Inactivation of Interleukin-8 by the C5a-Inactivating Protease From Serosal Fluid

By Suhail K. Ayesh, Yehudith Azar, Bernard M. Babior, and Yaacov Matzner

The complement fragment C5a and the cytokine interleukin-8 (IL-8) are proinflammatory peptides with potent chemotactic activity toward neutrophils. We have previously shown that C5a can be inactivated by a protease that is found in normal synovial and peritoneal fluids but is absent from serosal fluids obtained from patients with familial Mediterranean fever (FMF). We report here that serosal fluids can also eliminate the chemotactic activity of IL-8. The agent responsible for IL-8 elimination appears to be the C5a-inactivating protease, because the pure protease can inactivate IL-8, inactivation of IL-8 by normal peritoneal fluid is partly prevented by an antibody raised against the purified C5a-inactivating protease, and IL-8 is not inactivated by peritoneal fluids from patients with FMF. The ability of this protease to inactivate both, early (C5a) and late (IL-8) inflammatory mediators identifies it as a potentially significant regulator of inflammation.

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MATERIALS AND METHODS

Materials. Recombinant human IL-8 (monocyte-derived, 72 amino acid form, molecular weight [MW] 8,000) was purchased from Genzyme (Cambridge, MA) and dissolved in Dulbecco’s phosphate-buffered saline (PBS) containing 2.5 mg/mL BSA. Phenylmethylsulfonyl fluoride (PMSF), Blue Sepharose CL-6B, and arginine agarose were purchased from Sigma (St Louis, MO). Diethylaminoethyl (DEAE) cellulose (DE-52) was obtained from Whatman Biosystems (Maidstone, Kent, UK), Sephadex G-10 and G-100 from Pharmacia (Uppsala, Sweden), blind well Boyden chambers from Nucleopore Corp (Bethesda, MD), and corresponding filters (Membranafilter, 3 μm pore size) from Sartorius (Göttingen, Germany). All other chemicals were of reagent grade and were purchased from Sigma.

Peritoneal fluid. Peritoneal fluid was obtained from patients with ascites due to alcoholic or posthepatitic cirrhosis of the liver. Fluid samples were cleared by centrifugation (1,500g for 10 minutes at 4°C), divided into aliquots, and stored at −70°C. Before use, the fluid was decomplemented by heating at 56°C for 30 minutes. Purification of the investigated protease was performed along the following steps: ammonium sulfate precipitation, desalting over Seph-
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adex G-10, ion exchange chromatography using DE-52 column, blue Sepharose CL-6B, gel filtration with Sephadex G-100, and affinity chromatography using arginine agarose column. The purified protease showed a single band of approximately 55 Kd on SDS-PAGE. Samples of normal and FMF peritoneal fluid were obtained from healthy infertile women undergoing laparoscopy for suspected mechanical infertility.

Neutrophil chemotaxis. Human neutrophils were prepared by dextran sedimentation followed by hypotonic lysis and centrifugation over Ficoll Hypaque as described elsewhere, with slight modifications. Neutrophil chemotaxis was assayed by the leading front technique, with slight modifications, and calculated as the distance travelled in response to IL-8 minus random migration. In the assays, normal human neutrophils were placed in the upper compartment of the Boyden chamber, and the IL-8 solution, with or without added components, in the lower compartment. Incubations were performed at 37°C for 45 minutes.

Gel electrophoresis. SDS-PAGE was performed by the method of Laemmli. Samples of IL-8 (1 μg/10 μL) were preincubated with either 30 μL PBS or 30 μL of purified C5a-inactivating protease (35 μg/mL) for 20 hours at room temperature and then subjected to SDS-PAGE under reducing conditions on an 18% polyacrylamide gel, with staining with Coomassie Blue.

Statistical analysis. Results are expressed as mean ± 1 SE. The significance of difference was determined by the paired t-test.

RESULTS

The protection afforded by the C5a-inactivating protease against the development of an inflammatory reaction (see normal subjects v patients with FMF) suggested that this protease might have anti-inflammatory actions beyond its ability to destroy C5a. To obtain evidence on this point, we examined the effect of the protease on the chemotactic activity of IL-8. The initial experiments were performed with peritoneal fluid as the source of the C5a-inactivating protease and recombinant human IL-8 as the target. As shown in Fig 1, the chemotactic activity of IL-8 was rapidly lost when the interleukin was incubated with peritoneal fluid. Under the conditions of the experiment, IL-8 was extensively inactivated by 8 minutes, a rate comparable to that previously reported for the inactivation of C5a by peritoneal fluid. IL-8 inactivation increased with increasing concentrations of peritoneal fluid (Fig 2), and was readily apparent at IL-8 concentrations as high as 0.1 μg/mL (12 nmol/L) (Fig 3). These results too are similar to those previously obtained with C5a.

Several lines of evidence indicate that the activity in peritoneal fluid responsible for the loss of the chemotactic potency of IL-8 was the same as that which destroys C5a (Table 1). Similar to the C5a-inactivating agent, the activity that destroyed IL-8 was a serine protease, as indicated by the sensitivity of IL-8 inactivation to PMSF, and was stable to heating at 56°C for 30 minutes but not to boiling (Table 1, experiment 1). IL-8 inactivation was partly prevented by a polyclonal antibody that had been previously raised against the purified C5a-inactivating protease (Table 1, experiment 2). As with C5a, the chemotactic activity of IL-8 was not affected by peritoneal fluid from three patients with FMF (P < .01, Table 1, experiment 3).

The effect of the protease on the structure of IL-8 was investigated in an experiment in which the interleukin was treated with the purified protease and the product examined
toxemia after the administration of IL-1α, and probably in the lung during various inflammatory insults. IL-8 is secreted by many types of cells (blood monocytes, alveolar macrophages, endothelial cells, fibroblasts, and epithelial cells) and represents a long-lasting chemoattractant for neutrophils but not monocytes. Thus, IL-8 could play a major role in the long-term recruitment of neutrophils to inflamed areas in many tissues. This appears to be particularly important in diseases in which neutrophils appear to play a major pathogenetic role, such as the adult respiratory distress syndrome, reperfusion injury after myocardial infarction, and late complications of bacterial meningitis.

In conclusion, C5a and IL-8 are important, respectively, for the initiation and the maintenance of inflammatory reactions. We believe that the protease that inactivates both of these inflammatory mediators is likely to serve a significant function as a regulator of inflammation in serosal spaces and may offer the basis for a novel anti-inflammatory agent.

Table 1. Inactivation of IL-8 by Peritoneal Fluids

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Chemotaxin</th>
<th>Migration</th>
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<tbody>
<tr>
<td><strong>Experiment 1</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-8</td>
<td>44.6 ± 4.5</td>
<td>100</td>
</tr>
<tr>
<td>IL-8 + PMSF</td>
<td>46.1 ± 1.0</td>
<td>103</td>
</tr>
<tr>
<td>IL-8 + PF</td>
<td>19.5 ± 3.7</td>
<td>44</td>
</tr>
<tr>
<td>IL-8 + PMSF-treated PF</td>
<td>48.6 ± 3.5</td>
<td>109</td>
</tr>
<tr>
<td>IL-8 + PF (56°C)</td>
<td>19.5 ± 4.5</td>
<td>44</td>
</tr>
<tr>
<td>IL-8 + boiled PF</td>
<td>47.4 ± 6.7</td>
<td>106</td>
</tr>
<tr>
<td><strong>Experiment 2</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-8</td>
<td>51.3 ± 4.7</td>
<td>100</td>
</tr>
<tr>
<td>IL-8 + PF</td>
<td>11.7 ± 3.3</td>
<td>27</td>
</tr>
<tr>
<td>IL-8 + PF + nonimmune serum</td>
<td>14.7 ± 2.4</td>
<td>28</td>
</tr>
<tr>
<td>IL-8 + PF + antiprotease antiserum</td>
<td>34.9 ± 1.7</td>
<td>88</td>
</tr>
<tr>
<td>IL-8 + antiprotease antiserum</td>
<td>50.0 ± 8.2</td>
<td>97</td>
</tr>
<tr>
<td><strong>Experiment 3</strong></td>
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</tr>
<tr>
<td>IL-8</td>
<td>50.5 ± 4.1</td>
<td>100</td>
</tr>
<tr>
<td>IL-8 + normal PF</td>
<td>17.2 ± 7.2</td>
<td>34</td>
</tr>
<tr>
<td>IL-8 + FMF PF</td>
<td>54.2 ± 3.2</td>
<td>107</td>
</tr>
<tr>
<td>C5a</td>
<td>58.4 ± 3.9</td>
<td>100</td>
</tr>
<tr>
<td>C5a + normal PF</td>
<td>12.2 ± 6.8</td>
<td>21</td>
</tr>
<tr>
<td>C5a + FMF PF</td>
<td>56.8 ± 5.7</td>
<td>97</td>
</tr>
</tbody>
</table>

PMSF-treated peritoneal fluid was prepared by a published method, using 3 mmol/L PMSF and peritoneal fluid diluted 1:10 (vol/vol) in PBS+ in a total volume of 0.3 mL. Heated peritoneal fluid was incubated at 56°C for 30 minutes; boiled fluid was prepared by heating in boiling water for 3 minutes and then centrifuging at 800g for 10 minutes to remove denatured proteins. Peritoneal fluid was treated with antiserum or nonimmune serum diluted 1:30 in PBS as previously described. The peritoneal fluids (10% [vol/vol] in PBS+) were incubated with 50 ng/mL (6 nmol/L) IL-8 for 10 minutes at 37°C, after which chemotaxis was measured in Boyden chambers as described in Materials and Methods. All chemotaxis values were corrected for random migration, which was 40.5 ± 2.5, 47.1 ± 0.2, and 37.0 ± 3.8 μm, respectively, in experiments 1, 2, and 3. Results are expressed as the mean ± 1 SE for three determinations in experiments 1 and 2. Experiment 3 included three FMF patients and three healthy controls. Control experiments with the same three patients, using 10-9 mol/L rC5a as a chemoattractant, showed similar results.

Abbreviation: PF, peritoneal fluid.

DISCUSSION

The foregoing data suggest that a single anti-inflammatory protease in serosal fluid is able to inactivate both C5a and IL-8, two chemotactic factors with relatively limited homology. Precedent for this situation exists in the coagulation cascade, in which thrombin, for example, proteolytically activates several procoagulant molecules of widely varying structure.

IL-8 is recognized as an unusually stable inflammatory mediator, so this protease, which is able to rapidly and efficiently inactivate this chemotactic factor, could be important in its regulation.

IL-8 is involved in many diseases with neutrophil-mediated inflammatory component. It is found in skin lesions of patients with psoriasis, in synovial fluids and sera of patients with rheumatoid arthritis, and in the sera of patients with acute pancreatitis. Animal studies showed that IL-8 appears in the circulation during septic shock and sublethal endo-

Fig 3. Chemotaxis as a function of IL-8 concentration in the presence and absence of peritoneal fluid. Peritoneal fluid (3% [vol/vol] in PBS+) was incubated with IL-8 at the indicated concentrations for 10 minutes at 37°C, after which chemotaxis was measured. For each assay performed with peritoneal fluid (C), a control assay was performed under the same conditions except that the peritoneal fluid was replaced by an equal volume of buffer (O). The values for chemotaxis were corrected for random migration of 45.3 μm. The results are expressed as the mean ± 1 SE of three experiments. P < .01 for IL-8 concentrations less than 100 ng/mL.

by SDS-PAGE. Control IL-8 migrated as two bands that represented the interleukin monomer and dimer (Fig 4, lanes A) (as previously reported, IL-8 and its related molecules form aggregates in solution and on SDS-PAGE [M. Baggiolini, personal communication, 1992]). However, the protease-treated IL-8 showed only the monomer band, which was somewhat broader and migrated slightly more rapidly than control IL-8 (lane B). This result suggests that, similar to C5a, IL-8 is inactivated by limited proteolysis.
Fig 4. SDS gel electrophoresis of IL-8 treated with the purified C5a-inactivating protease or with PBS. For details see Materials and Methods. (A) PBS-treated sample; (B) protease-treated sample.

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