were used. We showed that the C-terminal peptide Glu<sup>208</sup>-Pro<sup>213</sup> had no effect on the inhibition of PMNL degranulation caused by angiogenin.

In addition we revealed the inhibitory activity of the tryptic angiogenin fragment GL-T-S-P-C-R-K-L-H-G-G-S-P-W-P-C-Q-Y-R, containing the disulfide bridge C<sup>94</sup>-C<sup>99</sup> of angiogenin. Compared to the native protein this tryptic fragment exhibited reduced but still significant inhibitory activity on degranulation of PMNL. The synthetic peptide L-H-G-G-S-P-W-P-C-Q-Y-R and the homologous peptide, containing Ser instead of Cys, had similar effects and showed dose-response curves similar to that of native angiogenin, with a maximum effect at approximate concentrations of 100 nM. Thus, a similar mechanism of action of the peptide and the whole molecule seemed reasonable to assure. Indeed, the fact that only 20% inhibition of degranulation could be obtained, even at high peptide concentrations, suggests that perhaps other factors like the threedimensional structure of the peptide, the truncated sequence, or another part of the protein sequence of angiogenin might be involved in the total effect of the protein.

The angiogenin residues His<sup>13</sup>, Lys<sup>14</sup>, and His<sup>129</sup> are essential for the ribonucleolytic activity of angiogenin (34), including the regions Gin<sup>1</sup>-Thr<sup>7</sup> (35) and Gin<sup>1</sup>-Arg<sup>20</sup> (36) and the residue Asp<sup>13</sup> (37). The ability of angiogenin to induce vascular growth relies on residue Asp<sup>13</sup> (38). In addition Asp<sup>13</sup> plays a role for the interaction with endothelial cells (38, 39). Our peptide, which displayed inhibitory activity in the PMNL degranulation test, does not contain any of those residues involved in the other activities described for angiogenin. This result supports the conclusion that a new functional site of angiogenin is responsible for the biological activity of the protein presented in this report.

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REFERENCES


bind to the membrane of different cell lines and to have the ability to intervene in cellular signal transduction (31).

No effects have so far been published on an interaction of angiogenin with PMNL. The new cellular effects described here could also be obtained with recombinant angiogenin, which inhibited the degranulation of PMNL in the same manner as the isolated protein DIP. Since DIP and angiogenin are identical, and angiogenin is a normal blood constituent, it can be expected that plasma ultrafilters of healthy persons containing proteins in the same molecular range as those found in plasma ultrafilters of patients with renal failure would also inhibit granulocyte protein secretion. This was observed for highly concentrated normal plasma ultrafilters. Angiogenin concentration in the plasma of healthy donors was determined to be in the range of 8-30 ng (19). This represents the concentration in which the inhibitory effect on degranulation of PMNL was shown to increase significantly. If this degranulation inhibiting activity of normal plasma is due to angiogenin, the question arises whether this protein has also a protective function against premature protein release from circulating PMNL.

Most plasma-derived molecules released during an inflammatory response, e.g. endotoxins, interleukins, complement factors, tumor necrosis factors, colony-stimulating factor, and interferons, are known to stimulate PMNL functions rather than inhibit them. Therefore, at first glance circulating angiogenin, which could impair the host's cellular defense, seems to be very unusual. However, a compound to counterbalance the stimulating agents during circulation of the cells in the vascular bed seems rather likely. Its efficacy is comparable to that of cytokines and lymphpokines, as the amounts required for maximum suppression of lactoferrin, collagenase, and gelatinase secretion are in the nanomolar range. Although many questions remain to be answered, attempts were made to unravel the mechanism of action of angiogenin. Suppression of the commonly observed effect of FNLPNTL on protein secretion is not due to competitive occupation of the appropriate receptor, as PMNL degranulation was restrained clearly below levels of untreated cells. Moreover, inhibition up to 40% was also caused by angiogenin alone. Chemotaxis along an FNLPNTL gradient, which was observed for PMNL pretreated with angiogenin, indicates that these cells are still able to recognize the chemotactic peptide. Destruction of microtubules by angiogenin could also be excluded, as Schettler et al. (32) recently reported that secretion of all classes of granula depend on intact microtubules. Thus, elastase would not be released if microtubules had been destroyed. However, elastase and lactoferrin discharge induced by cytochalasin B and FNLPNTL was not affected by angiogenin. This is also a strong indication for the importance of an intact system of microfilaments to exhibit the effect of angiogenin described in this report. The observation that phagocytosis and chemotaxis were unimpaired in the presence of angiogenin indicated a mainly intact cytoskeleton.

The cellular response of PMNL to angiogenin was as quick as to FNLPNTL alone, i.e. within seconds (33), as was revealed when cells were treated with a mixture of FNLPNTL and angiogenin. This investigation showed up to 64% inhibition of degranulation, compared to cells treated solely with the stimulant. The effect was significant but smaller than the corresponding effects in the other series of experiments, in which PMNL were pretreated with angiogenin and then stimulated with FNLPNTL. It is not clear at present whether or not this means that the signal transduction mechanism is similar to that of FNLPNTL, i.e. receptor-mediated.

<sup>2</sup>U. Hempelmann, unpublished data.
Inhibition of Degranulation of Polymorphonuclear Leukocytes