Immunoglobulin A1 Protease, an Exoenzyme of Pathogenic Neisseriae, Is a Potent Inducer of Proinflammatory Cytokines

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Summary

A characteristic of human pathogenic Neisseriae is the production and secretion of an immunoglobulin (Ig)A1-specific serine protease (IgA1 protease) that cleaves preferentially human IgA1 and other target proteins. Here we show a novel function for native IgA1 protease, i.e., the induction of proinflammatory cytokines such as tumor necrosis factor (TNF)-α, interleukin (IL)-1β, IL-6, and IL-8 from peripheral blood mononuclear cells. The capacity of IgA1 protease to elicit such cytokine responses in monocytes was enhanced in the presence of T lymphocytes. IgA1 protease did not induce the regulatory cytokine IL-10, which was, however, found in response to lipopolysaccharide and phytohemagglutinin. The immunomodulatory effects caused by IgA1 protease require a native form of the enzyme, and denaturation abolished cytokine induction. However, the proteolytic activity is not required for the cytokine induction by IgA1 protease. Our results indicate that IgA1 protease exhibits important immunostimulatory properties and may contribute substantially to the pathogenesis of neisserial infections by inducing large amounts of TNF-α and other proinflammatory cytokines. In particular, IgA1 protease may represent a key virulence determinant of bacterial meningitis.

Key words: N. gonorrhoeae • N. meningitidis • inflammation • tumor necrosis factor α • virulence factor

The Neisseriae are gram-negative, diplococcal bacteria which primarily colonize human mucosal surfaces. Besides several commensal species, the genus includes two human pathogens, N. gonorrhoeae (gonococci), the causative agent of gonorrhea, and N. meningitidis (meningococci), one major cause of bacterial meningitis. Both pathogens exclusively colonize or infect humans as their natural hosts. Gonococci induce a local inflammatory response of the urogenital mucosa (1). Meningococci are mostly harmless colonizers of the respiratory tract (e.g., nasopharynx). Under some not yet fully understood circumstances, meningococci disseminate from locally infected tissues into the blood stream and penetrate the blood–brain barrier to cause severe inflammations in the central nervous system (2).

Various bacterial components have been implicated in the pathogenesis of neisserial infections (3–6). Pili and Opa proteins contribute to the attachment and the invasion of different host cells, and the outer membrane porin protein P or PorB was found to prevent the microbicidal activities of phagocytes (7, 5) and to induce apoptosis in target cells (8). Circumstantial evidence related to the unique association of IgA1 proteases with human pathogenic bacterial species (9, 6) suggests that this enzyme plays a significant role in pathogenesis. IgA1 proteases are produced by a variety of gram-positive and gram-negative human pathogens (10–12). Among those, the IgA1 proteases of the pathogenic Neisseriae and various H. amophilus species are structurally closely related (13, 14). They undergo frequent horizontal exchange across the species barrier, thus forming a polymorphic class of proteins (15, 16). The gram-negative bacterial IgA1 proteases are derived from a polyprotein precursor molecule which directs enzyme secretion by an autocatalytic process. While the neisserial IgA1 proteases may include two additional secreted domains, an α-protein and a γ-peptide (13), the proteases of the two species probably exhibit similar functions.

IgA1 protease cleaves human IgA1 both in serum and at mucosal surfaces of the respiratory and urogenital tracts (10, 17). In particular, the neisserial IgA1 protease is a sequence-specific endopeptidase which cleaves single peptide bonds of distinct proline-rich consensus sequences that are found in the hinge region of human IgA1 but not IgA2 (10, 18). A consequence, the enzymatic activity or characteristic cleavage products of IgA1 molecules were found in nasopharyngeal and vaginal secretions of individuals infected with pathogenic N. Neisseriae (11, 19, 20). As intact IgA1 protease protects against microorganisms infecting mucosal tissues by prevention of microbial adherence, toxin neutralization,
and activation of Fc-α receptor–dependent phagocytosis, the cleavage of IgA1 has been postulated to impair IgA1-mediated effector functions (11, 12). However, most individuals have neutralizing antibodies against IgA1 protease that may interfere with the function of the enzyme (21–23). Thus, the role of IgA1 protease in neisserial pathogenesis remains obscure.

Inflammatory cytokines such as TNF-α, IL-1β, IL-6, and IL-8 are produced by activated monocytes/macrophages. They promote local inflammatory responses at the sites of microbial infections, and mediate adherence of leukocytes to the endothelial tissues and their transmigration by upregulating the expression of adhesion molecules (24–27). Elevated levels of TNF-α, IL-1, IL-6, and IL-8 have been found in the cerebrospinal fluid (CSF) and plasma of patients with meningitis (28–31) and were detected in the urine after the intraurethral challenge of patients with N. gonorrhoeae (32).

Besides LPS, several bacterial products that can stimulate cytokine synthesis in different cell types have been characterized (33). These cytokine-inducing agents include heat labile proteins, glycoproteins, lipoproteins, or peptides, which trigger cytokine synthesis by mechanisms distinct from LPS and therefore form an additional class of virulence factors termed modulins (34, 35).

The purpose of this study was to assess the immunogenic and immunomodulatory properties of the neisserial IgA1 protease. We demonstrated that purified IgA1 protease acts like a modulin by stimulating the release of TNF-α, IL-1β, IL-6, and IL-8 from PBMCs. These effects were independent of the proteolytic activity but required the native form of the enzyme.

Materials and Methods

Purification and Characterization of Recombinant IgA1 Protease. In this study, we used the recombinant form of IgA1 protease expressed in Esherichia coli strain H2053, which harbors plasmid pIP11 containing the iga gene of N. gonorrhoeae M511 with the temperature-inducible bacteriophage λ promoter Pλ, as described previously (18). In brief, the supernatant of a 4-liter stationary phase culture was recovered by centrifugation, supplemented with 0.1 M EDTA, and diluted 1:1 (vol/vol) with potassium phosphate buffer (20 mM, pH 7.0). The bacterial supernatant was applied to a cation exchange column. After washing, bound protein was eluted with 500 mM potassium phosphate, 8.6% glycerol. Fractions of 10 ml were collected, precipitated with ammonium sulfate, and then redissolved in gel filtration buffer. Gel filtration was performed with a preequilibrated Superdex column (Amersham Pharmacia Biotech). Protein-containing eluates were recognized by UV absorption, pooled, and precipitated with ammonium sulphate. Finally, the pellet was suspended in PBS and dialyzed against PBS, pH 7.4, and stored in aliquots at −70°C. Purity of the protein was assessed by SDS-PAGE with subsequent Coomassie staining showing the characteristic protein profile with the typical 109- and 106-kD protein bands as described previously (18). The protein concentration was determined using a commercial protein determination assay (Bio-Rad). Specificity for IgA1 protease was confirmed by immunoblotting using an IgA1 protease–specific rabbit antiserum (13). The cleavage activity of the recombinant IgA1 protease was determined with human IgA1 (20 ng/μl; Dakopatts) in Tris buffer (0.05 M Tris.HCl, 10 mM CaCl2, and 10 mM MgCl2, pH 7.5) at 37°C according to the method described previously (36). Heat denaturation (boiling for 10 min) completely destroyed the enzymatic activity. The enzymatic activity could also be blocked by addition of the specific peptide prolyl boronic acid inhibitor (37). 100 μM of the inhibitor (provided by A.G. Plaut, New England Medical Center, Boston, MA) abrogated cleavage of IgA at concentrations up to 10 ng per μl of IgA1 protease (data not shown).

Endotoxin Assays. The endotoxin contents of our IgA1 protease preparations were determined by Limulus amebocyte lysate assay (LAL; Haemochrom Diagnostic). All preparations contained <2 pg of endotoxin per μg protein. It is described that some proteins could interfere with the Limulus assay (38). To ensure that IgA1 protease is free of LPS, we have tested in addition the priming potency of IgA1 protease for neutrophils, which are as sensitive as the Limulus assay in detecting LPS. Different types of LPS can prime neutrophils to increase their oxidative response after stimulation with the agonist fMLP (Sigma Chemical Co.). (38). PM Ns preincubated with 10 μg/ml IgA1 protease for 30 min stimulated with 1 μM fMLP showed no alteration of the oxidative response compared with the control when measured as luminol-dependent chemiluminescence in a six-channel Bliomut LB 9505 (Berthold; data not shown).

C eils. PBMCs were isolated fromuffy coats or freshly citrated blood of adult, healthy volunteers by density gradient centrifugation over Ficoll/Isoaque (Amersham Pharmacia Biotech) and washed twice in Ca2+, Mg2+-free PBS. For some experiments, lymphocytes and monocytes were separated (6 × 107 cells/ml in PBS plus 0.5% BSA) by counterflow centrifugation (elutriation) using the Beckman (2×107) centrifuge system. Elutriation was performed at constant rotor speed with increasing flow rates, and lymphocytes were collected. To obtain purified T cells, the lymphocyte suspensions were positively sorted by anti-CD3 magnetic beads (MACS; Miltenyi Biotech). Purification of CD3+ T cells was always >95% as determined by flow cytometry. Monocytes were prepared from PBMCs either by adherence to plastic dishes (1 h, 37°C) or by negative selection using the MACS monocyte isolation kit (Miltenyi Biotech). The purity of CD14+ cells was always >85% as measured by flow cytometry using a PE-conjugated anti-CD14 antibody (Leu M3; Becton Dickinson). The remaining 15% consisted of CD3−, CD19−, and CD56− flow cytometric counting events.

The monotypic cell line MomoMac 6 (no. 124; American Type Culture Collection) was obtained from the German Collection of Microorganisms and Cell Cultures (Braunschweig, Germany) and maintained in 24-well plates under low endotoxin conditions (<10 pg/ml) in RPM I 1640 supplemented with 10% heat-inactivated FCS, 2 mM l-glutamine, 1% nonessential amino acids, 1 mM sodium pyruvate, and 9 μg/ml bovine insulin as described (39). The cell line normally expresses CD14 as controlled (40). Cell viability was assessed by trypan blue staining.

C el Viability. Cytotoxic effects of IgA1 protease preparations were excluded by staining of PBMCs with propidium iodide (10 μg/ml) and subsequent evaluation by flow cytometry. Cell viability of freshly isolated or cultured cells (24 h with 10 μg/ml IgA1 protease) was >95% in all experiments. Cell culture and Stimulation of Cytokine Release. All cell cultures were performed in RPM I 1640 supplemented with 20 nM...
Table I. Dose-dependent Cytokine Induction by Purified IgA1 Protease and LPS from *E. coli*

<table>
<thead>
<tr>
<th>Cytokine*</th>
<th>Control†</th>
<th>IgA1 protease (μg/ml)</th>
<th>LPS (ng/ml)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>0.1</td>
<td>1.0</td>
</tr>
<tr>
<td>IL-1β</td>
<td>&lt;0.01</td>
<td>0.02 ± 0.0</td>
<td>0.1 ± 0.0</td>
</tr>
<tr>
<td>IL-6</td>
<td>0.2 ± 0.0</td>
<td>0.3 ± 0.1</td>
<td>19.5 ± 0.5</td>
</tr>
<tr>
<td>IL-8</td>
<td>7 ± 0.5</td>
<td>15 ± 1.9</td>
<td>29.8 ± 3.6</td>
</tr>
</tbody>
</table>

* Cytokine levels in culture supernatants from PBMCs incubated for 18 h in the presence of different concentrations of IgA1 protease and *E. coli* LPS. Values are the means ± SD from triplicate wells. Amounts are given in ng/ml; IL-1β (detection limit 10 pg/ml), and IL-6 and IL-8 (detection limit 50 pg/ml).

† Culture medium was used as a control.
Statistical Analyses. The nonparametric Wilcoxon rank test was performed for comparison of cytokine data of PHA-, LPS-, and IgA1 protease-stimulated PBMCs. Statistical differences were considered significant at $P < 0.01$. Data from separate experiments were expressed as means ± SEM and were evaluated for statistical significance by Student’s $t$ test. Data from a single representative experiment are presented as means ± SD obtained by triplicate determination.

Results

Induction of Proinflammatory Cytokine Release from Human PBMCs by IgA1 Protease. To assess the production of the proinflammatory cytokines TNF-$\alpha$, IL-1$\beta$, IL-6, and IL-8, PBMCs from healthy human donors were incubated with purified IgA1 protease. LPS, PHA, and medium alone were used as control. Initial experiments showed that release of proinflammatory cytokines from PBMCs induced by IgA1 protease reached maximal levels between 12 and 24 h (data not shown). Furthermore, a rapid decrease in IgA1 protease-induced cytokine production could be observed after 24 h, and only small amounts of TNF-$\alpha$ were detectable after 72 h. Therefore, in subsequent experiments a single end-point of 18 h culture was chosen that allowed detection of TNF-$\alpha$ and other proinflammatory cytokines. IgA1 protease stimulated the release of proinflammatory cytokines by PBMCs in a dose-dependent manner (Fig. 1, and Table I). Significant amounts of TNF-$\alpha$ in the supernatants were already detectable in the presence of 1 $\mu$g/ml IgA1 protease, and highest levels were reached using 10 $\mu$g/ml. Similar dose–response patterns were obtained for other proinflammatory cytokines such as IL-1$\beta$, IL-6, and the chemokine IL-8. Nonstimulated PBMCs produced negligible amounts of TNF-$\alpha$, IL-1$\beta$, and IL-6, but some IL-8, which was probably induced by adherence as previously postulated by Kasahara et al. (40).

To determine whether the cytokine induction was due to LPS present in the preparation of the recombinant IgA1 protease from E. coli, we tested the potential of E. coli LPS to induce TNF-$\alpha$. The endotoxin content present in the protease preparation of 10 $\mu$g/ml did not exceed 20 pg/ml. As shown in Fig. 1, no significant cytokine production compared with the control was detected at 10–30 pg/ml LPS, indicating that endotoxin contamination was not the cause of cytokine induction by purified IgA1 protease. TNF-$\alpha$ synthesis in response to 10 $\mu$g/ml IgA1 protease reached the level synthesized by cells stimulated with a much higher dose of E. coli LPS (30 ng/ml). In subsequent experiments, low dose (30 pg/ml) and high dose (30 ng/ml) controls of LPS were always included.

The cytokine release of PBMCs showed similar variations between different donors upon stimulation with either IgA1 protease, LPS, or PHA. As shown in Fig. 2 (A–D), the median levels of the proinflammatory cytokines detected in the presence of 10 $\mu$g/ml IgA1 protease were approximately similar to those found after LPS stimulation and sometimes even higher than those incubated with the lectin PHA.

Heat denaturation abolished the cytokine-inducing capacity of IgA1 protease (data not shown). To test whether the
enzymatic activity is involved in that process, PBMCs were incubated in the presence of 100 μM of a specific IgA1 protease inhibitor (peptide prolyl boronic acid [37]). Although at this concentration the inhibitor completely blocked the cleavage of IgA1, it altered neither the LPS- nor the IgA1 protease–induced production of proinflammatory cytokines, but not the enzymatic activity of IgA1 protease is required for the induction of proinflammatory cytokines.

Cytokine release induced by IgA1 protease is augmented in the presence of T cells. IgA1 protease triggers a strong release of proinflammatory cytokines from PBMCs after only a short incubation period, indicating that monocytes might be the main producer of these mediators. To determine the source of cytokine release, we cultivated lymphocyte-depleted peripheral blood monocytes (purity varied between 85 and 95%). As demonstrated in Fig. 3, IgA1 protease induced significantly lower amounts of TNF-α, IL-1β, IL-6, and IL-8 compared with LPS, which readily stimulated TNF-α production in purified monocytes. Direct comparison with PBMCs revealed that IgA1 protease–stimulated PBMCs can elicit up to 20-fold higher amounts of these cytokines compared with the respective lymphocyte-depleted monocyte cultures, which was particularly true for IL-6 and IL-8 (data not shown; see also Fig. 2). We also tested the monocytic cell line, MonoM ac 6. This cell line showed phenotypic and functional characteristics of mature blood monocytes and produces IL-1, IL-6, and TNF-α upon stimulation with LPS or PMA (39, 41). Compared with LPS, we found a significantly lower production of these cytokines and IL-8 after stimulation with IgA1 protease (Table II).

Direct contact with T cells can augment monocyte cytokine production (42). To examine the necessity of cell contact for TNF-α release in the different types of stimulation, monocytes stimulated with IgA1 protease, LPS, or PHA were incubated with autologous active and fixed (15 min, paraformaldehyde at 0°C) lymphocytes at a monocyte/lymphocyte ratio of 1:2. In parallel, the lymphocytes and monocytes were incubated alone. Such lymphocytes and purified monocytes produced only low levels of TNF-α upon stimulation with 10 μg/ml IgA1 protease (Fig. 4). The same results were obtained after stimulation with PHA. The addition of viable lymphocytes increased the TNF-α secretion in nonstimulated, IgA1 protease–, and PHA-treated monocytes, whereas the addition of prefixed lymphocytes did not. Monocytes stimulated with IgA1 protease or PHA also showed a significantly stronger TNF-α induction in the presence of lymphocytes compared with the control. Only LPS was able to induce TNF-α in purified monocytes alone, and this was not further enhanced by lymphocytes.

To examine the particular role of T cells for IgA1 protease–induced TNF-α release, monocytes were incubated

Table II. Release of Proinflammatory Cytokines in the Monocytic Cell Line, MonoM ac 6

<table>
<thead>
<tr>
<th>Cytokine*</th>
<th>IgA1 protease (10 μg/ml)</th>
<th>LPS (30 ng/ml)</th>
<th>PHA (5 μg/ml)</th>
<th>PMA (100 ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNF-α</td>
<td>&lt;dl</td>
<td>0.03</td>
<td>0.12</td>
<td>&lt;dl</td>
</tr>
<tr>
<td>IL-1β</td>
<td>&lt;dl</td>
<td>0.02</td>
<td>0.06</td>
<td>&lt;dl</td>
</tr>
<tr>
<td>IL-6</td>
<td>&lt;dl</td>
<td>0.59</td>
<td>23.37</td>
<td>&lt;dl</td>
</tr>
<tr>
<td>IL-8</td>
<td>2.02</td>
<td>12.21</td>
<td>84.96</td>
<td>11.28</td>
</tr>
</tbody>
</table>

* Cytokine concentrations in culture supernatants are given in ng/ml. <dl, below detection limit.
1 MonoM ac 6 cells were incubated at 10⁶ cells/ml for 24 h, and supernatants were analyzed for cytokine production. Values are means from duplicate assays.
2 Corresponding culture medium was used as a control.
IgA1 Protease Stimulates Cytokine Production

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with autologous CD3+ T lymphocytes at a monocyte/lymphocyte ratio of 1:4. While T lymphocytes produced no or very low amounts of TNF-α, the monocytes released reduced levels of TNF-α in response to IgA1 protease compared with crude PBMCs. Such reduced TNF-α secretion of monocytes was enhanced in the presence of autologous purified T cells (Fig. 5). However, when the two cell populations were separated by a permeable membrane during the cell culture, the TNF-α release by monocytes was not increased in response to IgA1 protease (data not shown). These results suggest that direct contact between T cells and monocytes is necessary for a maximum of cytokine production by monocytes in response to IgA1 protease.

It is well known that lymphokines such as GM-CSF or IFN-γ can augment cytokine release in monocytes (43, 44). We found increased levels of GM-CSF in PBMC cultures after incubation with IgA1 protease for 18 h, whereas IgA1 protease failed to induce release of IFN-γ after that time (Fig. 6, A and B). Very low levels of IFN-γ were finally detectable after 4 d (data not shown). However, the IFN-γ levels in these cultures were always more than 100-fold lower than peak levels of IFN-γ in PHA-stimulated PBMC cultures. Furthermore, in the first 4 d of culture, neither cell proliferation nor increase of IL-2 receptor-positive lymphocytes could be measured after incubation with IgA1 protease (data not shown). These results suggest that viable T cells are required for the cytokine release by monocytes stimulated with IgA1 protease, but unlike PHA, IgA1 protease does not seem to provide a strong activation signal for T lymphocytes.

Effect of IgA1 Protease on IL-10 Production by PBMCs. IL-10 is a cytokine with immunosuppressive and antiinflammatory effects on monocytes. Reportedly, IL-10 synthesis is delayed relative to the synthesis of inflammatory cytokines TNF-α, IL-1β, and IL-6 (45). In contrast to the induction of proinflammatory cytokines, IgA1 protease failed to induce IL-10, whereas LPS as well as PHA could stimulate IL-10 production in PBMCs (Fig. 7). Kinetic experiments revealed that IgA1 protease does not induce IL-10 production in PBMCs within a period up to 96 h. In contrast, culture supernatants of PHA- or LPS-stimulated PBMCs contained high IL-10 levels at different time points (data not shown).

Figure 4. PBLs enhance TNF-α release of monocytes. PBMCs were separated by counterflow elutriation and adherence into monocytes (Mn) and lymphocytes (Ly) as described in Materials and Methods. After 1 h incubation, the autologous lymphocytes were added to the monocytes at a monocyte/lymphocyte ratio of 1:2. Fixed lymphocytes were treated with 2% paraformaldehyde (15 min at 0°C). TNF-α levels in the supernatants were measured 18 h after stimulation with 30 ng/ml LPS, 5 μg/ml PHA, or 10 μg/ml IgA1 protease.

Figure 5. Purified CD3+ T lymphocytes enhance TNF-α production of CD14+ monocytes. The autologous CD3+ T lymphocytes (T) were added to the monocytes (Mn) at a monocyte/lymphocyte ratio of 1:4. TNF-α levels in the supernatants were measured after 18 h of culture with 10 μg/ml IgA1 protease. The results shown here are mean values from three experiments from different donors ± SEM (△, ○, and ●).

Figure 6. GM-CSF (A) and IFN-γ (B) release of PBMCs in response to IgA1 protease (10 μg/ml), PHA (5 μg/ml), and LPS (30 ng/ml). The levels of these cytokines were determined in supernatants after 18 h. The results (means ± SD of three determinations) were confirmed by three independent experiments.

Figure 7. Western blot analysis of IL-10 expression in PBMCs. Total RNAs were extracted from PBMCs after 18 h incubation with 10 μg/ml IgA1 protease, 5 μg/ml PHA, or 30 ng/ml LPS and reverse transcribed to cDNA. Three independent experiments were performed.
can enhance the cytokine expression in monocytes synergistically in combination with GM-CSF (43, 44). Although GM-CSF was detected after IgA1 stimulation, the release of IFN-γ was low and the addition of anti-IFN-γ antibodies had no effect on the monokine production (data not shown). However, the fact that cocultivation with T cells separated from the monocytes by a permeable membrane could not enhance the production of proinflammatory cytokines indicates that T cells may also participate by a yet unknown contact-dependent mechanism or by nonclassical presentation of antigen (47–49). We also did not find binding of IgA1 protease to T cells, whereas CD14+ monocytes were clearly stained with FITC-labeled IgA1 protease (data not shown).

The profile of the cytokine response of PBMCs to IgA1 protease shows some similarities to LPS stimulation. Although the IgA1 protease was produced as a recombinant protein in E. coli, our preparations contained usually <2 pg LPS/μg protein. This LPS content could not sufficiently induce the observed cytokine production. Furthermore, effects which are typically triggered by LPS, such as priming of phagocytes for an enhancement of the oxidative burst, could not be confirmed in cells incubated with IgA1 protease (38; and data not shown). On the other hand, LPS does not require T cell engagement, suggesting that IgA1 protease functions by a different mechanism. Both molecules can have synergistic effects in vitro (data not shown) and may also act concertedly in vivo. Activated monocytes/macrophages also produce the antiinflammatory cytokine IL-10. This cytokine mediates important autoregulatory effects by downregulating inflammation, and its maximal production is delayed compared with the initial burst of proinflammatory cytokines (45). IL-10 production was augmented by stimulation with LPS or PHA, but not with IgA1 protease (50). Thus, IgA1 protease seems unique in stimulating an asymmetric synthesis of proinflammatory cytokines from monocytes and fails to induce strong regulatory feedback loops.

Besides the neisserial IgA1 protease reported here, other bacterial products have been found to induce proinflammatory cytokines. These components have been classified as a separate class of bacterial virulence factors termed modulins (51, 34). Examples include the listeriolysin from Listeria monocytogenes (52), the E. coli hemolysin (53), and the streptococcal pneumolysin (54). Other proteins with enzymatic activity, such as the urease of Helicobacter pylori (55) and the sphingomyelinase from Staphylococcus aureus (56), also modulate cytokine production. Only a few modulins, like the anthrax lethal toxin from Bacillus anthracis (34) and the clostridium toxin from C. difficile (57), act in the attomolar or femtomolar range, while most other modulins including IgA1 protease exhibit their activity in the nanomolar range. Some modulins, such the staphylococcal toxic shock syndrome toxin 1 (TSST-1) from S. aureus or the erythrogenic exotoxin (ET) from Streptococcus pyogenes possess superantigenic activity, thereby inducing T cells to proliferate and to produce cytokines (58). IgA1 protease is different from those superantigenic factors because it is not a T cell mitogen.

Discussion

IgA1 protease, a putative virulence factor of human pathogenic N. meningitis, was initially discovered as an enzyme that cleaves human IgA1 molecules, thus interfering with the biological functions of IgA1 (10). Here we report on a novel property of neisserial IgA1 protease, the induction of the proinflammatory cytokines TNF-α, IL-1β, and IL-6, and the chemokine IL-8 in PBMCs.

The stimulation of cytokine synthesis by IgA1 protease occurred in the nanomolar range and increased in a dose-dependent manner. The level of cytokine release was substantial but varied to some extent depending on the donor. The rapid cytokine induction within 24 h indicates a role of monocytes/macrophages in this process. However, compared with PBMCs, significantly lower amounts of TNF-α and IL-8 and practically no IL-6 and IL-1β were elicited by purified monocytes in response to IgA1 protease, whereas LPS induced a strong cytokine secretion that included IL-6 and IL-1β. The monocytic cell line MonoMac 6 also showed a low production of TNF-α, IL-1β, IL-6, and IL-8 in response to IgA1 protease. These results suggest that the presence of lymphocytes is required for full cytokine induction by IgA1 protease.

It is known that the activation of monocytes/macrophages and the release of monocyte-derived cytokines can be provoked by either direct cell–cell contact with activated antigen-specific T cells or secreted lymphokines (42–44, 46). This is consistent with our finding that IgA1 protease-induced TNF-α secretion by monocytes was enhanced by the addition of lymphocytes or CD3+ T cells to the cultures. To investigate the role of activated T cells in this process, we measured IFN-γ secretion, CD69, and IL-2 receptor expression, representing typical markers of activated T cells. Although CD69 was slightly upregulated, the low number of IL-2 receptor-positive cells suggests that no large T cell population was activated or that only partial activation occurred (46; and data not shown). The T cell cytokine IFN-γ...
The property of Neisseria IgA1 protease to induce proinflammatory cytokines may be highly relevant for the disease-causing properties of gonococci and meningococci. A hallmark of the pathophysiology in these diseases is the intense inflammatory response associated with the substantial release of proinflammatory cytokines. TNF-α, IL-1, IL-6, and IL-8 have been detected in local secretions and sera during mucosal infections with gram-negative species, including N. meningitidis and N. gonorrhoeae. Increased levels of IL-8 and TNF-α in particular in plasma and in urine, and smaller amounts of IL-1α and IL-6 were found in experimental gonorrhea after intraurethral application of gonococci (32).

High levels of these cytokines were also found in the CSF and in the serum during meningococcal inflammation. These mediators promote the inflammatory response with subsequent tissue damage and in some cases septic shock (28–30). The level of TNF-α in the CSF directly correlates with the severity of the damage to the blood–brain barrier (29).

The most important bacterial component of Gram-negative bacteria known to stimulate the release of inflammatory cytokines is LPS. Indeed, meningococcal LPS was found to be a major cytokine-inducing component in bacterial meningitis, since the cytokine profile and levels found in serum or CSF correlated with the LPS concentrations. However, Prins et al. (59) could not confirm the relationship between levels of cytokines and bacterial endotoxin produced by various strains of young meningitis patients, suggesting that factors other than endotoxin may be involved in cytokine induction during acute meningococcal septic shock. In vivo, LPS and IgA1 protease might be released together and act synergistically. Strikingly, though, the three major causative agents of bacterial meningitis, N. meningitidis, H. influenzae, and Streptococcus pneumoniae, all produce an IgA1 protease (11, 13, 14, 60). Therefore, IgA1 protease may constitute an underestimated virulence factor in sepsis and meningoccal infection.

Besides the cleavage of IgA1, several additional functions have been attributed to Neisseria IgA1 protease. IgA1 protease was recently shown to cleave LAMP-1, a lysosomal protein associated with the endosomal maturation (36, 61), but only one report suggested that, as a consequence, IgA1 protease facilitates the intracellular survival of pathogenic N. gonorrhoeae (61). Furthermore, other human proteins have been found to be proteolytic substrates in vitro. However, the biological relevance of their cleavage remains unclear. In a parallel study, we recently observed that IgA1 protease efficiently protects phagocytes from TNF-α-mediated apoptosis (28, 30). The observation is intriguing since Neisseria is capable of entering phagocytic cells by an active mechanism (62). Hence, the pathogens may have an interest in preventing lethal effects of TNF-α on their intracellular niches.

The induction of proinflammatory cytokines may contribute to the characteristic inflammatory reaction caused by the pathogenic Neisseria. Understanding the mechanism of IgA1 protease in the regulation of these cytokines has potential therapeutic significance and is currently in progress. Future research should reveal strategies for blocking this cytokine-inducing pathway in response to pathogenic Neisseria and probably to other IgA1 protease-secreting human pathogenic bacteria.

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