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Proteolytic Inactivation of Cytokines by *Pseudomonas aeruginosa*

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***Pseudomonas aeruginosa* alkaline protease and elastase are thought to contribute to bacterial invasiveness, tissue damage, and immune suppression in animals and patients infected with the bacterium. This study examined the ability of the two proteases to inactivate a number of cytokines that mediate immune and inflammatory responses. Human recombinant gamma interferon (rIFN-γ) and human recombinant tumor necrosis factor alpha were inactivated by both proteases. Murine rIFN-γ was relatively resistant to alkaline protease but was inactivated by elastase, and human recombinant interleukin-1α and recombinant interleukin-1β were resistant to the effects of both proteases. Western immunoblots suggested that cytokine inactivation by these proteases, where it occurred, required only limited proteolysis of the polypeptides. The ability of different *P. aeruginosa* strains to inactivate IFN-γ appeared to require the production of both proteases for optimum activity. These results indicate that in vitro cytokine inactivation by *Pseudomonas* proteases is selective, requires only limited proteolysis, and in certain instances reflects the cooperative effects of both proteases.**

Pseudomonas aeruginosa is an important pulmonary pathogen in conditions like cystic fibrosis (15, 42). It has been suggested that the ability of *P. aeruginosa* to establish itself in the respiratory tract may be promoted by its suppressive effects on pulmonary immune responses (2, 16, 40). The mechanisms of this immunosuppression are not entirely understood, but proteolytic enzymes secreted by the bacterium have been shown to degrade surface receptors on hematogenous cells (36, 43) and inactivate interleukin-2 (IL-2) and gamma interferon (IFN-γ) (16, 17, 41).

In the case of human IFN-γ, cytokine inactivation was caused by either *Pseudomonas* alkaline protease (AP) (17) or elastase (E) (16). Significant reductions in antiviral and immunomodulatory activities were associated with limited proteolysis of IFN-γ. Of particular interest were the synergistic effects on IFN-γ seen when both purified proteases were added to reaction mixtures (16). These results would predict that *Pseudomonas* strains that produce both enzymes should be particularly immunosuppressive, a property that may aid the bacterium in establishing initial colonization by significantly altering immune and inflammatory responses in infections like those seen in cystic fibrosis.

This study was undertaken to address three questions relating to the effects of *Pseudomonas* protease on cytokines. First, what are the specificities of these proteases relative to the inactivation of cytokines that might be involved in *Pseudomonas* infections? Second, is limited proteolysis of the type seen with human IFN-γ sufficient for the inactivation of other cytokines? Third, is the ability to inactivate cytokines a common property of *Pseudomonas* strains, and how does this property relate to their production of the two proteases?

MATERIALS AND METHODS

Human subjects. The studies reported here were approved by the Human Subjects Committee of the University of Kansas Medical Center, Kansas City, and informed consent was obtained from all subjects or their parents or guardians.

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***Pseudomonas* strains, bacterial filtrates, and proteases.** For this study, 19 strains of *P. aeruginosa*, including 13 from sputum samples from cystic fibrosis patients, were isolated from clinical specimens. Each bacterium was grown in tryptic soy broth at 37°C for 24 h, and then culture filtrates were prepared as previously described (17), except that a final dialysis against 5 mM Tris buffer (pH 8.0) was performed prior to filter sterilization. *Pseudomonas* AP (57 kilodaltons) and E (33 kilodaltons) (lots 9356012 and 9442008, respectively) were obtained from Nagase Co., Ltd. (Tokyo, Japan) and have been characterized previously (17, 35). Enzyme-active-site titrations were performed on stock protease solutions by the assay of Rinderknecht et al. (38).

Assays for proteases. Total protease activity of individual bacterial filtrates was determined by using the caseinolytic assay described by Bjerrum et al. (5). Briefly, 1% agar gels containing 0.5% casein were prepared by dissolving protease substrate tablets in 50 mM Tris buffer, pH 7.4, according to the instructions of the manufacturer (Bio-Rad Laboratories, Richmond, Calif.). Samples were allowed to diffuse from wells punched in the agar, and the diameters (D) of precipitation zones forming around the wells were measured after an overnight incubation at 37°C. A positive control sample was prepared by pooling equal volumes from each of the 19 filtrates. Values of D^2 for this sample were linearly related to the reciprocal of the dilution of the sample. Then, measurements of D for individual filtrates were made, converted to D^2, and expressed as a percentage of the D^2 value for the positive control. Thus, a test filtrate showing 100% caseinolytic activity had proteolytic activity equivalent to that of the positive control pool. When expressed in this fashion, the lower limit of assay sensitivity was 25%. The concentrations of the individual proteases in filtrates were determined by radioimmunoassay. *Pseudomonas* AP and E were labeled with $Na^{125}I$ by using Enzymobeads (Bio-Rad) to catalyze the iodination. Labeled protease (25 μl; approximately 10^4 cpm) was combined with 25 μl of test filtrate or unlabeled protease. These mixtures were then combined with 50-μl quantities of either anti-AP or anti-E antiserum, prepared as previously described (17). After incubation at 37°C for 1 h, bound radiolabeled antigen was separated from free antigen

with Formalin-killed *Staphylococcus aureus* (Igsorb; The Enzyme Center, Malden, Mass.) and counted by gamma spectrometry. Protease concentrations in unidentified samples were determined by extrapolation from a standard curve derived with purified AP or E. The lower limit of sensitivity of the assay was 50 ng/ml.

Cytokines and anticytokine antibodies. Unpurified natural IFN-γ was prepared as previously described (16) by stimulating immune blood lymphocytes with tuberculin purified protein derivative. Purified human and mouse recombinant IFN-γ (rIFN-γ; specific activities, 3×10^7 and 2.3×10^7 U/mg, respectively) and human recombinant tumor necrosis factor alpha (rTNF-α; specific activity, 6.2×10^8 U/mg) were kindly provided by Christine Czarniecki and Michael Shepard of Genentech, Inc. (South San Francisco, Calif.). Purified human recombinant IL-1α (rIL-1α; specific activity, 3.0×10^9 U/mg) and rIL-1β (specific activity, 2.8×10^9 U/mg) were a gift from Stephen Gillis (Immunex, Seattle, Wash.). Since some cytokines were provided in solutions containing bovine serum albumin (BSA) as a stabilizing agent, all protease-cytokine incubation mixtures were prepared in buffer containing 0.5% BSA. Rabbit antisera to human IFN-γ and TNF-α were purchased from Genzyme, Inc. (Boston, Mass.). Antisera to IL-1α and IL-1β were provided by S. Gillis. Stephen Russell provided hamster monoclonal antibodies to murine IFN-γ.

PAGE and Western immunoblots. The procedure employed for sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) has been described previously (16). For rTNF-α and rIL-1, 14% polyacrylamide gels were used; 13 to 18% polyacrylamide gradient gels were used for rIFN-γ samples. Cytokines were diluted in sample buffer and heated at 95°C for 5 min prior to electrophoresis. Following gel electrophoresis, the proteins were electrotransferred to nitrocellulose and the transfers were reacted with the appropriate anticytokine antibodies or antisera (see above). Western immunoblots were then developed as described previously (16), except for murine rIFN-γ immunoblots, which were developed with an alkaline phosphatase-conjugated second antibody.

Cytokine bioassays. In each of the bioassays, pretreatment of the indicator cells with either AP or E did not adversely affect the detection of a known quantity of the cytokine or the binding of radiolabeled cytokine to those cells. Likewise, protease-treated BSA or protease-treated serum (sources of carrier proteins present in the reaction mixtures) did not inhibit the bioassays.

Antiviral activities of the human interferons were determined as previously described (17). For the measurement of murine IFN-γ antiviral activity, a plaque reduction assay using L929 cells and vesicular stomatitis virus was employed. In both cases, 1 U of antiviral activity was defined as the amount of test sample necessary to inhibit cytopathic effect or plaque numbers by 50% under standard assay conditions (17).

For determining the effects of *Pseudomonas* culture filtrates on IFN-γ, a source of natural IFN-γ was prepared by stimulating immune mononuclear leukocytes with tuberculin purified protein derivative. Serial twofold dilutions of the test bacterial filtrates were added to this IFN-γ sample, and the mixtures were incubated for 20 h at 37°C. Then, residual antiviral activity was determined, and inhibitory activity was expressed in inhibitory units per milliliter. One inhibitory unit was defined as the volume of test filtrate necessary to reduce antiviral activity of the IFN-γ sample by 50%, calculated by linear regression. All samples tested showed some measurable inhibitory or proteolytic activity or both.

IL-1 activity was determined by the costimulator assay described by Kurt-Jones et al. (25) using the D10.G4.1 helper T-cell line (21). D10.G4.1 cells were incubated in Click EHAA (Earle-Hanks amino acid) medium containing 5% fetal calf serum, 2 mM L-glutamine, penicillin-streptomycin, 1.2 μg of concanavalin A per ml, and serial dilutions of the test samples. After 48 h cultures were labeled with 1 μCi of [^{3}H]thymidine and harvested 16 to 18 h later. One unit of IL-1 activity was defined as the amount of test sample necessary to induce a half-maximum proliferative response of the cells, compared with a sample of rIL-1β (3×10^3 U/ml), which served as a positive control.

TNF-α bioactivity was assayed on murine L929 cells by a modification (8) of the MTT assay of Mossman (31). Briefly, test samples were serially diluted and added to microtiter plate cultures of dactinomycin (actinomycin D)-treated L929 cells for 24 h. Then, the cell monolayers were washed and cellular dehydrogenase activity of residual viable cells was determined by the MTT assay. One unit of TNF-α activity was defined as the amount of sample necessary to cause half-maximum killing of L929 cells, by using cells treated with 3 M guanidine hydrochloride as a positive control.

For all bioassays, datum reduction was performed by using a computer program that calculated the units per milliliter of each sample from titration curves. First, the program converted all absorbance or plaque assay readings to a logit function ($y/[1 - y]$, where y = fraction of the maximum response, which was determined for each test sample). Then, by linear regression, the program fit a straight line to a plot of the logit function versus log sample volume. Finally, a sample volume was calculated from this plot for $y/[1 - y] = 1$, which defined the volume of test sample containing 1 U of activity (i.e., 50% maximum activity).

RESULTS

To determine the relative sensitivities of various cytokines to *Pseudomonas* AP and E, human and murine rIFN-γs, human rTNF-α, and human rIL-1α and IL-1β were treated with the proteases under similar conditions. Recombinant cytokines were used for these experiments so that evidence of proteolysis could be visualized by SDS-PAGE. All cytokines were prepared at concentrations of 10 to 100 μg/ml in 10 mM phosphate buffer, pH 7.4, containing 0.5% BSA. The cytokines were combined with an equal volume of protease in the same buffer at enzyme-to-substrate weight ratios of 0.004 to 1.0. After 4 h of incubation at 37°C, reaction mixtures were divided into two samples. One sample was heated in sample buffer and analyzed by SDS-PAGE; the second sample was diluted in medium containing 10% fetal calf serum and tested for residual bioactivity by assays described in Materials and Methods.

Results of these experiments are reported in Fig. 1. Each experiment was performed at least three times, and representative results are shown. Each of the untreated cytokines (lanes 1) appeared principally as a 15- to 17-kilodalton monomer, but some cytokine preparations (e.g., human and murine rIFN-γ) also showed residual undissociated dimers. Changes in the mobility of the monomer form following proteolysis were always reflected in similar changes in the dimer.

The cytokines differed significantly in their sensitivities to AP and E. Human rIFN-γ was inactivated by both prote-

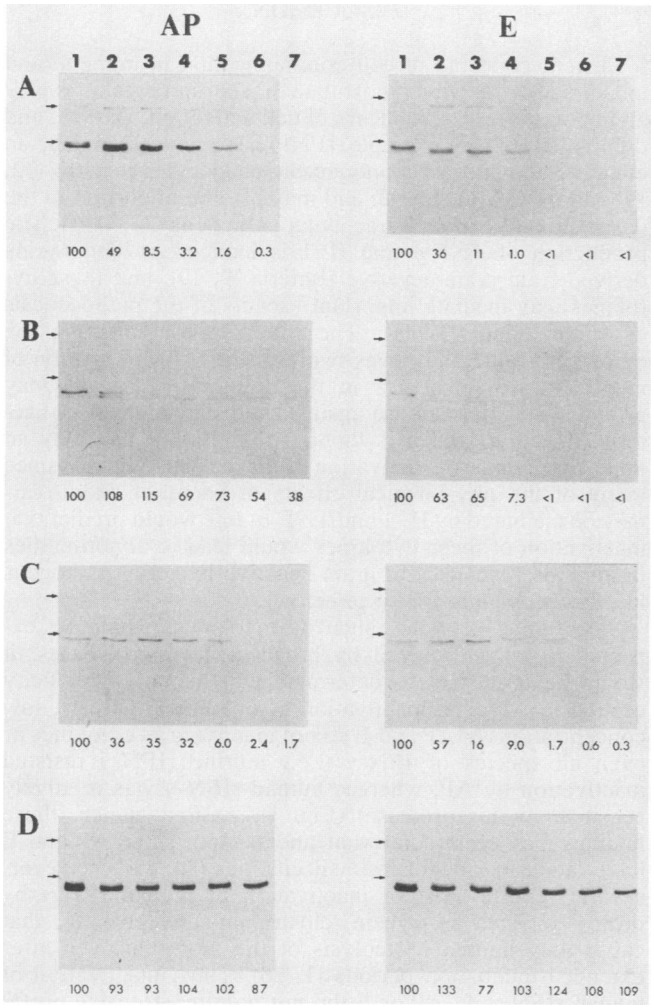

FIG. 1. Degradation and inactivation of various cytokines by *Pseudomonas* AP or E. Each cytokine was treated with either AP or E at various protease-to-cytokine ratios. Shown here are Western immunoblots using the appropriate anticytokine antisera or monoclonal antibodies. Samples were incubated either without protease (lanes 1) or with the indicated protease at an enzyme-to-substrate weight ratio of 0.004 (lanes 2), 0.012 (lanes 3), 0.037 (lanes 4), 0.11 (lanes 5), 0.33 (lanes 6), or 1.0 (lanes 7). Numbers below each lane indicate the residual bioactivity for that sample expressed as a percentage of the activity of cytokine not exposed to protease. (A) Human rIFN-γ; (B) murine rIFN-γ; (C) human rTNF-α; (D) human rIL-1α. The arrows to the left of each immunoblot denote the migration of 39- and 17-kilodalton molecular mass markers.

ases, and its loss of bioactivity was accompanied by small reductions in its apparent molecular mass (Fig. 1A). Approximately 90% inactivation occurred at enzyme-substrate ratios of 0.01. Although these reaction mixtures contained 0.5% BSA, the results are consistent with those of earlier experiments in which no carrier protein was present (16, 17). Peptides with molecular weights of less than 5,000 were not detected, which is consistent with the inability to resolve small peptides on SDS-polyacrylamide gels. At very high protease concentrations, faint or no rIFN-γ bands were seen, suggesting that the cytokine was completely degraded.

The bioactivity of murine rIFN-γ was enhanced slightly by low concentrations of AP (Fig. 1B). In contrast, murine rIFN-γ was inactivated at low E-rIFN-γ ratios. Despite these differences, both AP-treated and E-treated murine

TABLE 1. Effects of *Pseudomonas* AP and E on cytokine bioassays

Incubation mixture[a]	Preincubation of protease and cytokine	Residual bioactivity (% control)
Human rIFN-γ	−	100
Human rIFN-γ + AP	−	59[b]
Human rIFN-γ + E	−	88
Human rIFN-γ	+	100
Human rIFN-γ + AP	+	3[c]
Human rIFN-γ + E	+	4[c]
Murine rIFN-γ	−	100
Murine rIFN-γ + AP	−	156[b]
Murine rIFN-γ + E	−	91
Murine rIFN-γ	+	100
Murine rIFN-γ + AP	+	22[c]
Murine rIFN-γ + E	+	17[c]
Human TNF-α	−	100
Human TNF-α + AP	−	85
Human TNF-α + E	−	119
Human TNF-α	+	100
Human TNF-α + AP	+	10[c]
Human TNF-α + E	+	16[c]

[a] Protease-cytokine incubation mixtures (weight ratio = 0.037) were prepared exactly as described for Fig. 1 and were incubated either at 37°C for 4 h or assayed immediately.
[b] $P < 0.05$ by Student's t test.
[c] $P < 0.01$ by Student's t test.

rIFN-γs showed altered migration on gels, suggesting that the cleavage sites for the two proteases are different. At high E-rIFN-γ ratios (>0.11), no bands were resolved by SDS-PAGE, again suggesting that complete degradation of the cytokine had occurred.

Human rTNF-α was also susceptible to inactivation by both AP and E, although slightly higher protease-substrate ratios were required than those shown to be effective for inactivating human rIFN-γ (Fig. 1C). Greater than 90% inactivation of TNF-α bioactivity occurred without an observable change in the migration of the cytokine on gels or loss of staining intensity of the TNF-α band (i.e., lane 5 versus lane 1 in Fig. 1C).

Human rIL-1α was resistant to proteolysis by either protease (Fig. 1D). Neither a reduction in bioactivity nor an alteration in the mobility of the cytokine on SDS-PAGE was observed. Similar findings were made with human rIL-1β (data not shown).

Although cytokine proteolysis seemed a likely mechanism for these effects (16, 17), it was important to determine whether the proteases affected the assays employed for detection of cytokine bioactivity. Pretreatment of the cells used in the bioassays with these proteases did not inhibit the detection of a standard rIFN-γ sample (data not shown), indicating that the enzymes did not directly affect the bioassays. Likewise, mock digests prepared by incubating the proteases with BSA but no cytokine had no significant effect on the bioassays. To further address this possibility, AP or E was combined with either human or murine rIFN-γ or human rTNF-α at weight ratios shown to cause inactivation of these cytokines (i.e., 0.037) (Fig. 1). Half the samples were diluted immediately with RPMI 1640 medium containing 10% fetal calf serum and added directly to the bioassays. The remaining samples were incubated at 37°C for 4 h prior to dilution and testing. Residual bioactivities are shown in

TABLE 2. Correlation between IFN-γ inhibitory activities and protease concentrations of culture filtrates from 19 *Pseudomonas* strains

Pseudomonas strain	IFN-γ inhibitory activity (U/ml)[a]	Protease concn (μg/ml)[b]		Caseinolytic activity (% control)[c]
		AP	E	
1	1,679	8.9	46.9	753
2	1,370	12.8	39.2	877
3	1,146	10.2	11.4	623
4	1,095	15.1	9.9	543
5	1,006	8.2	10.0	898
6	975	7.3	11.0	543
7	775	10.0	48.0	709
8	696	6.0	8.8	623
9	504	0.8	1.1	25
10	500	13.7	9.1	665
11	405	13.2	44.7	800
12	0	9.6	1.0	25
13	0	7.3	1.3	25
14	0	3.1	0.2	25
15	10	7.6	0.2	25
16	15	5.3	0.3	25
17	10	8.2	0.2	25
18	10	8.7	0.2	25
19	10	NT[d]	NT	25

[a] IFN-γ inhibitory activity is expressed in units/ml, where 1 U is defined as the volume of test filtrate necessary to reduce antiviral activity by 50%. These values were derived from a linear regression plot of volume of test sample versus residual antiviral activity.
[b] Determined by radioimmunoassay.
[c] See Materials and Methods.
[d] NT, Not tested.

Table 1. In each case, inactivation of the cytokine by AP or E was significantly greater following preincubation of the enzyme and substrate. AP partially inactivated human rIFN-γ without preincubation, which may reflect the somewhat slower rate of inhibition of the protease by protease inhibitors present in serum, when compared with E (16). Overall, these results are consistent with the conclusion that proteolysis of the cytokines was probably essential for a full reduction in their bioactivities.

These data would predict that the capacity that different *Pseudomonas* strains have for inactivating cytokines should correlate with the overall production of proteases by the strains. To test these predictions, we examined filtrates prepared from 19 different *P. aeruginosa* strains for AP and E concentrations, total proteolytic activity, and the ability to inactivate natural human IFN-γ. This cytokine was selected because of its susceptibility to both *Pseudomonas* proteases (Fig. 1) (16). The IFN-γ sample used in this experiment was a culture supernatant fluid prepared by stimulating immune human mononuclear leukocytes with tuberculin purified protein derivative and contained 850 antiviral units per ml. Inactivation of human IFN-γ is a property common to many *Pseudomonas* strains (Table 2). This activity showed a strong positive correlation with the caseinolytic activity of the individual filtrates ($r = 0.83$; $P < 0.001$). Ability to inactivate IFN-γ was not significantly correlated with AP concentration ($r = 0.37$; $P > 0.1$). Although a significant correlation also existed between IFN-γ inactivation and E concentration ($r = 0.64$; $P < 0.01$), inspection of the data for individual filtrates (e.g., strain 1 versus strain 7 versus strain 8) suggested that E did not play an exclusive role in IFN-γ inactivation.

DISCUSSION

Current concepts of cell communication in immune and inflammatory responses postulate that certain cytokines play an important role as inducers of cell activation, growth, and differentiation. For example, IFN-γ has been shown to be an effective activator of mononuclear phagocytes in vitro (32, 39) and in vivo (7, 12, 32) and may also be important in the destruction of certain intracellular pathogens (7, 33, 44). The production of TNF-α and IL-1 is induced by endotoxins derived from gram-negative bacteria (9, 19), and these cytokines may mediate important aspects of the pathogenesis of endotoxemia (3, 11). The rationale for selecting the cytokines studied here was twofold. First, the activation of mononuclear phagocytes in the pulmonary airways may provide an initial defense against the establishment of bacterial infection, and it is thought that IFN-γ may play an important role in the activation of these cells. Second, since many of the physiological effects of bacterial endotoxins may be mediated by IL-1 and TNF-α, one would predict that inactivation of these cytokines would lead to abnormalities in immune responses to gram-negative bacterial pathogens like *Pseudomonas* spp. (see below).

Since small proteins, such as cytokines, would be expected to be hydrolyzed by broad-spectrum proteases, it would be important to determine whether any specificity existed in AP or E inactivation of cytokines. Clearly, low concentrations of AP and E do not inactivate all cytokines or even all species of IFN-γ (i.e., murine rIFN-γ resisted inactivation by AP, whereas human rIFN-γ was relatively sensitive to this protease) (Fig. 1). At first glance, these findings may seem somewhat unexpected, since AP and E have rather broad substrate specificities (14, 29). However, several reports exist of inactivation of human IFN-γ by proteases, such as trypsin, clostripain, and pronase, that cause only limited proteolysis of the molecule even after extended incubation periods (1, 26). Thus, inactivation of human rIFN-γ by AP or E did not require extensive proteolysis but rather was associated with a slight overall reduction in apparent molecular mass. This was also found to be true of murine rIFN-γ. With human TNF-α, no apparent change in migration of the protein on gels was observed. These results do not preclude the extensive hydrolysis of the proteins by attack of less-accessible peptide bonds given higher enzyme concentrations or prolonged incubations, but they suggest that many cytokines bear relatively protease-sensitive domains that are required for their bioactivities.

We believe that the reduction in the bioactivities of human and murine IFN-γs and human TNF-α reported here resulted from the proteolysis of IFN-γ and TNF-α by AP or E or both. The loss of bioactivity and the loss of the parent forms of the cytokine proteins visible on gels showed similar dose-response relationships. The effects of AP and E on rIFN-γ and rTNF-α required preincubation of the enzyme-substrate mixture; very little effect was seen if the reaction mixtures were tested immediately. Finally, AP and E had no apparent direct effects on the IFN-γ or TNF-α bioassays under the conditions employed in this study.

The ability to inactivate natural IFN-γ with a filtrate prepared from a protease-deficient *Pseudomonas* strain (PAO-1-1641) was reconstituted by the addition to the filtrate of purified AP and E (data not shown). That reconstitution of this filtrate with both proteases yielded more activity than the addition of either protease alone is consistent with the conclusion that the two proteases act synergistically (16). Also consistent with this conclusion are the data derived by

comparing a large number of *Pseudomonas* strains that differed widely in terms of IFN-γ inhibitory activity (Table 2). This property correlated positively with E concentrations, but an even higher correlation was observed between IFN-γ inactivation and total caseinolytic activity. Although these results would argue for a dominant role for E (as compared with AP) in inactivation of human IFN-γ, E concentrations were less predictive of this trait than was total proteolytic activity.

Treatment of cytokines with AP or E was performed in the presence of either BSA or human serum, either of which includes potential substrates for the proteases. Proteolysis and inactivation of human rIFN-γ in the presence of BSA (Fig. 1) required somewhat greater protease concentrations than were required in the absence of the carrier protein (16), but overall patterns of inactivation and cleavage were nearly identical (data not shown). The degree of cytokine inactivation by a given concentration of protease varied somewhat between different experiments. This was more apparent with AP than with E (e.g., Fig. 1B versus Table 1). We attribute this property of AP to the relative instability of the enzyme with storage following rehydration, even at −70°C. For this reason, we have characterized substrate susceptibility to proteolysis by AP and E in relative terms.

Unlike TNF-α, human rIL-1α and rIL-1β appeared to be resistant to the effects of *Pseudomonas* AP and E. The cytokines showed neither evidence of proteolysis on gels nor any reduction in their comitogenic activity for T cells, even at high enzyme-to-substrate weight ratios. These findings are consistent with earlier reports demonstrating that natural human and mouse IL-1s are relatively resistant to inactivation by trypsin, chymotrypsin, and papain (24, 27). IL-1 and TNF-α share a number of properties that relate to inflammatory responses, including induction of fever (11), stimulation of acute-phase protein synthesis by hepatocytes (30), and inhibition of lipoprotein lipase (4, 20). However, differences in the biologic effects of the two cytokines do exist (10), leading to speculation as to the potential effects of depletion of one but not the other by an inactivating enzyme. One example of a potential cytokine imbalance relates to the relative abilities of human TNF-α and IL-1 to stimulate the synthesis of certain complement components by fibroblasts. Factor H destabilizes the alternative pathway C3 convertase, C3bBb, and thereby serves to regulate complement activation. Whereas TNF-α can induce C3, factor B, and factor H production by human fibroblasts, only C3 and factor B production are stimulated by IL-1 (18). Inactivation of TNF-α, but not IL-1, by *Pseudomonas* proteases would be expected to yield an imbalance in the synthesis of these components and a dysregulation of complement activation. Since TNF-α also differs qualitatively or quantitatively from IL-1 in terms of its shock-inducing potential (34), lymphocyte comitogenic activity (22, 37), effect on hematopoiesis (6, 28), effect on neutrophil oxidative metabolism (13), and cytotoxic range on tumor target cells (23), differences in proteolytic sensitivities between the two cytokines would be expected to substantially alter host immune responses.

Whether proteases derived from *P. aeruginosa* can regulate the activity of cytokines in vivo is unknown. Although the potential effects of protease inhibitors or excess competing substrates have not, as yet, been determined, the potential for proteolytic inactivation of certain cytokines during infections is clearly demonstrated here. Cytokine inactivation by the *Pseudomonas* proteases AP and E is selective and appears to require limited proteolysis rather than extensive degradation of these cytokines. Synergistic effects of the two proteases appear to occur. Thus, the potential for selective biological inactivation of cytokines by many *P. aeruginosa* strains exists, particularly during the early phase of pulmonary infection when the composition of airway fluids is relatively free of antiprotease antibodies and plasma-derived protease inhibitors.

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