Proteolytic Cleavage of ICAM-1 by Human Neutrophil Elastase

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Proteolytic Cleavage of ICAM-1 by Human Neutrophil Elastase

Benoit Champagne,* Pierre Tremblay,* André Cantin, † and Yves St. Pierre2*

Human leukocyte elastase (HLE) participates in tissue destruction in a number of inflammatory disorders, including rheumatoid arthritis and cystic fibrosis. Since HLE has been shown to bind to Mac-1, and ICAM-1 plays a key role during the recruitment and the activation of leukocytes at inflected sites, we investigated the capacity of HLE to cleave ICAM-1. Flow-cytometric analyses showed a dose-dependent cleavage of ICAM-1 by HLE on different human cell lines. The cleavage was completely inhibited by α1-antitrypsin, a natural HLE protease inhibitor. The ability of HLE to degrade ICAM-1 was further confirmed by electrophoretic analysis using a soluble form of ICAM-1 (D1-D5). Enzymatic removal of N-linked glycosylation did not significantly modulate ICAM-1 cleavage by HLE, while removal of sialic acid residues partially reduced the sensitivity of ICAM-1 to HLE. We further showed that spumon of cystic fibrosis patients contains high levels of HLE activity capable of cleavage of cell surface ICAM-1. The cleavage induced by incubation of cells with the sputum sample was totally inhibited by α1-antitrypsin and the specific peptidic HLE inhibitor N-methoxy succinyl-Ala-Ala-Pro-Val-chloromethyl ketone. Moreover, the cleavage of ICAM-1 was concomitant to that of CD4 at the surface of the same cell, at the same amplitude, and at all HLE concentrations. The capacity of HLE to modulate the expression of ICAM-1 on the surface of leukocytes by proteolytic cleavage brings support to the hypothesis that overproduction of HLE can cause severe immunologic lung disorders by affecting intercellular adhesion. The Journal of Immunology, 1998, 161: 6398–6405.

Several membrane molecules with various functions are cleaved by endogenous proteases and then released into the extracellular medium in the form of soluble fragments (reviewed in Refs. 1 and 2). Such shedding has been proposed as a mechanism responsible for the detachment of migrating leukocytes from the endothelium during extravasation. The constant flow of reports of new molecules removed from the cell surface by enzymatic cleavage and the presence of their soluble counterparts in blood plasma that tends to increase in concentration in many inflammatory states (3) indicate that this process occurs both in physiologic and pathologic conditions. Since many adhesion molecules were reported to be shed from the surface of leukocytes by proteolytic cleavage, this down-regulation mechanism could have consequences in immunologic processes involving leukocyte interaction with other cells and with constituents of the extracellular matrix. In most cases, however, the enzymes involved are unknown.

Among the cellular proteases that are potentially taking part in the physiologic cleavage of leukocyte Ags are the various neutral serine proteases that are stored in the primary granules of neutrophils (PMN)3 and released following their activation. Human leukocyte elastase (HLE; PMN elastase, E.C. 3.4.21.11) and cathepsin G (E.C. 3.4.21.20) are the major serine proteases secreted by PMN; they were reported to play important roles in PMN-mediated proteolytic events. These two proteases were also found on the plasma membrane of resting and activated PMN as noncovalently membrane-bound forms. Their expression is not restricted to PMN, and both soluble and membrane-bound forms were also reported on monocytes and lymphocytes (4–6). HLE received the closest attention over the last two decades, since it was found to participate in tissue destruction in a number of inflammatory disorders, including rheumatoid arthritis, cystic fibrosis (CF), glomerulonephritis, and emphysema. In addition to its capacity to digest numerous macromolecules of the extracellular matrix, HLE can also cleave specific leukocyte Ags, such as CD4 and CD8 (7), CD43 (8), and thrombin receptor (9). However, to cleave these receptors in vivo, favorable microenvironmental conditions are needed, so as to limit the rapid inhibition of HLE by naturally occurring protease inhibitors. Indeed, it was demonstrated in vitro that extracellular proteolysis may occur at physiologic protease inhibitor concentrations during close contact between PMN and a substrate-coated surface, therefore confining the proteolytic activity to the immediate pericellular zone of PMN contact, where this temporary microenvironment protects the catalytic activity of HLE from inhibition (10–12). The importance of proteases in cellular adhesion is also supported by the recent observations that receptors of adhesion molecules are capable of binding HLE. Thus, Cai and Wright (13) demonstrated that Mac-1 was one of the ligands present on the cell surface of PMN that could bind HLE. They also showed that the expression level of HLE on PMN was inversely proportional to their degree of adhesiveness, and that a mAb against HLE prevented their detachment from fibrinogen-coated surfaces, indicating that HLE was involved in PMN-mediated interactions and could potentially act as a regulating factor on Mac-1-mediated adhesion. It is thus possible that proteases such as HLE could be important for the detachment of PMN, by cleaving Mac-1 ligand. A role for HLE during the infiltration of neutrophils

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4Abbreviations used in this paper: PMN, polymorphonuclear; α1-AT, α1-antitrypsin; CF, cystic fibrosis; HLE, human leukocyte elastase; MFI, mean fluorescent intensity; MMP-9, matrix metalloproteinase-9; 1-antitrypsin; 1-AT, 1-antitrypsin; N-methoxy succinyl-Ala-Ala-Pro-Val-chloromethyl ketone; sICAM-1, soluble ICAM-1.

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was reported by Woodman et al. (14), in which the infiltration of PMN into inflamed vessels, observed by in vivo intravital microscopy, was significantly abrogated with a low m.w. inhibitor of HLE. Since ICAM-1-mediated interactions were shown to be critical for the firm attachment of PMN to endothelial cells, and since Mac-1 was able to bind HLE, we have, in the present work, tested the sensitivity of ICAM-1 to HLE-mediated proteolytic cleavage.

Materials and Methods

Reagents and Abs

HLE was obtained from Calbiochem (La Jolla, CA). The α1-antitrypsin (α1-AT) and N-methoxysuccinyl-Ala-Ala-Pro-Val-chloromethyl ketone (ZMAVPCK) were obtained from Sigma (St. Louis, MO). MMP-9 (gelatinase B) was purified from supernatants of the human monocytic cell line THP-1 by affinity chromatography on gelatin-Sepharose, and its proteolytic activity was confirmed by fluorescence-activated substrate conversion, as previously described (15). Flavobacterium meningosepticum recombiant N-glycosidase F and Vibrio cholerae neuraminidase were obtained from Boehringer Mannheim (Laval, Quebec, Canada). Purified human sICAM-1 was kindly provided by Boehringer Ingelheim Pharmaceuticals (Ridgefield, CT). Sputum samples were obtained from six patients, four female, two male; age 17–31 yr during stable disease. All patients had Pseudomonas aeruginosa in their sputum. None of the patients were receiving antibiotics at the time of sputum collection. Sputum samples were mixed 1:1 (weight:volume) with PBS, vortexed, and centrifuged at 15,000 × g for 15 min. The supernatants were stored at −70°C until analysis. SYPRO-RED was obtained from Molecular Probes (Eugene, OR). Tissue culture reagents were obtained from Life Technologies (Mississauga, Ontario, Canada). The FITC-conjugated anti-ICAM-1 (B-H17 mAb) was obtained from BioSource International (Camarillo, CA). The phycocerythrin-conjugated anti-CD4 (clone MT-310) was obtained from Dako (Carpenteria, CA), and the anti-HLA-DR (clone CR3/43) was obtained from Boehringer Mannheim (Laval, Canada). The RR1/1 (anti-ICAM-1) was a gift from Dr. T. A. Springer (Center for Blood Research, Boston, MA) CR3/43 (anti-HLA-DR) and FITC-conjugated goat antirabbit IgG were obtained from Boehringer Mannheim and Sigma, respectively.

Cell cultures

The U937 promonocytic and Raji B lymphocyte cell lines were obtained from Dr. D. Oth (Institut Armand-Frappier). MT-2 and MT-4 T lymphocyte cell lines were obtained from Dr. P. Talbot (Institut Armand-Frappier). Cells were maintained in vitro culture in RPMI 1640 supplemented with 10% FCS, 2 mM glutamine, 10 mM HEPES buffer, 100 U/ml penicillin, and 100 µg/ml streptomycin.

HLE treatments

Cells were washed three times in complete RPMI without serum (RPMI-S) and seeded in 96-well plates. The wells contained 80 μl of cells (2 × 10^6 total) resuspended in RPMI-S and 10 μl of protease-containing solution. When specified, 10 μl of α1-AT (1 mg/ml) was preincubated 15 min (37°C) with 10 μl of HLE (200 μg/ml) before addition to cells. The volume was completed with RPMI-S to 100 μl and cells incubated at 37°C for the indicated times. After incubation, cell surface expression of ICAM-1 was measured by flow-cytometric analysis using specific fluorescent antibodies. ICAM-1-mediated interactions were shown to be critical for the firm attachment of PMN to endothelial cells, and since Mac-1 was able to bind HLE, we have, in the present work, tested the sensitivity of ICAM-1 to HLE-mediated proteolytic cleavage.

Measure of HLE activity in sputum samples

Cells were washed twice with ice-cold PBS containing 1% (v/v) BSA and 0.01% (v/v) sodium azide (PBA) and then incubated for 25 min on ice with predetermined concentrations of Abs. For indirect staining, cells were washed twice after binding of the first mAb and incubated again with a saturating concentration of fluorescein-conjugated goat anti-mouse IgG for 30 min on ice. After mAb binding, cells were washed twice with PBA and resuspended in 400 μl of PBS containing 0.01% of sodium azide. Samples were kept at 4°C in the dark and analyzed using a Coulter XL-MCL (Coulter Electronics, Hialeah, FL). Between 5,000 and 10,000 cellular events were analyzed for each sample.

Results

Cleavage of ICAM-1 by HLE

U-937 cells were incubated in serum-free condition with exogenous HLE, and ICAM-1 expression was measured by flow cytometry with a FITC-labeled anti-ICAM-1 mAb. Treatment of U-937 cells for 2 h with 20 μg/ml of HLE significantly reduced ICAM-1 expression at the cell surface, as indicated by the reduced intensity of staining of HLE-treated cells (MFI = 3.7) compared with untreated cells (MFI = 13.7) (Fig. 1, A and B). The effect of HLE treatment on ICAM-1 expression was dose dependent (Fig. 1, C). At higher doses, up to 90% reduction of ICAM-1 expression could be achieved within 2 h. Addition of α1-AT, a potent inhibitor of elastase activity, completely inhibited the ability of elastase to reduce ICAM-1 expression, strongly arguing in favor of a catalytic mechanism. Furthermore, complete inhibition of HLE-reduced ICAM-1 expression was observed in the presence of human serum (10% v/v), a rich source of protease inhibitors, including α1-AT (data not shown). The sensitivity of ICAM-1 to HLE was further confirmed using other cell lines expressing ICAM-1, such as the MT-2 (Figs. 2B and 2A) and MT-4 T-lymphocyte cell lines (data not shown), and on the Raji B-lymphocyte cell line (Fig. 2A).

To examine the specificity of cell surface ICAM-1 as an HLE substrate, different approaches were used. In a first series of experiments, we investigated the cleavage of ICAM-1 in the presence of increasing concentrations of purified human gelatinase B.
(MMP-9, E.C. 3.4.24.35), a 92-kDa matrix metalloproteinase expressed by many cell types including leukocytes. The dose-dependent catalytic activity of MMP-9 was first confirmed by flow cytometry using immobilized fluorescent gelatin on microspheres, as previously described (15) (Fig. 2B). We found no evidence of cleavage of ICAM-1 by MMP-9 when it was tested at equivalent molar concentrations (i.e., 60 μg/ml) used for HLE (i.e., 20 μg/ml) (Fig. 2A). In a second series of experiments, we compared the sensitivity of ICAM-1 and HLA-DR, both expressed at high levels on the surface of MT-2 cells. We found that HLE treatment (20 μg/ml) reduced the expression of ICAM-1 by 50–60%, but not that of HLA-DR (Fig. 2C). Finally, we found that preincubation of U-937 cells with anti-ICAM-1 RR1/1 mAb, specific for the first Ig domain of ICAM-1, inhibited the ability of HLE to cleave ICAM-1 (Fig. 2D).

To determine the kinetic characteristics of ICAM-1 cleavage by HLE, MT-2 cells were incubated with HLE (20 μg/ml) for different periods of time (15 min to 4 h). We found that cleavage of
ICAM-1 by HLE was time dependent (Fig. 3A). When HLE-treated cells were seeded back in culture after removal of HLE from culture medium, ICAM-1 was gradually reexpressed upon normal culture condition (Fig. 3B).

**In vitro cleavage of purified human sICAM-1**

To establish that ICAM-1 is cleaved directly by HLE, we investigated the proteolysis of purified human ICAM-1 in vitro. This soluble form ICAM-1 (sICAM-1) contains the five extracellular Ig-like domains. SDS-PAGE analysis confirmed that sICAM-1 was cleaved by HLE in a dose-dependent manner. As shown in Fig. 4, HLE treatment of ICAM-1 resulted in the appearance of a major diffuse band of approximately 45 kDa. The diffuse pattern of the bands was due to extensive glycosylation of ICAM-1 (16, 17). The 30-kDa band could be HLE as it corresponded to its molecular mass of 29.5 kDa. The fact that the 30-kDa band appeared less intense in the sample containing HLE alone compared with that coincubated with ICAM-1 may be the consequence of an autoca-
talysis of elastase in absence of ICAM-1. A longer HLE treatment (3 h) resulted in complete digestion of sICAM-1, as no proteolytic fragments were detected (data not shown). Repeated attempts to identify the cleavage site(s) by amino acid analysis of proteolytic fragment bands were, however, inconclusive, probably because of the heavy glycosylation of ICAM-1.

**Role of posttranslational modification of ICAM-1 in cleavage by HLE**

ICAM-1 is differently glycosylated depending on cell type, and this is reflected in molecular mass that varies between 76 and 114 kDa. The complete deglycosylation of ICAM-1 results in a protein of 55–60 kDa corresponding to the molecular mass predicted by its mRNA sequence (18). We next investigated the possible influence of N-linked carbohydrates on cleavage by HLE. For this purpose, sICAM-1 was pretreated with N-glycosidase F (molecular mass 35.5 kDa), and then subjected to HLE treatment. As shown in Fig. 5, the N-glycosidase treatment resulted in the appearance of...
a ladder of bands, consistent with the heterogeneous deglycosylation pattern of ICAM-1. The thin band of approximately 50–60 kDa most likely represented the complete deglycosylated form of ICAM-1. After a 1-h treatment with HLE, both native (glycosylated) and deglycosylated forms of ICAM-1 were sensitive to proteolysis by HLE. At 4 h, however, although HLE treatment resulted in complete digestion of native ICAM-1, a significant amount of the deglycosylated forms of ICAM-1 was still detectable upon incubation with HLE, suggesting that removal of negatively charged residues from ICAM-1 may slightly slow the proteolysis of ICAM-1, possibly by interfering with the binding of the positively charged HLE to its substrate. The diffuse 45-kDa band, previously described on Fig. 5, appeared very lightly on this gel following a 1-h HLE treatment on native forms of ICAM-1. Minor bands of low molecular mass (10–30 kDa), which represent other ICAM-1 proteolytic fragments, were still detectable after 1- and 4-h HLE treatments on native and deglycosylated forms of ICAM-1. From these observations, we conclude that enzymatic removal of N-linked sugar residues did not significantly influence ICAM-1 sensitivity to HLE.

Additional experiments were conducted to evaluate the possible role of sialic acid in proteolysis of ICAM-1 by HLE. Previous studies showed that CD43, an extensively O-glycosylated protein, is insensitive to certain proteases such as trypsin and calpain, unless the cells are first treated with sialidase (8). To determine whether the removal of sialic acid on ICAM-1 could influence its sensitivity to HLE, purified sICAM-1 and ICAM-1-expressing U-937 cells were pretreated with neuraminidase (sialidase) before exposure to HLE. Sialic acid removal treatment induced a band shift on purified sICAM-1, indicative of the successful removal of sialic acid residues; it did not, however, prevent the cleavage of ICAM-1, although it consistently reduced its sensitivity to HLE (Fig. 6A). Similar results were obtained by flow cytometry on U-937 cells, where enzymatic removal of sialic acid residues from the cell surface induced a slight, but reproducible, decrease of ICAM-1 proteolysis (Fig. 6B). These data corroborate previous finding on CD43, in which sialidase treatment did not increase CD43 sensitivity to HLE (8).

Cleavage of ICAM-1 in sputum samples

It is well known that PMN activities, such as phagocytosis and oxygen burst, are impaired in lungs of patients with CF (19). Sputum of CF patients have been shown to contain very high concentrations of HLE, which is not detectable in bronchoalveolar lavage of healthy individuals (7). To determine whether the concentration of PMN elastase is produced in sufficient amounts to significantly modulate the cleavage of ICAM-1 from the cell surface of leukocytes, we incubated U-937 cells with sputum samples obtained from patients with CF. We found that the ICAM-1 expression was reduced significantly by incubation of cells in sputum samples, even when they were diluted 1 in 40 (i.e., in unsaturating conditions) (Fig. 7A), and correlated with the amount of elastase-specific activity found in these fluids (Fig. 7C). When diluted 1/4, almost complete cleavage of ICAM-1 was observed for all samples (i.e., >95%, data not shown). The cleavage of ICAM-1 in the sputum was HLE specific since addition of MSAAPVCK (a specific HLE inhibitor) completely inhibited the effect. Moreover, the sensitivity of ICAM-1 to cleavage by HLE was concomitant to that of CD4 (Fig. 7B), previously identified as a substrate for HLE (7). Interestingly, the extent of cleavage of ICAM-1 and CD4 followed closely the severity of the disease, as sputum samples SP-20 and 21 were obtained from patients with a mild condition; SP-40 was obtained from a patient with moderate clinical manifestations; and SP-30, SP-4, and SP-27 were obtained from patients that are now deceased, or were at the limit of respiratory failure. These data provide direct evidence that sufficient concentrations of catalytically active HLE are present in sputum of CF patients to rapidly down-regulate the expression of cell surface ICAM-1.

Discussion

In the present work, we have established and characterized the proteolytic cleavage of ICAM-1 by HLE. First, by immunofluorescence flow cytometry on different human cell lines, we demonstrated the dose-dependent cleavage of ICAM-1 by HLE, which was completely inhibited by the presence of α1-AT, a natural HLE protease inhibitor. Furthermore, a mAb (RR1/1) directed against the N-terminal domain 1 of ICAM-1 almost completely blocked ICAM-1 proteolysis by HLE, suggesting that HLE must first bind to the domain 1 of ICAM-1. The ability of HLE to degrade ICAM-1 was confirmed by electrophoretic analyses using sICAM-1 (D1-D5). We also showed that HLE cleavage of ICAM-1 was not significantly affected by the posttranslational modifications. Finally, we showed that the sputum samples of CF patients were capable of degrading cell surface ICAM-1, and that this cleavage was inhibited by α1-AT and MSAAPVCK.
Over the years, several studies on ICAM-1 have revealed a complex structure-function relationship derived from posttranscriptional and posttranslational modifications that could potentially affect the cleavage of ICAM-1 by HLE. The ability of ICAM-1 to form homodimers (20), the expression of isoforms resulting from alternative splicing (21), and the presence of different cell-specific glycosylation patterns (16, 17) could all affect the processing of ICAM-1 by HLE. Furthermore, other extrinsic factors, such as heteropolymerization of ICAM-1 with other molecules, expression of membrane protease inhibitors to HLE, expression of HLE receptors, and the presence of other HLE substrates may limit the efficiency of ICAM-1 cleavage at the cell surface. The question as to whether the glycosylation of ICAM-1 significantly affects its proteolysis by HLE was considered in the present work. Since it has been shown that Mac-1 binding to ICAM-1 is regulated by N-linked glycosylation of ICAM-1 (16), the heterogeneity of glycosylation found on ICAM-1 may then shield specific preferential cleavage site(s) for HLE. Our in vitro experiments with differential glycosylated forms of sICAM-1, obtained by enzymatic removal of N-linked sugar residues, did not show any significant change in ICAM-1 sensitivity for HLE following deglycosylation. Although these experiments may not necessarily simulate natural differential glycosylation patterns in different cell types, other studies using purified ICAM-1 obtained from different cell types expressing distinct glycosylation patterns of ICAM-1 will be needed to further elucidate the influence of posttranscriptional modifications in a more physiologic context, including experiments that address the sensitivity of ICAM-1 isoforms to proteolytic cleavage by HLE.

One of the most important roles for ICAM-1 was shown to be its ability to mediate PMN adhesion to vascular endothelium. Although the cleavage of ICAM-1 by elastase in vivo is likely to be inhibited by the presence of high concentrations of protease inhibitors, most notably in intravascular situations, the concentration of elastase may not have to be necessarily high to achieve cleavage of ICAM-1. As the excessive proteolytic activity encountered is primarily delimited by a high enzyme:inhibitor ratio, it is possible that the presence of HLE inhibitors is counterbalanced by a local concentration of HLE that outnumbers inhibitor molecules, allowing proteolysis to occur in evanescent pericellular zones (11). Moreover, during cell-cell adhesion, reorientation of the microtubule-organizing center polarizes and directs secretion of granule content to the region of the surface of the congener cell (22). Consistent with this hypothesis, ICAM-1 and Mac-1 have both been shown to cluster at the interface during cell-cell contact (23, 24). Several studies have also reported that HLE is expressed at the cell surface of many types of leukocytes as a noncovalently membrane-bound protease (5, 25). Recently, Cai and Wright (13) have shown that HLE was a ligand for Mac-1, and that an Ab against HLE prevents detachment of PMN from fibrinogen-covered surfaces, suggesting a new mechanism that could be used by cells to regulate Mac-1 adhesiveness. Our data bring support to this model. Interestingly, other studies have shown that β2 integrins play a major role in the secretion of HLE by PMN. In fact, it has been reported that binding of sICAM-1 to PMN, or the cross-linking of β2 with a mAb induced high secretion levels of HLE (26, 27).

Mac-1/ICAM-1-mediated PMN cellular interactions could then induce the release of HLE via the cross-linking of CD18 to allow the cleavage of ICAM-1 when intercellular contacts are no longer required. Since D1 has previously been shown to be the region containing the binding site for LFA-1 (28), it appears likely that LFA-1/ICAM-1-mediated cell-cell adhesion would also be affected following the proteolysis of membrane-bound ICAM-1 by HLE. Similarly, binding of rhinoviruses to ICAM-1 will also be affected by proteolysis of ICAM-1 by HLE. Since our in vitro experiments with sICAM-1 revealed that HLE cleaved ICAM-1 at multiple sites, it is also likely that binding of Mac-1 to ICAM-1, which is mediated by the third domain of ICAM-1 (16), will also be affected by HLE treatment. The observation that cleavage of sICAM-1 by HLE first generates a large fragment of 45 kDa, corresponding approximately to the molecular mass of three Ig-like domains, supports the possibility that Mac-1 binding is also affected. Our observation, however, that binding of RR1 mAbs, specific for D1, inhibits cleavage of ICAM-1, suggests that contact between LFA-1 and ICAM-1 will prevent degradation of ICAM-1 by HLE. Identification of the putative cleavage sites of ICAM-1 will help to clarify this issue. Furthermore, the physical association of HLE to Mac-1 would potentially enhance and facilitate the proteolysis of ICAM-1. The extracellular expression of proteases such as HLE could be a general mechanism used for the locomotion and the deadhesion of cells bound to other cells and/or to macromolecules of the extracellular matrix. However, whether Mac-1-bound elastase is still enzymatically active toward ICAM-1, or whether HLE...
needs to be released from CD11b/CD18 to cleave ICAM-1 on the opposing cell also remains to be tested.

Several immunologic lung disorders are characterized by the incapacity of the local immune response to clear effectively bacterial infections, rendering patients highly prone to chronic infections and antibiotic treatments (19). In CF, the immunologic defect has been shown to be related to abnormal production of active HLE that cleaves Igα and the C3b receptor on neutrophils, thereby reducing phagocytosis of pathogens (29, 30). Our results now show that abnormal HLE expression will significantly affect ongoing intercellular adhesion between leukocytes that involves ICAM-1 and Mac-1. CD11b/CD18 receptor regulates cellular activation of PMN and their ability to mediate phagocytosis of particles that are bound via CR1 and the integrins on leukocytes (32). Interestingly, sICAM-1 has been reported in CF patients (33, 34). In CF, sICAM-1 is mainly found in clinically well patients, whereas during acute exacerbation, the level of sICAM-1 dropped significantly. Given our data on the capacity of elastase to cleave ICAM-1 at several sites, this sICAM-1 drop may be due to increased elastase activity during exacerbation (33). In other pathologic conditions, such as purulent peritonitis, abdominal exudate was shown to contain an average of 68 μg/ml of HLE (35). These peritoneal fluids have abnormally high levels of sICAM-1 that correlate with the number of PMN during episodes of infection. However, given the large number of conditions associated with abnormally high levels of sICAM-1, and given the recent isolation of alternative spliced mRNA encoding sICAM-1 (36), it is too early to conclude to what extent the activity of HLE is responsible for the presence of circulating forms of ICAM-1. Whether other proteases that are released by PMN, such as cathepsin G, can also cleave ICAM-1 remains an interesting possibility that will be addressed in future experiments. In CF, however, cathepsin G would probably not be involved in the cleavage of ICAM-1 since: 1) the concentration of cathepsin G in broncho-alveolar lavage (BAL) fluids is most likely too low to expect significant cleavage of ICAM-1 (37), and 2) our data showed that the specific HLE-inhibitor MSAAPVCK almost completely inhibited the cleavage of ICAM-1 in the sputum of CF patients.

As expected, we found that MHC class II molecules were resistant to cleavage by HLE. This was expected since MHC class II molecules, before being expressed at the surface of lymphocytes, must resist harsh environment along an intracellular pathway that encounters endosomal compartment that favors proteolysis of native Ags into antigenic peptides. The fact that ICAM-1, as well as CD4, are both cleaved, will significantly affect the functional recognition of MHC/peptide complexes by T cells. Our data showed indeed that cleavage of ICAM-1 and CD4 occurs at the surface of the same cell, at the same amplitude, and at any given concentrations of HLE. It remains possible that cleavage of other accessory molecules will further affect that delivery of coactivator signals necessary for activation of T cells. Whether an anergic state is induced upon cleavage of these accessory signals involved in T cell–APC interactions remains an interesting possibility that is currently being investigated.

In summary, we showed that ICAM-1 is sensitive to proteolytic cleavage by the serine protease HLE, which is ubiquitously expressed on leukocytes. Because the β2 integrin Mac-1 is able to bind HLE specifically and because the release of HLE seems to be regulated through β2, our results suggest that intercellular binding mediated by Mac-1/ICAM-1 interactions could be regulated and abrogated by HLE-mediated proteolytic cleavage of ICAM-1. This regulatory pathway could be instrumental in various phenomena involving intercellular contacts, such as extravasation and leukocyte activation. Whether other receptors are also cleaved upon exposure to HLE remains to be tested, but the cleavages of CD4, CD8, and ICAM-1 most likely are sufficient to explain, in a large part, the severe immunologic disorders found in lung diseases. Our results will help to better understand the immunologic abnormalities in lung diseases, and bring support to novel approaches that could overcome damages induced by overproduction of HLE.

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