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Effect of proteolytic enzymes on neutrophil FcγRII activity

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SUMMARY

Previous studies have demonstrated that the treatment of neutrophils with proteolytic enzymes markedly reduces the expression of receptor III for the Fc portion of IgG (FcγRIII), but it does not affect the number of FcγRII on the cell surface. In the present study, we analysed the effect of proteolytic enzymes on functional responses of neutrophils induced by immune complexes (IC). Our results showed that treatment with pronase or chymotrypsin markedly increased the binding of IgG-coated erythrocytes (IgG-E) to neutrophils, as well as their capability to display IgG-mediated functions such as antibody-dependent cellular cytotoxicity (ADCC) and chemiluminescence (CL) induced by IgG-E, responses that have been shown to be completely dependent on FcγRII. A similar enhancing effect was observed, in all cases, after neutrophil treatment with neuroaminidase. We also studied the effect of proteolysis on neutrophil activation induced by other types of IC. It was found that pronase and chymotrypsin significantly enhanced CL responses induced by soluble IC (sIC) but did not modify the responses induced by either precipitating IC (pIC) or soluble IC prepared with cationized antibodies (catIC). On the other hand, neuroaminidase significantly enhanced CL induced by either sIC, pIC or catIC. Taken together, our data suggest that the activity of FcγRII can be up-regulated by proteolysis. However, this effect appears to be strongly dependent on the characteristics of the IC employed as stimulus.

INTRODUCTION

The interaction of receptors for the Fc portion of IgG molecules (FcγR) with immune complexes (IC) triggers a variety of cell responses, such as phagocytosis, antibody-dependent cellular cytotoxicity (ADCC), and secretion of inflammatory mediators.[1,2] Three classes of human FcγR have been defined, both at protein and complementary DNA level: FcγRI (CD64), FcγRII (CD32) and FcγRIII (CD16).[1–4]

Recent studies have analysed the effect of proteolysis on the activity of FcγRII expressed by human monocytes. It has been demonstrated that monocyte treatment with pronase or trypsin strongly enhances the ability of FcγRII to bind IgG-coated erythrocytes (IgG-E). Subsequently, it has been shown that not only the binding of IgG, but also IgG-mediated functions such as IgG-induced release of tumour necrosis factor-α (TNF-α) and ADCC, are increased by proteolysis.[5,6] Considering the fact that in areas of inflammation proteases are abundantly present, it has been proposed that monocyte FcγRII might function as a 'stand-by' Fc receptor for IgG: a receptor with low affinity for complexed IgG under normal conditions, but whose affinity and functional activity are increased by proteolytic enzymes released locally during an immune response.[5–7]

Human neutrophils constitutively express two low-affinity FcγR: FcγRII and FcγRIII. While FcγRII is a transmembrane molecule, FcγRIII is linked via a glycosylphosphatidylinositol anchor to the cell membrane.[1–4] It has been demonstrated that neutrophil treatment with proteolytic enzymes does not affect the number of FcγRII on the cell surface, while it markedly reduces the expression of FcγRIII.[8,9] The effect of proteolysis on the ability of neutrophils to display FcγR-dependent functions, however, has been poorly studied. Tosi & Berger[9] have showed that neutrophil treatment with elastase does not affect either the ingestion of IgG-coated particles or superoxide anion production induced by multivalent IgG complexes. On the other hand, during the course of a study to characterize the properties of IC prepared with cationized antibodies (catIC), we have observed that neutrophil treatment with pronase or chymotrypsin markedly increases neutrophil cytotoxicity induced by catIC.[10] To clarify the effect of proteolysis on the activity of FcγRII, in the present work we studied different neutrophil responses induced by distinct FcγRII agonists.

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MATERIALS AND METHODS

Effector cells

Peripheral blood polymorphonuclear leucocytes (PMN) were isolated from heparinized human blood samples by

Ficoll–Hypaque gradient centrifugation (Ficoll, Pharmacia, Uppsala, Sweden; Hypaque, Winthrop Products Inc., Farmasa Farmaceútica, Buenos Aires, Argentina)[11] and sedimentation in dextran. Contaminating erythrocytes were removed by hypotonic lysis. After washing, the cells were resuspended in the desired medium. Cell suspensions contained 95–98% neutrophils.

Reagents

Pronase E (from *Streptomyces griseus*), α-chymotrypsin (type II, from bovine pancreas), *N*-formyl-methionyl-leucyl-phenyl-alanine (FMLP), phorbol myristate acetate (PMA), zymosan, platelet-activating factor (PAF), arachidonic acid (AA) and neuroaminidase (type VI from *Clostridium perfringens*) were obtained from Sigma (St Louis, MO).

Treatment with proteolytic enzymes

Neutrophils (5×10^6/ml, in isotonic saline solution) were incubated in the presence or absence of pronase (200 μg/ml), chymotrypsin (1 mg/ml) or neuroaminidase (0·25 U/ml), for 30 min at 37°. After four washes, cells were resuspended in the desired medium. Viability of cells was always higher than 95%.

Anti-FcγR antibodies

Monoclonal antibodies (mAb) 3G8 (IgG1) $F(ab')_2$, which recognizes human FcγRIII, and IV3 (IgG2b) and IV3 Fab, which recognize human FcγRII, were obtained from Medarex Inc. (West Lebanon, NH). Unless otherwise stated, in inhibition experiments mAb anti-FcγR (at a final concentration of 15 μg/ml) were incubated with neutrophils for 30 min at 4°.

Cationization of human IgG

Human IgG (Sigma) was cationized as previously described using 1-ethyl-3-(3-dimethylamino-propyl) carbodiimide HCl (EDAC; Sigma) as activator and ethylene diamine (Sigma) as a nucleophile to replace carboxyl groups.[12] The reaction was carried out in a beaker in an ice bath. Briefly, 0·5 ml of ethylene diamine was added to 10 ml of 0·1 M sodium acetate buffer (pH 4·75) containing 4 mg of human IgG. The pH was then re-adjusted to 4·75. Then, 300 mg of EDAC was added and the reaction maintained at pH 4·75 for 20 min. The unreacted reagents were removed by exhaustive dialysis against phosphate-buffered saline (PBS; pH = 7·4). The pI of proteins was determined by isoelectric focusing. The use of a broad range (pI 3–10) of standards (Sigma) allowed direct determination of the pI from the gel. Isoelectric points showed a range of 5·8–8·2 for untreated human IgG and 8·1–9·2 for cationized human IgG.

Preparation of immune complexes

Precipitating IC (pIC) and soluble IC (sIC) were prepared using human IgG (Sigma), as antigen and specific rabbit IgG antibodies to human IgG.[13] Soluble cationized IC (catIC) were prepared with cationized human IgG, as antigen and specific rabbit IgG antibodies.[10] Precipitating IC were formed at the equivalence zone and sIC and catIC were formed at fivefold antigen excess, based on equivalence points determined by quantitative precipitin curves. In all cases, antigen and antibody were incubated for 1 hr at 37° and 18 hr at 4°. After this period, IC were centrifuged at 3000 *g* for 10 min, and the precipitate or the supernatant was recovered.

EA rosetting

Rosetting was performed employing 15 μl of neutrophil suspension (at 5×10^6/ml) in RPMI-1640 with 5% fetal calf serum (FCS) and 100 μl of chicken erythrocytes (CRBC) (2% v/v in RPMI-1640 with 5% FCS) sensitized with subagglutinating amounts of rabbit IgG anti-CRBC. After incubation for 45 min at 4°, rosetting (EA rosettes) was evaluated by a microscopic method. At least 100 cells were scored in each experiment.

Antibody-dependent cellular cytotoxicity

ADCC was performed as previously described[14] by reacting 2×10^5 neutrophils (suspended in RPMI-1640 with 5% FCS) with 2×10^5 ^{51}Cr-labelled CRBC and different concentrations of rabbit IgG anti-CRBC. After incubation for 2 hr at 37°, the culture plate was centrifuged, the radioactivity of supernatants and pellets measured and the percentage of lysis determined. Spontaneous release was always less than 3%.

Chemiluminescence assays

Luminescence response of neutrophils was measured with a whole-blood Lumi-aggregometer (Chrono-Log, Havertown, PA) at 1000 rev/min and 37°, in the presence of luminol (Sigma) (10^{-7} M), as previously described.[13] Briefly, 1×10^6 neutrophils were suspended in 400 μl of Hank's solution supplemented with 2% FCS. After stimulation, light emission was continously registered during 10 min. Data are expressed as the maximum response observed during this period, in relative chemiluminescence units (URCL). One chemiluminescence (CL) unit was defined as a 1 cm shift of the light emission signal on the paper recorder.

Statistical analyses

Significance of differences between means were calculated using Student's *t*-test.

RESULTS

Proteolysis increases the binding of IgG-coated erythrocytes to neutrophils

Table 1 shows the effect of pronase and chymotrypsin treatment on the ability of neutrophils to bind CRBC sensitized with suboptimal amounts of rabbit IgG anti-CRBC (IgG-E). Proteolysis greatly enhanced not only the percentage of neutrophils that bound to IgG-E but also the number of IgG-E that bound to each neutrophil. In no case was rosette formation observed using unsensitized erythrocytes.

The role of FcγRII and FcγRIII in IgG-E binding to untreated neutrophils was investigated employing mAb 3G8 $F(ab')_2$ fragments (anti-FcγRIII), and mAb IV3 and IV3 Fab' fragments (anti-FcγRII). It was found that the low levels of EA rosettes induced by suboptimal concentrations of IgG anti-CRBC antibodies were markedly reduced by either mAb 3G8 $F(ab')_2$ or mAb IV3 (Table 1). In addition, after employing IV3 Fab fragments an inhibition of 61 ± 5% (mean ± SEM, $n = 4$) was observed. These data suggest that, when the erythrocyte surface is coated with low amounts of IgG antibodies, both FcγR types, FcγRII and FcγRIII, are involved in the binding of

Table 1. Effect of proteolysis on EA rosette formation

Treatment	E	EA	IV3	3G8 $F(ab')_2$	% rosettes	No. of erythrocytes in each rosette	*n*
None	+	–	–	–	0	0	13
None	–	+	–	–	29 ± 4	3 ± 0	29
None	–	+	+	–	7 ± 4	1 ± 1	4
None	–	+	–	+	4 ± 2	2 ± 1	5
Pronase	+	–	–	–	0	0	13
Pronase	–	+	–	–	91 ± 3	10 ± 2	24
Pronase	–	+	+	–	9 ± 6	2 ± 1	4
Pronase	–	+	–	+	85 ± 7	7 ± 1	5
Chymotrypsin	+	–	–	–	0	0	13
Chymotrypsin	–	+	–	–	85 ± 4	7 ± 1	6
Chymotrypsin	–	+	+	–	10 ± 0	3 ± 0	3
Chymotrypsin	–	+	–	+	56 ± 2	6 ± 0	3

Neutrophils (5×10^6/ml, in isotonic saline solution) were incubated in the presence or absence of pronase (200 μg/ml) or chymotrypsin (1 mg/ml), for 30 min at 37°. After washing, cells were treated with mAb anti-FcγR IV3 or 3G8 $F(ab')_2$ for 30 min at 4°. The cells were washed and EA rosette assays were performed as described in the Materials and Methods. Data are expressed as the arithmetic mean ± SEM from *n* donors.

IgG-E. In a separate set of experiments we employed higher amounts of IgG anti-CRBC (sevenfold higher than those used in the experiments described in Table 1), in order to obtain high levels of EA rosettes, comparable to those observed with protease-treated neutrophils. Under these experimental conditions, the binding of IgG-E to untreated neutrophils (% EA rosettes = 83 ± 6, mean ± SEM, $n = 5$) was markedly decreased by mAb 3G8 $F(ab')_2$ (percentage inhibition = 79 ± 6, $n = 5$) and slightly reduced by mAb IV3 or IV3 Fab fragments (percentage inhibition = 28 ± 4 and 15 ± 3, respectively, $n = 5$). Taken together, these data suggest that FcγRIII plays a critical role in IgG-E binding to untreated neutrophils. On the other hand, the role of FcγRII appears to be strongly conditioned by the amount of IgG that coats the erythrocyte surface.

The IgG-E binding to pronase-treated neutrophils was not modified by mAb 3G8 $F(ab')_2$, but it was dramatically inhibited by mAb IV3 (Table 1). Moreover, IV3 Fab fragments were found to inhibit rosette formation in a concentration-dependent fashion (percentage EA rosette inhibition induced by 15 μg/ml, 5 μg/ml and 1·5 μg/ml of IV3 Fab = 87 ± 6, 63 ± 6 and 27 ± 4, respectively, $n = 4$). On the other hand, rosette formation by chymotrypsin-treated neutrophils was slightly decreased by mAb 3G8 $F(ab')_2$ and markedly inhibited by mAb IV3 (Table 1) or IV3 Fab fragments (percentage EA rosette inhibition induced by 15 μg/ml, 5 μg/ml and 1·5 μg/ml of IV3 Fab = 81 ± 5, 47 ± 4 and 18 ± 5, respectively, $n = 4$). These data indicate that IgG-E binding to protease-treated neutrophils is mainly dependent on FcγRII.

Proteolysis increases ADCC and chemiluminescence responses induced by IgG-coated erythrocytes

In order to determine whether proteolysis is also able to increase IgG-triggered responses, we analysed the ability of neutrophils to mediate ADCC. Our results (Table 2) indicated that proteolysis markedly enhanced neutrophil-mediated ADCC. With both treated and untreated neutrophils, ADCC was almost completely abrogated by mAb IV3 (Table 2). Fab fragments of mAb IV3 were also able to inhibit ADCC. The percentages of inhibition induced by 15 μg/ml, 5 μg/ml and 1·5 μg/ml of IV3 Fab were 84 ± 5, 58 ± 5 and 16 ± 2 for untreated neutrophils, 83 ± 6, 66 ± 4 and 21 ± 3 for pronase-treated neutrophils, and 79 ± 5, 48 ± 5 and 18 ± 3, respectively, for chymotrypsin-treated neutrophils (mean ± SEM, $n = 5$ in all cases). On the contrary, $F(ab')_2$ fragments of mAb 3G8 did not modify ADCC levels (Table 2).

It is well-established that oxygen-reactive intermediates (IRO) produced during phagocytic cell activation can act on

Table 2. Effect of proteolysis on ADCC

Treatment	IgG anti-CRBC (ng)	IV3	3G8 $F(ab')_2$	%ADCC	*n*
None	3	–	–	5 ± 1	17
None	10	–	–	21 ± 3	17
None	10	+	–	5 ± 2	6
None	10	–	+	24 ± 5	6
Pronase	3	–	–	49 ± 3	14
Pronase	10	–	–	63 ± 4	14
Pronase	10	+	–	11 ± 3	6
Pronase	10	–	+	71 ± 6	6
Chymotrypsin	3	–	–	33 ± 4	5
Chymotrypsin	10	–	–	51 ± 5	5
Chymotrypsin	10	+	–	12 ± 4	3
Chymotrypsin	10	–	+	48 ± 6	3

Neutrophils (5×10^6/ml, in isotonic saline solution) were incubated in the presence or absence of pronase (200 μg/ml) or chymotrypsin (1 mg/ml), for 30 min at 37°. After washing, cells were treated with mAb anti-FcγR IV3 or 3G8 $F(ab')_2$ for 30 min at 4°. The cells were washed and ADCC assays were performed as described in the Materials and Methods, employing two different amounts of rabbit IgG anti-CRBC. Data are expressed as the arithmetic mean ± SEM of triplicates from *n* donors.

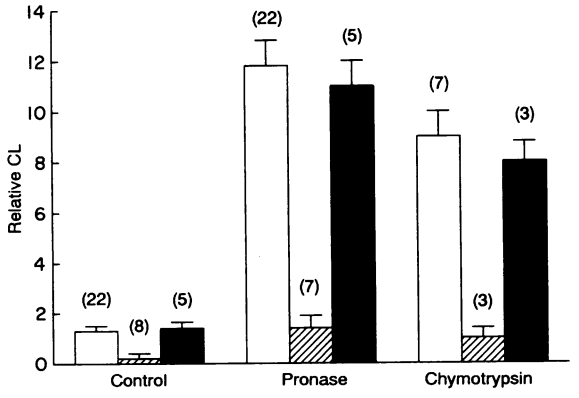

Figure 1. Effect of proteolysis on CL responses induced by IgG-sensitized erythrocytes. Neutrophils (5×10^6/ml, in isotonic saline solution) were incubated in the presence or absence of pronase (200 μg/ml) or chymotrypsin (1 mg/ml), for 30 min at 37°. After washing, cells were incubated with mAb anti-FcγR IV3 (□), 3G8 F(ab')$_2$ (■) or with culture medium alone (□) for 30 min at 4°. Cells were washed again and CL assays were performed as described in the Materials and Methods, employing chicken erythrocytes sensitized with subagglutinating amounts of rabbit IgG anti-CRBC as stimulus. Data are expressed as the arithmetic mean ± SEM of duplicates from *n* (between parentheses) donors.

molecules containing regions of high electron density, with resulting excitation and photon emission upon relaxation, thereby producing CL.[15–17] Since CL can, therefore, be regarded as an overall indicator of the oxidative burst activity of phagocytic cells, we next evaluated the effect of proteolysis on light emission mediated by stimulated neutrophils. Using untreated or protease-treated neutrophils, we found that proteolysis enhanced CL responses triggered by IgG-E (Fig. 1). This response was not modified by 3G8 F(ab')$_2$ and was almost completely abolished by IV3, either in untreated or protease-treated neutrophils (Fig. 1). The Fab fragments of mAb IV3 were also able to markedly reduce CL responses mediated by either untreated or protease-treated neutrophils (percentage inhibition > 83 in all cases, $n = 3$).

Effect of neuroaminidase on neutrophil-mediated EA rosette formation, ADCC and CL responses induced by IgG-coated erythrocytes

Proteolytic enzymes may act by a sialidase-'like' mechanism, inducing a partial loss of surface glycoproteins and, consequently, decreasing both the sialic acid content and the negative charge of the cell surface. Therefore, we examined the effect of neuroaminidase on neutrophil responses induced through FcγR. Our results (Table 3) showed that neuroaminidase increased the binding of IgG-E to neutrophils as well as ADCC and CL, in a similar fashion to pronase or chymotrypsin. IgG-E binding to neuroaminidase-treated neutrophils was significantly inhibited either by mAb 3G8 F(ab')$_2$ (percentage inhibition = 71 ± 7, $n = 4$) or by mAb IV3 Fab (percentage inhibition = 39 ± 5, $n = 4$). On the other hand, ADCC and CL were markedly suppressed by mAb IV3 Fab (percentage inhibition = 87 ± 6 and 74 ± 6, respectively, $n = 4$) but were not modified by mAb 3G8 F(ab')$_2$ (percentage inhibition < 10, in all cases).

Table 3. Effect of neuroaminidase on rosette formation, ADCC and CL induced by IgG-coated erythrocytes

	Untreated	Neuroaminidase	*n*
EA rosette (%)	26 ± 6	95 ± 5	7
ADCC (%)	4 ± 2	38 ± 4	6
CL (URCL)	1 ± 1	15 ± 3	8

Neutrophils (5×10^6/ml, in isotonic saline solution) were incubated in the presence or absence of neuroaminidase (0·25 U/ml), for 30 min at 37°. The cells were washed and EA rosette, ADCC and CL assays were performed as described in the Materials and Methods. The assay of ADCC was carried out employing 3 ng rabbit IgG anti-CRBC. Data are expressed as the arithmetic mean ± SEM of triplicates or duplicates from *n* donors.

Effect of proteolysis on neutrophil-mediated CL responses induced by different types of IC

IC constitute a heterogeneous group of FcγR agonists that differ not only in their ability to trigger distinct cellular responses but also in the mechanisms by which they induce cell activation.[1–4,14,18,19] These observations prompted us to perform subsequent studies to determine the ability of protease-treated neutrophils to be activated by different types of IC: sIC, pIC and catIC. As shown in Fig. 2, neutrophil treatment with pronase or chymotrypsin significantly enhanced CL responses induced by sIC but did not modify light emission induced by either pIC or catIC. This was true for a wide range of sIC, pIC and catIC concentrations (not shown). In order to determine if differences in FcγR requirements could explain these results, we analysed the role of FcγRII and FcγRIII in CL responses induced by each agonist. Our results indicated that light emission mediated by untreated neutrophils induced by pIC and sIC was significantly inhibited by either mAb IV3 (percentage inhibition = 60 ± 4 and 64 ± 4, $n = 5$,

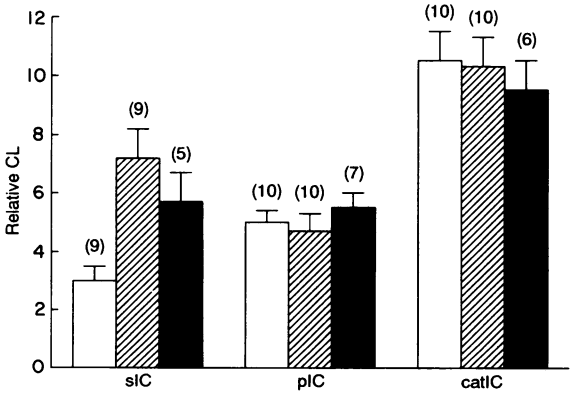

Figure 2. Effect of proteolysis on CL responses induced by different types of IC. Neutrophils (5×10^6/ml) were incubated with 200 μg/ml of pronase (▨), 1 mg/ml of chymotrypsin (■) or with isotonic saline solution (□), for 30 min at 37°. After washing, CL assays were performed as described in the Materials and Methods, employing sIC (50 μg/ml), pIC (5 μg/ml) or catIC (5 μg/ml) as stimuli. Data are expressed as the arithmetic mean ± SEM of duplicates from *n* (between parentheses) donors.

respectively), IV3 Fab' (percentage inhibition = 47 ± 6 and 56 ± 4, $n = 4$, respectively) or mAb 3G8 F(ab')$_2$ (percentage inhibition = 52 ± 6 and 54 ± 5, respectively, $n = 4$). On the other hand, light emission induced by catIC was markedly suppressed by either mAb IV3 (percentage inhibition = 92 ± 6, $n = 6$) or IV3 Fab (percentage inhibition = 77 ± 9, $n = 4$), while it was not modified by mAb 3G8 F(ab')$_2$ (percentage inhibition = 6 ± 4, $n = 6$). It was also observed that CL responses induced by either sIC, pIC or catIC, mediated by protease-treated neutrophils, were almost completely abrogated by mAb IV3 or IV3 Fab' (percentage inhibition > 86 in all cases).

Finally, we examined the effect of neuroaminidase treatment on the ability of these IC to induce neutrophil-mediated CL responses. The results indicated that light emission induced by either sIC (50 μg/ml), pIC (5 μg/ml) or catIC (5 μg/ml) (2 ± 1, 4 ± 2 and 8 ± 3 URCL, respectively, $n = 5$) was significantly enhanced by neuroaminidase treatment (8 ± 2, 9 ± 1 and 14 ± 3, respectively, $n = 5$). Light emission mediated by neuroaminidase-treated neutrophils induced by pIC and sIC was significantly inhibited by either mAb IV3 Fab (percentage inhibition = 68 ± 7 and 74 ± 6, $n = 4$, respectively) or mAb 3G8 F(ab')$_2$ (percentage inhibition = 49 ± 6 and 44 ± 3, respectively, $n = 4$). On the other hand, CL induced by catIC was markedly reduced by mAb IV3 Fab (percentage inhibition = 83 ± 5, $n = 5$) while it was not inhibited by mAb 3G8 F(ab')$_2$ (percentage inhibition = 7 ± 4, $n = 6$).

Effect of proteolysis on neutrophil-mediated CL responses induced by different agonists

In order to analyse the effect of proteolysis on neutrophil activation induced by different agonists, we studied their ability to induce CL responses. Table 4 shows that the responses induced by FMLP, PAF and zymosan were dramatically inhibited after proteolysis. In contrast, those induced by PMA or AA, which do not require the specific interaction with cell surface receptors, were not modified.

DISCUSSION

Neutrophils express constitutively two low-affinity FcγR: FcγRII (10,000 sites/cell) and FcγRIII (100,000 sites/cell).[1–4] The role of each one in neutrophil activation by IC is still unclear, but it appears to be strongly dependent on the characteristics of the stimulus.[1–4,18,19] The present study demonstrates that the activity of neutrophil FcγRII can be up-regulated by cell proteolysis. This treatment also induces a dramatic reduction in the expression of FcγRIII.[8,9] Both phenomena should be considered in order to evaluate the real impact of proteolysis on neutrophil FcγR-dependent functionality.

Our results also indicate that the enhancing effect on FcγRII activity induced by proteolysis is strongly dependent on the characteristics of the IC employed as stimulus. In fact, while CL responses induced by IgG-E and sIC were markedly increased after proteolysis, the responses induced by pIC or catIC were not modified. We have also observed that different responses, induced by a given IC, appear to be distinctly affected after neutrophil proteolysis. In this regard, we have demonstrated previously that neutrophil treatment with pronase or chymotrypsin markedly increases cytotoxicity induced by catIC.[10] These results contrast with our present data, indicating the inability of protease treatment to increase CL responses induced by catIC. Similar contrasting findings were observed employing sIC as stimulus. Proteolysis did not increase neutrophil cytotoxicity[10] but markedly increased CL responses induced by sIC.

Not all proteases appear to be able to enhance FcγRII activity. Tosi & Berger[9] have shown that neutrophil treatment with elastase does not increase either the ingestion of IgG-coated particles or the production of superoxide anion induced by multivalent IgG complexes. In agreement with these results, we have observed that neutrophil treatment with elastase (10–100 μg/ml) is unable to enhance EA rosette formation, as well as IgG-E-induced responses such as ADCC and CL (A. S. Trevani, unpublished results). Further studies will be required to define the ability of the different proteases, present at inflammatory sites, to modulate neutrophil FcγRII activity.

Membrane sialic acid, including acetylated derivatives of aminohexoses and *N*-acetylneuroaminic acid is, at least in part, responsible for the negative surface charge of PMN.[20] Previous reports have indicated that partial removal of sialic acid from the phagocytic cell surface, by treatment with neuroaminidase, enhances both the binding of IgG-sensitized erythrocytes to human monocytes and the release of superoxide anion from phagocytosing neutrophils.[21,22] In agreement with these reports, we observed that neuroaminidase treatment of neutrophils increased not only the binding of IgG-E but also IgG-E-triggered functions such as ADCC and CL. All these effects could be explained by a decrease of surface negative charge of sialidase-treated cells and a concomitant diminished repulsion between phagocytic and target cells. A similar mechanism could account, at least in part, for the enhancing effect on FcγRII activity induced by proteolysis. Proteolytic enzymes may induce a partial loss of neutrophil surface glycoproteins and, in this way, decrease both the sialic acid content and the negative charge of the cell surface. It is noteworthy, however, that neuroaminidase, but not proteases, was able to increase CL responses triggered by pIC and catIC. Taken together, these data would suggest that the effect of proteolysis on neutrophil FcγRII activity cannot be merely explained by a sialidase-'like' effect. Specific mechanisms whereby proteases can modulate FcγRII functionality remain to be elucidated.

Table 4. Effect of proteolysis on CL responses induced by different agonists

Treatment	FMLP (25 nM)	PAF (0·5 μM)	Zymosan (150 μg/ml)	PMA (5 nM)	AA (50 μM)
None	9 ± 1	12 ± 1	8 ± 1	15 ± 2	7 ± 1
Pronase	3 ± 1	3 ± 1	1 ± 0	16 ± 2	7 ± 1
Chymotrypsin	2 ± 1	2 ± 1	2 ± 1	15 ± 2	6 ± 1

Neutrophils (5×10^6/ml, in isotonic saline solution) were incubated in the presence or absence of pronase (200 μg/ml) or chymotrypsin (1 mg/ml), for 30 min at 37°. After washing, CL assays were performed as described in the Materials and Methods. Data are expressed as the arithmetic mean ± SEM of duplicates from n donors.

The present study demonstrates that the activity of neutrophil FcγRII can be up-regulated by proteolysis. Taking into account the high levels of proteases in areas of inflammation, the results presented here support the notion that this mechanism could be relevant, *in vivo*, for the regulation of neutrophil FcγRII activity. It is noteworthy that the increase in neutrophil responses observed after proteolysis appears to be restricted to FcγRII-dependent functions. In fact, light emission mediated by neutrophils stimulated by FMLP, PAF or zymosan was dramatically inhibited by treatment with either pronase or chymotrypsin. Further studies will be required to establish the net effect of cell proteolysis on the proinflammatory activities of phagocytic cells.

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