Mechanism of Formation of Human IgE-binding Factors (Soluble CD23): III. Evidence for a Receptor (FceRII)-associated Proteolytic Activity

By M. Letellier,* T. Nakajima,* G. Pulido-Cejudo,* H. Hofstetter,‡ and G. Delespesse*

From the *Laboratory for Allergy Research, Notre-Dame Hospital, University of Montreal, Montreal, Quebec, Canada H2L 4M1, and ‡Ciba-Geigy Ltd., Department of Biotechnology, CH-4002 Basel, Switzerland

Summary

There is mounting evidence that FceRII (CD23) and its soluble fragments (IgE-binding factors [BFs] or soluble CD23) have pleiotropic activities. IgE-BFs are formed mainly by the proteolytic cleavage of surface FceRII; they are first released as 37- and 33-kD unstable molecules that are subsequently transformed into 25-kD IgE-BFs. In this study, purified and radioiodinated 37-kD IgE-BFs as well as 45-kD FceRII were used as substrates to identify the proteases leading to the formation of 25-kD IgE-BFs. These substrates generate 25-kD IgE-BFs when incubated with several FceRII-bearing cells, including CH01-7 cells (transfected with FceRII cDNA); by contrast FceRII- cells, including CHO control cells, have no effect. Highly purified unlabeled native 37-kD and recombinant 29-kD IgE-BFs also cleave labeled 45-kD FceRII into 25-kD IgE-BFs. The proteolytic activity of these purified IgE-BFs is specifically removed by immunoprecipitation with an antibody against IgE-BFs. These data strongly suggest that FceRII and some of its soluble fragments play an active role in the proteolytic mechanism generating IgE-BFs. They are supported by the observation that IgE-BFs released by CH01-7 cells are cleaved exactly at the same sites as B cell-derived IgE-BFs. Taken collectively, the results are compatible with an autoproteolytic process.

The low affinity receptor for IgE (FceRII) is implicated in protective immunity and in immunoregulation. The FceRII on eosinophils, monocytes, and platelets is involved in IgE antibody-dependent cellular cytotoxicity against parasites (1, 2). On B lymphocytes, FceRII is identical to CD23 (3, 4), a B cell differentiation antigen selectively expressed on sIgM/sIgD double-bearing lymphocytes. In a recent study on mouse B cells, FceRII was shown to mediate IgE-dependent presentation of antigen to T cells (5). FceRII is a 45-kD glycoprotein containing one N-linked carbohydrate chain of the complex type and several O-linked chains (5–9). The cDNA coding for the FceRII from RPMI 8866 cells, a human lymphoblastoid B cell line, has been cloned (10–12) and used to express IgE-binding molecules on the surface of CHO cells (11). This receptor displays an unusual orientation with the COOH terminus being extracellular and also displays a striking homology to animal C-type lectins and to a new family of adhesion proteins (13).

The FceRII is cleaved into biologically active soluble fragments, some of which retain the ability of binding to IgE, i.e., IgE binding factors (IgE-BFs) (14, 15). Soluble FceRII fragments were reported to have pleiotropic activities, including (a) the regulation of the in vitro synthesis of human IgE (15–18), (b) the control of B and T cell proliferation (19–22), (c) the induction in synergy with IIr1 of the differentiation of early thymocytes (23), and (d) the inhibition of monocyte migration (24). IgE-BFs are mainly produced by the cleavage of cell surface FceRII (25). However, a small fraction of the IgE-BFs may also be formed intracellularly by proteolytic cleavage of newly synthesized FceRII (26). IgE-BFs exist in different molecular forms with apparent Mᵦ of 37,000, 33,000, and 25,000 (9, 27–30). The NH₂-terminal sequences of these IgE-BFs were determined and matched with the sequence deduced from the FceRII cDNA in order to localize their respective proteolytic cleavage site (28). Recently we reported that IgE-BFs are first produced as 37-kD (and 33-kD) IgE-BFs which are then cleaved into 25-kD IgE-BFs.

1 Abbreviation used in this paper: IgE-BFs, IgE binding factors.
Materials and Methods

Reagents. Carrier-free Na\(^{125}\)I was obtained from Amersham Corp. (Arlington Heights, IL); Affigel 10, SDS-PAGE standards (10,000–100,000 mol wt), Coomassie Brilliant Blue R-250, SDS, acrylamide, \(N,N'\)-methylene-bis-acrylamide, \(N,N,N',N'\)-tetramethyl-ethylenediamine (TEMED), Tris, glycine, ammonium persulfate, 2-ME, and molecular weight markers for gel filtration were purchased from Bio-Rad Laboratories (Richmond, CA); PMSF, benzamidine hydrochloride, e-aminocaproic acid, iodoacetamide, Hepes, NaCl, and FCS were obtained from Sigma Chemical Co. (St. Louis, MO). RPMI 1640 and HBSS were purchased from Flow Laboratories (McLean, VA). MEM, trypsin-EDTA, and FCS were obtained from Gibco Laboratories (Grand Island, NY); HB101 culture medium was from Hana Biologics (Berkeley, CA) and lactoperoxidase was from Calbiochem-Behring Corp. (San Diego, CA). Immobilon™-P transfer membranes were purchased from Millipore Corp. (Bedford, MA).

Cell Culture. RPMI 8866 and RPMI 8226 cells were cultured in RPMI 1640 medium supplemented with 5 mM t-glutamine, 10% FCS, 50 IU penicillin, and 50 mg/ml streptomycin. The CHO cells were maintained in MEM medium supplemented with 5% FCS, penicillin-streptomycin, and t-glutamine.

Antibody and Immunoadsorbents. The mAb against FceRII used in this study has been described: 207.25.A.4.4/30: IgG1,: FCS, penicillin-streptomycin, and L-glutamine.

Optimization and Application of Immunoadsorbents. The mAb against FceRII was affinity-purified from CHO serum-free culture supernatant by immobilized mAb-laden Affi-gel; after overnight rotation at 4°C, the gel was washed with 30 ml of D-PBS containing 0.5% NP-40 and with 30 ml of D-PBS containing 200 µg/ml BSA. Bound material was eluted with 0.2 M glycine-HCl, pH 2.3, containing 200 µg/ml BSA. The eluted material was neutralized with 2 M Tris, pH 9.0, and dialyzed against D-PBS.

Purification of 125I-labeled FceRII. A total of 1 ml of cell lysate from radioiodinated RPMI 8866 cells was mixed with 200 µl of mAbER 30-Affi-gel; after overnight rotation at 4°C, the gel was washed with 30 ml of D-PBS containing 0.5% NP-40 and with 30 ml of D-PBS containing 200 µg/ml BSA. Bound material was eluted with 0.2 M glycine-HCl, pH 2.3, containing 200 µg/ml BSA. The eluted material was neutralized with 2 M Tris, pH 9.0, and dialyzed against D-PBS.

Electrophoresis and Autoradiography. SDS-PAGE was done on 15%–25% slab gels according to Laemmli (32). Samples containing 5% (vol/vol) 2-ME were boiled for 2 min and applied on gels. The gels were stained with 0.25% (wt/vol) Coomassie Brilliant Blue, destained, and dried. Autoradiography was performed at -70°C by using XAR-5 x-ray film (Eastman Kodak Co., Rochester, NY).

Purification of 29- and 37-kD IgE-BF's. For the purification of 37-kD IgE-BF's, the supernatant from RPMI 8866 cells cultured for 4 h in serum-free HB101 medium at 2 × 10⁶ cells/ml in the presence of iodoacetamide (20 mM) was adsorbed on 1 ml of mAbER 30-Affi-gel at a flow rate of 2 ml/h. The gel was then washed with 50 ml of D-PBS containing 0.05% NP-40 and 5 mM iodoacetamide. The bound material was eluted with 3.5 M MgCl₂, pH 4.8, made 0.05% NP-40, and dialyzed against PBS containing 0.05% NP-40 and iodoacetamide (5 mM). The dialyzed material was further purified on 1 ml of freshly prepared mAbER 30-Affi-gel. This gel was washed with D-PBS containing 0.05% NP-40 and the 37-kD IgE-BF's were eluted with 3.5 M MgCl₂, pH 4.8, made 0.05% NP-40. The eluted material was subsequently dialyzed against D-PBS containing 0.05% N-P40 and concentrated by centrifugation at 6,000 g on an IEC refrigerated centrifuge using Centricon 10 microconcentrators (Amicon Corp., Danvers, MA). This procedure was also used to isolate 29-kD and 25-kD IgE-BFs from the supernatant of CHO-29 and CHO 20.3 cells, respectively, with the exception that iodoacetamide was omitted in every step. In some experiments, the affinity-purified 29-kD IgE-BFs were further purified by HPLC ion-exchange chromatography exactly as previously described (27).

Fractionation of IgE-BFs by Gel Filtration on HPLC. 25 µl of concentrated culture supernatants were applied on a BIOSIL TSK 250 column (Bio-Rad) and eluted with a buffer containing 0.05M Na₂SO₄ and 0.02M NaH₂P0₄, pH 6.8, at a flow rate of 0.5 ml/min. The column was calibrated by using the Bio-Rad gel filtration standards. Fractions (0.25 ml) were tested by RIA for IgE-BFs as described elsewhere (14). The results are expressed in units/milliliter by reference to a standard preparation. 1 U of IgE-BFs corresponds to 150 pg of 25-kD IgE-BFs.

IgE-BFs Amino Acid Sequence Analysis. IgE-BFs were isolated from the culture supernatant of CHO1-7 cells by affinity chromatography and fractionated by PAGE in the presence of SDS as described. The gel was then electroeluted to Immobilon™-P transfer membrane according to the Millipore tech protocol TP006 and TP017. Briefly, the gels were soaked in transfer buffer (25 mM Tris, 192 mM glycine, 15% methanol [vol/vol], pH 8.3) for 30 min and the Immobilon membrane was pre-wet in 100% methanol and soaked in transfer buffer for 30 min. The gel was sandwiched between the Immobilon membrane and sheets of filter paper, assembled into a Bio-Rad transblot cell apparatus and electroeluted for 5 h at 50 V in transfer buffer. The membrane was stained with 0.2% Coomassie brilliant blue R-250 in 45% methanol, 10% acetic acid, and destained in 90% methanol, 7% acetic acid. The stained bands were excised from the membrane and loaded into a sequencer cup of a gas-phase sequencer (No. 470A; Applied Biosystems, Inc., Foster City, CA). The phenylthiohydantoin derivatives of the amino acid of interest were generated and sequenced.
acids obtained by Edman degradation were identified by reverse-phase HPLC using an Altex 5 μm ultrasphere-ODS column (33).

Expression of FcεRII, 37-, 29-, and 25-kD IgE-BFs in CHO Cells. The construction of the expression plasmid pSVd-ER, and the selection of cell line CHO (1-7) expressing human FcεRII have been described (11). In order to express 37-, 29-, and 25-kD IgE-BFs, plasmid pSVd-ER was modified. First, a 2.3-kb Dfr selection marker, derived from plasmid pSVneo2911 (34) by digestion with Sall and XhoI, was integrated into the Sall site of pSVd-ER next to the ampicillin gene. Second, the SV40 enhancer/promoter of pSVd-ER was replaced by the 540-bp Accl-HindIII fragment of the SV40-MCMV virus P1 (35). Third, direct secretion of 37-, 29-, and 25-kD IgE-BFs was achieved by removal of the leader and NH2-terminal coding sequences of FcεRII cDNA up to amino acid residues 75, 118, and 147, respectively, and subsequent replacement by an artificial DNA segment coding for the signal sequence of the avian influenza virus hemagglutinin (36). These modifications yielded the following plasmids: pCAL12Mdfr, pCAL8Mdfr and pCAL10Mdfr, which were used to transfected Dfr- CHO DUOX-B1 cells. After 14 d of Dfr selection, single colonies were picked and expanded, and colonies that produce 37-kD IgE-BFs (CHO-37), 29-kD IgE-BFs (CHO-29), and 25-kD IgE-BFs (CHO-20.3) at high level were selected. These cell lines were maintained in MEM medium lacking ribonucleosides and deoxyribonucleosides.

Cleavage of 125I-labeled 45-kD FcεRII by Purified Recombinant 29-kD IgE-BFs. In typical experiments 60 pmol of recombinant 29-kDa IgE-BFs (in 50 μl D-PBS, 0.05% NP-40) was incubated with 50 μl (5,000 cpm) of radiiodinated 45-kD FcεRII dissolved in D-PBS in a final volume of 100 μl. After 17-24 h of incubation at 37°C, samples were taken into 80 A1 of SDS-PAGE electrophoresis buffer consisting of 63 nM Tris-HCl, pH 6.8; 2% SDS; 10% glycerol; 5% 2-ME, and were boiled for 2 min. In controls samples the 125I-labeled 45-kD FcεRII was incubated with D-PBS containing 0.05% NP-40. SDS-PAGE was then performed in 12% polyacrylamide gels at 10 mA during 17 h.

Results

Purified 125I-labeled FcεRII Is Cleaved into 25-kD IgE-BF after Interaction with FcεRII-bearing Cells. In the course of our studies on the degradation of 37-kD IgE-BFs into 25-kD IgE-BFs we noticed that affinity-purified and radiolabeled 45-kD FcεRII was degraded into 25-kD IgE-BFs when incubated with FcεRII+ cells (RPMI 8866, HC-EBV transformed cell lines, U937 macrophage cell line), whereas the same preparation remained stable when incubated with FcεRII- cells (RPMI 8226, U266 myeloma cells). One such experiment is shown in Fig. 1 a where 125I-labeled 45-kD FcεRII was incubated with an NP-40 lysate of either FcεRII+ (RPMI 8866 cells) or FcεRII- cells (RPMI 8226). As seen, the 45-kD labeled FcεRII was cleaved into a 25-kD molecule only by the NP-40 lysate of RPMI 8866 cells.

To further examine whether the ability of a given cell line to cleave 125I-labeled FcεRII into IgE-BFs is related to the expression of surface FcεRII, we compared CH01-7 cells (transfected with FcεRII cDNA) with CHO control cells. As seen, the CHO1-7 cell line cleaves both the 45-kD FcεRII (Fig. 1 b) and the 37- and 33-kD IgE-BFs (Fig. 1 c) into 25-kD IgE-BFs while CHO control cells have no effect. These experiments indicate that the expression of FcεRII is required for a given cell line to cleave 125I-FcεRII. This may be ex-
explained either by a receptor-associated proteolytic activity of FcεRII or by the FcεRII-dependent activation of an unknown protease. The latter should be ubiquitous since it is expressed on several human as well as hamster cells.

Moreover, several serine protease or thiolesterase (SH-protease) inhibitors failed to inhibit this cell-associated proteolytic activity. Indeed, with the exception of iodoacetamide, none of the following inhibitors could inhibit the formation of 25-kD IgE-BFs by RPMI 8866 cells: e-amino caproic acid (50 mM), benzamidine hydrochloride (10 mM), PMSF (1 mM), leupeptin (100 μg/ml), cystatin (10 μg/ml), soybean trypsin inhibitor (100 μg/ml), pepstatin (10 μg/ml), TLCK (4 mM), TPCK (4 mM), HgCl₂ (10 μM), 2-ME (1 mM), and EDTA (10 mM). In these experiments, RPMI 8866 cells were incubated for 2 h in the presence of the protease inhibitors. The IgE-BFs in the culture supernatants were measured by RIA and their molecular weight was determined by HPLC gel filtration. In keeping with our previous studies, cells cultured in the presence of iodoacetamide released a 37-kD instead of 25-kD IgE-BFs.

The Proteolytic Activity Is Associated with the 37-kD IgE-BFs. To further analyze the cleavage of 37-kD IgE-BFs into 25-kD IgE-BFs by FcεRII-bearing cells, a functional recombinant 37-kD IgE-BFs was expressed in CHO cells after stable transformation with a mammalian expression vector containing the coding region of FcεRII corresponding to amino acids 77-321. Surprisingly, the 24-h culture supernatant of these transformed CHO cells (CHO-37) contained a 25-kD IgE-BFs and not a 37-kD IgE-BFs as expected. The latter was detected only when CHO-37 cells were cultured in the presence of iodoacetamide. The instability of 37-kD IgE-BFs suggested that these may be endowed with the proteolytic activity ascribed to the intact FcεRII. In the next series of experiments we examined the ability of several native or recombinant IgE-BFs to cleave 125I-labeled 45-kD FcεRII. Fig. 2 shows that both purified native 37-kD IgE-BFs as well as a recombinant 29-kD IgE-BFs are capable of cleaving the labeled receptor into 25-kD IgE-BFs. The 29-kD IgE-BFs were purified from the supernatant of CHO cells transfected with a cDNA coding for the amino acids 120-321 of the FcεRII. Contrary to the recombinant 37-kD IgE-BFs, the 29-kD IgE-BFs are rather stable in the absence of iodoacetamide. In several similar experiments, we could hardly detect any proteolytic activity of either native or recombinant 25-kD IgE-BFs. The possibility that the proteolytic activity associated with the purified IgE-BFs might be due to a contaminating protease was not supported by the following experiments. First, immunoprecipitation of the 37-kD IgE-BFs by anti-FcεRII mAb completely abrogates the proteolytic activity (Fig. 2 c). Second, 25-kD and 37-kD IgE-BFs were purified from parallel culture supernatants of RPMI 8866 cells by using identical purification procedure. The only difference was that RPMI 8866 cells were supplemented with iodoacetamide for the preparation of 37-kD IgE-BFs. When reacted with radiiodinated 45-kD FcεRII, only the 37-kD IgE-BFs have proteolytic activity (data not shown).

Iodination Procedure of FcεRII Destroys the Proteolytic Activity. The above experiments were made possible because, in contrast to the unlabeled molecules, radiolabeled 45-kD FcεRII or 37-kD IgE-BFs are stable when incubated for 24 h at 37°C. Since the labeled molecules may still be cleaved, it may be predicted that the iodination procedure destroys the proteolytic activity. To examine this possibility, purified 37-kD IgE-BFs were iodinated with cold iodine (0.1 mM)

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**Figure 2.** (a) Cleavage of FcεRII by purified 37-kD and 29-kD IgE-BFs. 50 µl of 125I-labeled FcεRII (5,000 cpm; about 6 pmol) were incubated for 24 h at 37°C with 50 µl of PBS (lane A), 50 µl of affinity chromatography purified 37-kD IgE-BFs (0.3 nmol) (lane B), and 50 µl of recombinant 29-kD IgE-BFs (0.3 nmol) purified by affinity chromatography followed by HPLC ion-exchange chromatography (lane C). The mixtures were analyzed by SDS-PAGE and autoradiography. SDS-PAGE molecular weight markers are indicated at the side. (b) SDS-PAGE analysis of the IgE-BFs (29-kD) purified from the culture supernatant of CHO-29 cells (lane A). Lane B is an empty well. The gel was stained with silver nitrate. (c) 125I-labeled FcεRII were incubated with PBS (lane A), supernatants from 37-kD IgE-BF preparation (lane B), and immunoprecipitated with anti-FcεRII mAb (lane B), or antiprolactin mAb (unrelated mAb of the same IgG subclass) (Lane C). A preparation of 37-kD IgE-BFs (0.3 nmol in 100 µl) was supplemented with 2 µg of mAbER 30 or antiprolactin mAb. After a 4-h incubation at 4°C, the mixtures were precipitated with 30 µg of affinity-purified rabbit anti–mouse IgG. The supernatants (50 µl) were incubated for 24 h at 37°C with 50 µl of 125I-labeled FcεRII (5,000 cpm; ~6 pmol).
Figure 3. Effect of iodination procedure on the cleavage of FceRII. 50 μl of 125I-labeled FceRII (5,000 cpm; ~6 pmol) were incubated for 24 h at 37°C with 50 μl of PBS (lane A), 50 μl of iodinated 37-kDa IgE-BFs (0.3 nmol) (lane B) and 50 μl of 37-kDa IgE-BFs (0.3 nmol) (lane C). The mixtures were analyzed by SDS-PAGE and autoradiography. SDS-PAGE molecular weight markers are indicated at the side.

by using the same lactoperoxidase method as for cell surface radiiodination and their proteolytic activity was compared to that of control 37-kDa IgE-BFs preparations treated exactly the same way except that lactoperoxidase was omitted (Fig. 3). The results show that iodinated 37-kDa IgE-BFs were unable to cleave the 125I-labeled FceRII while 37-kDa IgE-BFs cleaved 125I-FceRII into 25-kD IgE-BFs. The inactivation of the proteolytic activity associated with the 37-kD IgE-BFs by the iodination procedure is caused by lactoperoxidase oxidation rather than by the iodination of the molecules. Indeed, treatment of 37-kD IgE-BFs with lactoperoxidase alone abrogates the proteolytic activity (data not shown).

Similar observations were also made with intact RPMI 8866 cells iodinated with cold iodine and incubated with 125I-FceRII in the presence of cycloheximide to inhibit the synthesis of new receptor molecules (data not shown).

CHO Cells Expressing FceRII Produce IgE-BFs Identical to Those Released from RPMI 8866 Cells. Our interpretation of the above results is that the cleavage of FceRII and its soluble 37-kD IgE-BFs into 25-kD IgE-BFs is mediated by either an autoproteolytic mechanism or by an FceRII-dependent ubiquitous protease. If this view is correct it may be predicted that CHO cells expressing recombinant 45-kD surface FceRII should release IgE-BFs that are identical to those of B cell origin. In the next series of experiments we first ascertained that recombinant FceRII on CHO cells has the same structure as on B cells (Fig. 4). We then analyzed the IgE-BFs released by CHO1-7 cells. These IgE-BFs were purified by affinity chromatography, fractionated by SDS-PAGE, and then transferred to immobilon™-P transfer membrane by electrophoretic blotting. The NH2-terminal sequence of the first four residues was determined on the excised 25-kD stained band as described. A double sequence was obtained corresponding to Leu-Arg-Met-Glu and Met-Glu-Leu-Gln. Matching these sequences with those deduced from the FceRII cDNA indicates that the 25-kD IgE-BFs from CHO1-7 cells starts with leucine at position 148 or methionine at position 150 of the FceRII, which are exactly the same two proteolytic cleavage sites as for the 25-kD IgE-BFs isolated from RPMI 8866 cells culture supernatant (10-12). Moreover, in additional experiments, we observed that in the case of RPMI 8866 cells, the 25-kD IgE-BFs are derived from soluble 37-kD IgE-BF precursors. Indeed, when CHO1-7 cells are cultured in the presence of iodoacetamide, the molecular mass of IgE-BFs

Figure 4. SDS-PAGE analysis of the affinity chromatography-purified radiolabeled FceRII from RPMI 8866 (lanes A and B) and CHO1-7 (lanes C and D) cells. (Lanes A and C) KSCN eluates; (lanes B and D) glycine eluates. Similar amount of radioactivity was applied to each lane. SDS-PAGE molecular weight markers are indicated at the side.

Figure 5. Fractionation by gel filtration of the supernatant from CHO1-7 cells cultured for 6 h in HB101 medium in the presence (●) or absence of 20 mM iodoacetamide (○). The supernatants were concentrated 50-fold and fractionated by HPLC on a TSK 250 column.
is shifted from 25 to 37-kD as determined by HPLC gel filtration (Fig. 5).

Discussion

IgE-BFs are mainly produced by the proteolytic cleavage of surface FceRII (9, 25). The rate of this process is reduced by IgE, whereas it is increased by treatment of FceRII-bearing cells with tunicamycin, indicating that the N-linked carbohydrate chain stabilizes the receptor (25). We recently reported that IgE-BFs are first released as 33–37-kD molecules which are subsequently transformed into 25-kD IgE-BFs (17). The latter are much more stable and they are detected in the supernatants of 2–3 wk cultures in human sera and even in urine (37).

In the course of our initial studies on the cloning of FceRII cDNA, we noticed that after purification to homogeneity, 45-kD FceRII molecules were unstable and degraded into 37-, 33-, 29-, and 25-kD fragments. In contrast, most preparations of radiolabeled receptors isolated from surface radioiodinated cells were rather stable (in the absence of inhibitors), even when incubated for 24 h or more at 37°C. Such preparations were therefore used as a substrate for the identification of the proteolytic enzymes that cleave FceRII. In this study, we first observed that the ability of a given cell line to cleave 125I-labeled 45-kD FceRII or 37-kD IgE-BFs is dependent upon the expression of FceRII. Indeed, 125I-labeled 45-kD FceRII and its 37-kD fragments are transformed into 25-kD IgE-BFs when incubated with several FceRII-bearing cells (including the transfected CH01-7 cells), while the same preparations remain stable when incubated with several FceRII− cells (including CHO control). There are two possible explanations for these observations: (a) FceRII is degraded by a receptor-associated proteolytic mechanism (where one FceRII molecule could cleave adjacent identical molecules), or (b) it is degraded by a protease that is both ubiquitous and activated by FceRII. These two possibilities are also supported by the observation that CH01-7 cells, expressing functional recombinant IgE receptor, release IgE-BFs identical to those released by B cells (i.e., they have the same molecular weight and the same NH2-terminal end cleavage sites). To examine these alternatives it was necessary to test the ability of purified unlabeled FceRII to cleave radioiodinated FceRII. Because it is difficult to obtain convincingly pure preparations of 45-kD FceRII, the proteolytic activity of soluble IgE-BFs was examined. The instability of 37-kD IgE-BFs suggested that it might be endowed with such activity. Indeed, highly purified 37-kD IgE-BFs proved to be capable of cleaving radiolabeled 45-kD FceRII, as well as radiolabeled 37-kD IgE-BFs, into 25-kD IgE-BFs. Moreover, recombinant and stable 29-kD IgE-BFs but not native or recombinant 25-kD IgE-BFs displayed the same activity. Three observations militate against the possibility that the proteolytic activity of the purified 37- and 29-kD molecules was due to a co-purified protease. First, the 24-h culture supernatant of FceRII-bearing B cells (containing 25-kD IgE-BFs) has no activity (28). Second, immunoprecipitation of the 37-kD IgE-BFs specifically removed the proteolytic activity of the preparation. Third, when 37 and 25 kD were isolated from the culture supernatants of the same cell line using identical purification procedures, only the 37-kD molecules were capable of cleaving the labeled 45-kD FceRII. Taken collectively, the present data suggest, but do not demonstrate, that IgE-BFs are produced by an autoproteolytic mechanism. This possibility is currently examined more directly by (a) using synthetic peptides as substrate, and (b) comparing the proteolytic activity of several FceRII mutants, aiming to localize the putative active site(s).

Autoproteolysis was recently described as a mechanism to activate or inactivate an increasing number of biologically active proteins in bacteria (38, 39), virus (40–42), and eucaryotes (43–47). For example, in polymorphonuclear leukocytes, calpain (a protease) is derived from its inactive precursor by autoproteolysis (43–44). Other examples include cathepsin D (45) and human collagenase (46). The current observations of an FceRII-associated proteolytic activity raise the intriguing possibility that this receptor or its 37-kD fragment may be involved in the cleavage of other biologically relevant molecules. In this regard, it is worth noting that some differentiation antigens were recently shown to display protease activity: examples include CD10 (or CALLA, endopeptidase −24.11) (47, 48), CD13 (aminopeptidase N) (49), and CD26 (dipeptidyl peptidase IV) (50).

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Address correspondence to Dr. G. Delespesse, Notre-Dame Hospital, Research Center, 1560 Sherbrooke Street East, Montreal, Quebec, Canada H2L 4M1.

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