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INTRACELLULAR CLEAVAGE OF NEWLY SYNTHESIZED LOW AFFINITY FCER RECEPTOR (FCER2) PROVIDES A SECOND PATHWAY FOR THE GENERATION OF THE 28-kDA SOLUBLE FCER2 FRAGMENT

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It has been reported that the 45-kDa low affinity FCER (FCER2) on B cells is cleaved spontaneously from the cell surface to release a 28-kDa soluble fragment (sFCER2). This study demonstrates an additional mechanism by which B cells generate this fragment. Data from 35S methionine pulse-chase experiments with the FCER2 bearing human B lymphoblastoid cell line, RPMI 8866, and immunoprecipitations of cell lysates and culture supernatants with an FCER2 specific mAb, mAb 25, demonstrates the existence of a cell-associated 28-kDa FCER2 fragment. This fragment was shown by partial amino(NH2)-terminal sequence analysis to be identical to the previously described 28-kDa sFCER2. The resistance to cell treatment with trypsin indicated that it was located intracellularly. Its appearance early in the biosynthesis of the FCER2 (within a 10-min pulse), before the FCER2 reached the cell surface, suggested that some of this fragment was generated intracellularly. Neutralization of acidic organelles with NH4Cl inhibited the formation of this intracellular fragment, strongly suggesting that it was a product of intracellular cleavage of the FCER2. Finally, this 28-kDa intracellular fragment was shown to be released into the culture supernatant, suggesting an intracellular mechanism by which the cells generate sFCER2.

FCER2 is expressed on subpopulations of B lymphocytes (1), monocytes (2), macrophages (3), eosinophils (4), and platelets (5). The FCER2 of B cells has been shown to be identical to the B cell activation antigen CD23/blast-2 (6-10). Recent evidence suggests that FCER2 of B cells may play a role in transmembrane signal transduction and subsequent cell proliferation. For example, ligation of cFCR with selected mAb or polyclonal antibodies enhances the entry of phorbol ester-activated human tonsil B lymphocytes into the S phase of the B cell cycle (11, 12). It has also been suggested that the 12-kDa B cell growth factor is an endogenous ligand for the FCER2 (13, 14).

The biochemical characteristics of FCER2 on the B cell have been extensively studied (15-17). The B lymphoblastoid cell line RPMI 8866 expresses a 45-kDa FCER2 at its plasma membrane. Cell surface receptors are cleaved to shed a 25- to 30-kDa sFCER2 into the culture supernatant (15, 16). Studies using normal tonsil lymphocytes also suggest that the sFCER2 is derived from the membrane-bound receptor, because the loss of FCER2 from the surface of these cells in culture parallels the appearance of sFCER2 in the culture supernatant (16).

Although the precise function of the sFCER2 remains unknown, it has nonetheless been demonstrated that it can act as a growth factor for EBV-infected B lymphoblasts and receptor-stimulated B cells (18), and may be related to the previously described 25- to 30-kDa B cell-derived BCGF (19-21). In view of the potential importance of sFCER2 as a B cell growth factor, it is essential to delineate the potential sources for this fragment. It has been shown previously that sFCER2 can be generated by cleavage of FCER2 from the plasma membrane (15, 16). In this study, we demonstrate an intracellular pathway for the formation of the sFCER2. Pulse-chase metabolic labeling experiments showed that a fragment of FCER2 identical to shed sFCER2 is formed intracellularly by proteolytic cleavage of newly synthesized FCER2. This intracellular fragment of FCER2 is eventually secreted into the extracellular medium. The data indicate that proteolytic processing of the receptor can occur inside the cell.

MATERIALS AND METHODS

Cell lines. mAb, Ig, RPMI 8866, a human FCER2(+) B lymphoblastoid cell line, and CEM-6, a FCER2(-) human lymphoblastoid T cell line were kindly provided by Dr. K. Ishizaka (The Johns Hopkins University, Baltimore, MD). mAb 25, an IgG1, murine mAb raised against RPMI 8866 and specific for FCER2 on B cells (7), was kindly provided by Drs. J. Benchereau and J. de Vries (UNICET Laboratory for Immunological Research, Dardilly, France). This mAb is also known to precipitate sFCER2. The IgE (PS) myeloma protein was kindly provided by Dr. K. Ishizaka (The Johns Hopkins University, Baltimore, MD).

Surface labeling of cells with [35S]methionine and immunoprecipitation of FCER2. RPMI 8866 cells were surface radiolabeled by the lactoperoxidase method (22) at 4°C. Cells (30 x 106) were washed twice with PBS, pH 7.4, resuspended in 400 μl PBS, and 0.5 mCi [35S]Na (sp. act. 15.8 mCi/μg of iodine; Amersham, Arlington Heights, IL) was added. This was followed by addition of 10 μl lactoperoxidase (140 U/ml; Sigma Chemical Co., St. Louis, MO), and 10 μl H2O2 (0.0075% in PBS). The mixture was agitated with three further additions of H2O2 (10 μl) at 5-min intervals. After 20 min, the reaction was quenched by the addition of 10 ml PBS containing potassium iodide (20 mM) and tyrosine to saturation, followed by two washes in ice cold PBS. The cell membranes were solubilized at 4°C in 1 ml TEA
buffer (10 mM triethanolamine, 0.15 M NaCl, and proteolytic enzyme inhibitors: 1 mM FMSF, 1 mM EDTA, 10 mM 1,000, and 1 μg/ml aprotinin, antipain, leupeptin, and pepstatin, and (Sigma), pH 7.8), containing 1.5% NP-40. Cell lysates were clarified by centrifugation at 16,000 \times g for 20 min at 4°C. These lysates, and corresponding culture supernatants containing the above mentioned proteolytic enzyme inhibitors, were incubated overnight with 10 μl of 100-fold concentrated Staphylococcus aureus (Pharmacia Fine Chemicals, Piscataway, NJ). The precipitations of the precleared cell lysates or culture supernatants were carried out by incubating with 10 μg of 

methionine and immunoprecipitation of \( \text{FcrR}_2 \). RPMI 8866 cells were washed three times with HBSS and incubated at 1 × 10^7/ml for 2 h in methionine-free RPMI medium (GIBCO Laboratories, Grand Island, NY) supplemented with 10% FBS. The procedure with Trypan blue exclusion test showed that the cell viability was >99%. For the incubation, 0.05% saponin (ICN Biochemicals, Irvine, CA) in pre-warmed (37°C, methionine-free RPMI containing 10% FBS (1 ml/ml). After an equal volume of culture medium containing 10% FBS was added. Cells were lysed and immunoprecipitated with 10% dialyzed FBS. For the chase period, cells were resuspended in warm medium (37°C), and cultured for various time intervals. At the end of the pulse-chase period, cells were washed twice in PBS at 4°C. Cell membrane solubilization and pre-clearance of cell lysates were carried out as described in the above section for [125I]surface labeling of cells. Immunoprecipitation was carried out with preformed complexes of rabbit anti-mouse IgG and 3 μl of mouse ascites containing specific activity of 25 μCi/mg IgG for 2 h at 4°C (24). The immunoprecipitates were taken up in 200 μl of 0.6% sodium deoxycholate in TEA buffer and washed by centrifugation at 16,000 \times g for 1 min at 4°C on a discontinuous gradient, which consisted of an upper layer of 10% sucrose in TEA buffer containing 0.5% NP-40 on a lower layer of 20% sucrose in TEA buffer without detergent. Finally, the immune complexes were washed once in TEA buffer containing 0.5% NP-40.

In experiments to determine the effect of the weak base NH\(_4\)Cl, on the generation of intracellular \( \text{FcrR}_2 \) fragment, RPMI 8866 cells were pre-incubated with 50 mM NH\(_4\)Cl for 45 min in methionine-free medium before and during the 45-min pulse labeling with [\(^{35}\)S]methionine.

Partial amino(NH\(_2\))-terminal sequence analysis of 28-kDa cell-associated \( \text{FcrR}_2 \) fragment. The partial NH-tertiary sequence analysis of the 28-kDa cell-associated \( \text{FcrR}_2 \) fragment was performed by an Applied Biosystems (Foster City, CA) 470 amino acid sequencer equipped with on-line phenylthiohydantoin-amino analysis using the regular program 03RPTH. In two separate experiments, 30 and 60 min pulse-labeled samples were specifically labeled with 0.5 μl per lane containing 10 μCi/ml of [\(^{35}\)S]methionine (sp. act. 1326 Ci/mM; Amersham Corp., Arlington Heights, IL); and 1 mM/cm containing 10 μCi/ml of [\(^{35}\)S]methionine (sp. act. 32 Ci/mM; Amersham). In each experiment cells were labeled for 16 h in 5 ml of RPMI 1640 medium made up in the absence of the corresponding radiolabeled amino acid (RPMI select amine kit: GIBCO), supplemented with 10% dialyzed FBS. Cells were lysed and immunoprecipitated as described in the above section for the metabolic labeling with [\(^{35}\)S]methionine and immunoprecipitation of \( \text{FcrR}_2 \). This fraction was analyzed on SDS-PAGE, and protein transferred by electroblotting onto a polyvinylidene difluoride membrane as previously described (25).

Trypsination of cells. [\(^{125}\)I]- or [\(^{35}\)S]methionine-labeled cells (10 × 10^5) were washed twice with ice cold PBS, and subsequently incubated with either 1 μl trypsin-EDTA solution (0.5 trypsin and 0.2 g EDTA/liter of HBSS; GIBCO) or Ca\(^{2+}\)/free HBSS containing 5 mM EDTA for 60 min on ice. At the end of the incubation, an equal volume of culture medium containing 10% FBS was added. Cells were pelleted and washed three times in PBS.

[\(^{125}\)I]-radiolabeled \( \text{FcrR}_2 \) [\(^{35}\)S]methionine-labeled \( \text{FcrR}_2 \) fragment from 24-h culture supernatants of [\(^{125}\)I]-labeled RPMI 8866 cells was isolated by adsorption to a mAb 25 Sepharose 4B affinity column. The protein was eluted using 0.1 M glycine, pH 3. The eluate was neutralized with Tris to pH 8 and divided to two equal aliquots. One aliquot, trypsin (Calbiochem-Behring Corp., Malvern, PA) bound to Protein A Sepharose 4B (Pharmacia Fine Chemicals, Piscataway, NJ), and the other served as a control. After a 30-min incubation at 37°C, 50 μg of BSA in PBS was added to neutralize trypsin and act as a carrier protein. The proteins were acelone precipitated (9/1 v/v) at −20°C for 5 h and subsequently lyophilized. The digested material and control were analyzed by SDS-PAGE and subjected to autoradiography.

**Cell permeabilization with saponin.** A total of 10 million [\(^{35}\)S]methionine-labeled RPMI 8866 cells was treated with 0.05% saponin in PBS for 15 min at 4°C. The cells were pelleted by centrifugation and solubilized in TEB buffer containing 1.5% NP-40 at 4°C. The cell lysate and the saponin-containing supernatant were separately each immunoprecipitated with mAb 25 and analyzed on SDS-PAGE.

**RESULTS**

**Twenty-eight kDa \( \text{FcrR}_2 \) exists in cell-associated form.** Using pulse-chase experiments, Figure 1 shows that mAb 25 specifically immunoprecipitated two polypeptides from RPMI 8866 cells pulsed with [\(^{35}\)S]methionine for 10 min, then chased for periods up to 120 min. An \( \text{FcrR}_2 \) precursor protein of 41 kDa was detected as early as 5 min after pulsing (Fig. 1A), and increased in intensity with a 10-min pulse (Fig. 1B). With chase periods of 30, 60, and 120 min, this protein was processed to a broad band of 41 to 45 kDa. A second 28-kDa band (Fig. 1, arrow), which was absent at the 5-min pulse, became detectable at 10 min. The intensity of this band increased over the chase period of 30 min, and was stable thereafter. The m.w. of the backbone of the polypeptide recognized by mAb 25 was assessed by pre-incubating cells with tunicamycin to prevent co-translational addition of high mannose oligosaccharides. Under these conditions, the m.w. of the major precipitated band was reduced from 41 to 39 kDa (Fig. 1C). This value corresponds closely to the m.w. of \( \text{FcrR}_2 \) (26) calculated from the published amino acid sequence.

Interestingly, tunicamycin did not alter the m.w. of the second 28-kDa band. This observation is consistent with the known structure of \( \text{FcrR}_2 \). The predicted cleavage site between amino acids 149 and 150 is nearer the glycosylated asparagine residue (amino acid 63). The 28-kDa secreted form would not therefore be glycosylated. The sharp 45-kDa band which is consistently present in all lanes including the control lane is most likely actin. In addition, a number of non-specific faint bands were seen with mAb 25 immunoprecipitates and were identical to those seen in the control lane (ns) containing immunoprecipitates using normal mouse IgG.

We next assessed the structural relationship between the cell-associated 28-kDa polypeptide and the \( \text{FcrR}_2 \) that is shed into the supernatant (27). To show that the 28-kDa polypeptide immunoprecipitated from the cell lysates and is the same protein as \( \text{FcrR}_2 \), the partial NH-tertiary sequence of [\(^{35}\)S]methionine- and [\(^{35}\)H]valine-labeled cell-associated polypeptide was determined and compared with the known sequence of the shed \( \text{FcrR}_2 \) (26). Analysis of the first 15 amino acid residues revealed methionine in position 1 and valine in positions 5 and 10. This partial sequence aligns with the known NH-tertiary sequence of \( \text{FcrR}_2 \) (Fig. 2). Taken together, these data indicate that the 28 kDa molety of \( \text{FcrR}_2 \) exists in a cell-associated form and is identical to the previously described shed \( \text{FcrR}_2 \) (26).

**Cell-associated \( \text{FcrR}_2 \) fragment is intracellular.** To determine the location of the cell-associated 28-kDa \( \text{FcrR}_2 \) fragment, its susceptibility to trypsin degradation was assessed. To this purpose, RPMI 8866 cells were pulse-labeled for 2 h with [\(^{35}\)S]methionine and chased for
Figure 1. Pulse-chase analysis of the synthesis and processing of FcεR2. RPMI 8866 cells were labeled with 35S methionine for 5 min (A) and 10 min (B). Cells were washed with methionine containing RPMI 8866 supplemented with 10% FBS and chased for a further period of 30, 60, and 120 min. In a third experiment (C), cells were pulse labeled for 10 min and chased for 60 min in the presence or absence of tunicamycin. In all lanes, cell lysates were immunoprecipitated with mAb 25 and analyzed on a 12% SDS-PAGE under non-reducing conditions. Control lane (ns) represents immunoprecipitation of cell lysates using normal mouse IgG.

Figure 2. Partial NH2-terminal sequence analysis of the 28-kDa cell-associated FcεR2 fragment. RPMI 8866 cells were labeled with 35S methionine (---), and 3H valine (-----) in separate experiments. The data presented are radioactivity (counts/min (cpm)) released at each sequencer cycle. * indicates peaks corresponding to residues in the sFcεR2. The one-letter amino acid abbreviation of the first 15 NH2-terminal of the previously described sFcεR2 is represented below the horizontal axis.

24 h, then treated with trypsin for 1 h on ice before immunoprecipitation. Figure 3A shows that the 45-kDa FcεR2 is trypsin sensitive, whereas the metabolically labeled 28-kDa FcεR2 fragment (Fig. 3 arrow) is resistant to trypsin digestion. The resistance of the cell-associated 28-kDa FcεR2 fragment to trypsin degradation contrasted with the trypsin sensitivity of the 28-kDa sFcεR2 present in the culture supernatant. Figure 3B shows that 125I-labeled sFcεR2 isolated from the 24-h culture supernatant of 125I-labeled RPMI 8866 cells is degraded after treatment with trypsin. Taken together these data show that the cell-associated FcεR2 fragment is protected from trypsin digestion, which strongly suggests that it is located intracellularly.

Evaluation of membrane and extracellular sources for intracellular FcεR2 fragment. In addition to the mechanism described above, the generation of the 28-kDa intracellular FcεR2 fragment may result from 1) endocytosis of cell surface FcεR2, followed by intracellular cleavage; and/or 2) uptake of shed sFcεR2.

To determine whether cell surface FcεR2 was constitutively endocytosed and cleaved to the 28-kDa fragment intracellularly, RPMI 8866 cells were surface-iodinated on ice, and then incubated at 37°C in culture medium to allow membrane receptor endocytosis. At this point, cell viability assessed by trypan blue exclusion was 90%. The cells were subsequently treated with trypsin, lysed, and the cell lysates assessed for the presence of labeled receptor or its 28-kDa cleaved fragment that were protected from trypsin treatment. Figure 4A shows that at time 0, the 125I surface-labeled 45-kDa FcεR2 band which was immunoprecipitated by mAb 25 completely disappeared after trypsin treatment. Figure 4B shows that at time 0 the 125I surface-labeled 45-kDa FcεR2 band which was immunoprecipitated by mAb 25 completely disappeared after trypsin treatment of the cells. After 37°C incubation of surface-iodinated cells for as long as 120 min (to allow internalization of the protein) before trypsin treatment, no 45-kDa FcεR2 or 28-kDa FcεR2 fragment was detectable.

These results indicated that the incubation at 37°C did not protect surface-labeled FcεR2 from trypsin digestion, and therefore suggest that FcεR2 was not constitutively endocytosed. Furthermore, neither ligand binding by IgE nor by FcεR2-specific mAb 25 (10 μg/ml, 37°C for 2 h) induced any detectable endocytosis of the FcεR2 (data not
Trypsin the generation of the 28-kDa polypeptide. Figure 5 shows cells. After detected in the corresponding cell supernatants. Taken that the formation of intracellular FctR, fragment (Fig. 4A) shows that both the 45- and 28-kDa moieties are present in the control cells. After saponin treatment, the transmembrane 45-kDa FceR, is still associated with the cell lysate (lane B), whereas the 28-kDa intracellular FceR, fragment is completely released into the supernatant (lane C). Actin, an intracellular protein, is also shown to be readily released into the supernatant. These results suggest that the intracellular FceR, fragment is present in a soluble form.

Intracellular FceR, fragment is shed into cell supernatant. Inasmuch as the intracellular FceR, fragment was in a soluble form, we next determined whether the cells released intracellular FceR, fragment into the culture supernatant. Then 10-min 35S methionine pulse-labeled RPMI 8866 cells were chased for 4 h to “chase” all the labeled receptor to the plasma membrane, then trypsinized to remove all labeled FceR, from the cell surface. Figure 7 (left panel) shows that after trypsin treatment, no 45-kDa FceR, could be recovered from the cell lysates; only the 28-kDa FceR, fragment remained associated with the cell lysates. The cells were then recultured for 16 h and the supernatants of control and trypsin-treated cells were assessed for the presence of 28-kDa sFceR,. Figure 7 (right panel) shows that a labeled 28-kDa sFceR, fragment was present in the culture supernatants of both control and trypsin-treated cells. Inasmuch as trypsin-treated cells lacked labeled 45-kDa FceR, the data strongly suggest that intracellular labeled 28-kDa FceR, was secreted into the supernatant of these cells. Densitometric scanning of the 28-kDa band in 16-h supernatants of control and trypsin-treated cells revealed that approximately one-third of the sFceR, was derived from intracellularly generated FceR, fragment (data not shown).

**DISCUSSION**

It has been described previously that cell surface FceR, on B cells is cleaved spontaneously to release 28-kDa sFceR, (15, 16). This study demonstrates an intracellular pathway for the formation of the 28-kDa FceR, fragment. Our data suggest that the 28-kDa FceR, fragment can be formed intracellularly by cleavage of some of the newly synthesized FceR,. This product is eventually secreted into the medium to contribute to sFceR,. The possibility that sFceR, was taken up by the cells was assessed by incubating 10 x 10^6 unlabeled RPMI 8866 cells at 37°C for 3 h with 125I-labeled sFceR,. Culture supernatants (Sup.) were collected after overnight incubation of 125I surface-labeled RPMI 8866 and immunoprecipitated with mAb 25. The immunoprecipitates were then left untreated (−) or were digested with trypsin. 20 μg/ml 37°C, 30 min (+), then subjected to 12% SDS-PAGE analysis. No significant degradation was observed in control (−) cells, whereas digestion of the labeled sFceR, (Fig. 4A) shows that after trypsin treatment, no 45-kDa FceR, could be recovered from the cell lysates; only the 28-kDa FceR, fragment remained associated with the cell lysates. The cells were then recultured for 16 h and the supernatants of control and trypsin-treated cells were assessed for the presence of 28-kDa sFceR,. Figure 7 (right panel) shows that a labeled 28-kDa sFceR, fragment was present in the culture supernatants of both control and trypsin-treated cells. Inasmuch as trypsin-treated cells lacked labeled 45-kDa FceR, the data strongly suggest that intracellular labeled 28-kDa FceR, was secreted into the supernatant of these cells. Densitometric scanning of the 28-kDa band in 16-h supernatants of control and trypsin-treated cells revealed that approximately one-third of the sFceR, was derived from intracellularly generated FceR, fragment (data not shown).

**Mechanism of generation of intracellular FceR, fragment.** Acidic organelles were possible sites for FceR, cleavage. Cells were therefore treated with NH,Cl to assess whether neutralization of acidic organelles affected the generation of the 28-kDa polypeptide. Figure 5 shows that the formation of intracellular FceR, fragment (Fig. 5, arrow), and the processing of the 41kDa FceR, to mature 41- to 45-kDa protein was partially inhibited by NH,Cl treatment, whereas the synthesis of the FceR, precursor protein remained unaffected. The inhibition of formation of the 28-kDa intracellular fragment indicates that it may indeed be formed by intracellular cleavage of newly synthesized FceR, in an acidic compartment.

**Intracellular FceR, fragment is in soluble form.** We next investigated whether the intracellular FceR, fragment was in soluble form of membrane associated. RPMI 8866 cells were labeled with 35S methionine for 2 h and chased for 16 h, then permeabilized with 0.05% saponin for 15 min on ice to release soluble, i.e., non-membrane associated intracellular molecules. Fig. 6 (lane A) shows that both the 45- and 28-kDa moieties are present in the control cells. After saponin treatment, the transmembrane 45-kDa FceR, is still associated with the cell lysate (lane B), whereas the 28-kDa intracellular FceR, fragment is completely released into the supernatant (lane C). Actin, an intracellular protein, is also shown to be readily released into the supernatant. These results suggest that the intracellular FceR, fragment is present in a soluble form.

**Figure 3.** A. Trypsin treatment of 35S methionine labeled RPMI 8866 cells. After 1 h 35S methionine pulse label and 4-h chase, cells were trypsin (0.05%) treated for 60 min on ice. The cell lysates (Lys) of untreated (−) and trypsin-treated cells (+) were immunoprecipitated with mAb 25 and analyzed in a 12% SDS-PAGE under non-reducing conditions. B. Trypsinization of 125I labeled sFceR,. Culture supernatants (Sup.) were collected after overnight incubation of 125I surface-labeled RPMI 8866 and immunoprecipitated with mAb 25. The immunoprecipitates were then left untreated (−) or were digested with trypsin. 20 μg/ml 37°C, 30 min (+), then subjected to 12% SDS-PAGE analysis.
**Figure 4.** A. Assessment of endocytosis of the FcRα. RPMI 8866 cells were surface-labeled at 4°C, incubated at 37°C in culture medium for the time periods of 0 and 120 min and then trypsinized on ice. In all lanes cell lysates were immunoprecipitated with mAb 25 and analyzed on a 12% SDS-PAGE under non-reducing conditions. B. Assessment of cellular uptake of soluble FcRα. RPMI 8866 cells and CEM-6 cells were incubated with affinity-purified 125I-FcRα for 3 h at 37°C. Subsequently, the cell lysates and the culture supernatants were analyzed by SDS-PAGE.

**Figure 5.** Inhibition of the generation of intracellular FcRα fragment by NH₄Cl. RPMI 8866 cells were exposed to 50 mM NH₄Cl in culture medium 45 min before and 45 min during ³⁵S methionine pulse labeling. mAb 25 immunoprecipitates of cell lysates of NH₄Cl untreated (−) and treated (+) cells were analyzed on a 12% SDS-PAGE.

**Figure 6.** Permeabilization of ³⁵S methionine-labeled RPMI 8866 cells. RPMI 8866 cells that were ³⁵S methionine pulse labeled for 2 h and chased 16 h, were treated with 0.05% saponin in PBS for 15 min on ice. The untreated cells (lane A), saponin-treated cells (lane B), and supernatant containing saponin (lane C), were mAb 25 immunoprecipitated and analyzed on a 12% SDS-PAGE under non-reducing conditions.

Observations indicated that this fragment was generated intracellularly. De novo synthesis of the 28-kDa fragment seemed unlikely because FcRα B cells analyzed by Northern blot contain only a single band of mRNA coding for the receptor [27]. Thus, the data strongly suggest that at
least part of the intracellular sFceR$_2$ fragment is derived from the cleavage of FceR$_2$ before surface expression. In this regard, Keegan and Conrad (28) have observed that sFceR$_2$ is released by tunicamycin-treated murine B cells in the absence of detectable surface expression of FceR$_2$. This observation is compatible with intracellular generation of FceR$_2$ fragment from newly synthesized FceR$_2$. In addition to newly synthesized FceR$_2$, potential sources of intracellular FceR$_2$ fragment include recycling membrane FceR$_2$ and uptake of sFceR$_2$ from the culture medium. We could find no evidence for uptake of sFceR$_2$ or evidence for recycling using a trypsin protection assay (Fig. 4). However, FceR$_2$ is reported to share sequence similarities with the asialoglycoprotein receptor (26) which is a receptor that recycles rapidly between endosomes and the plasma membrane (29). Thus, it is possible that a very rapidly recycling membrane FceR$_2$ could have been missed in our assay in which case intracellular cleavage of recycling surface FceR$_2$ may play an important role in the generation of soluble FceR$_2$.

The generation of the intracellular FceR$_2$ fragment was inhibited by NH$_4$Cl (Fig. 5). Weak bases such as NH$_4$Cl and chloroquine raise intravesicular pH (30) and result in the disruption of endosome, lysosome, and trans-Golgi function (31–33). Several secretory proteins have been shown to be products of intracellular proteolytic cleavage of precursor proteins. Maturational cleavage of proinsulin to insulin has been shown to be a post-Golgi event which occurs in acidic clathrin-coated secretory vesicles (34). The transmembrane polymeric IgR is also cleaved to form to the secretory component of polymeric Ig during the process of transcytosis (35).

The intracellular FceR$_2$ fragment was released completely from cells by treatment with saponin (Fig. 6) showing that it was not bound to intracellular membranes. In addition, we demonstrated that the intracellular FceR$_2$ fragment was eventually secreted into the culture supernatant. This was shown by the detection of labeled 28-kDa sFceR$_2$ in the culture supernatants of trypsin-treated $^{35}$S methionine-labeled RPMI 8866 cells which were chased to a point when all labeled parent FceR$_2$ had reached the cell surface (Fig. 7, left panel). Because trypsin treatment removed all labeled 45-kDa FceR$_2$ from the cells, the 28-kDa sFceR$_2$ detected in the culture supernatant of the trypsin-treated cells could only be derived from the intracellularly cleaved receptor (Fig. 7, right panel). It should be noted, however, that trypsin treatment decreased cell viability (from 90 to 75%). Thus, it is possible that after 16 h, the 28-kDa FceR$_2$ detected in the supernatants of trypsin-treated cells may be partially due to leakage from damaged cells.

Taken together, the results of this study identify intracellular cleavage of newly synthesized FceR$_2$ as a pathway for the generation of sFceR$_2$. The mechanisms that lead to intracellular cleavage of FceR$_2$ is still unclear. From the known amino-acid sequence of this receptor (26), the cleavage occurs at an arginine-methionine site, and therefore may result from the activity of a serine proteinase.

Interestingly, a family of arginine-specific serine proteinases is known to be involved in the processing of precursors of growth factors, e.g., β nerve growth factor and epidermal growth factor-binding protein (36). This may be of significance when considering the generation of the sFceR$_2$, because this soluble receptor has been reported to have autocrine growth-promoting activity (18). The precise proteolytic enzyme implicated in the processing of FceR$_2$, the location of this enzyme, and the mechanisms which may regulate its activity require further investigation.

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