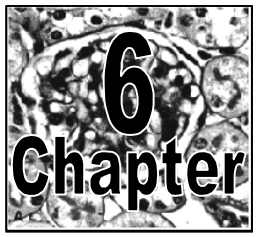

Crosslinking of the human Fc receptor for IgA (CD89) triggers FcR gamma-chain dependent shedding of soluble CD89 [#]

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SUMMARY

CD89/FcαRI is a 55-75 kD type I receptor glycoprotein, expressed on myeloid cells, with important immune effector functions. At present, no information is available on the existence of soluble forms of this receptor. We developed an ELISA for the detection of soluble CD89 forms (sCD89) and investigated the regulation of sCD89 production. PMA/ionomycin stimulation of monocytic cell lines (U937, THP-1 and MM6), but not of neutrophils, resulted in release of soluble CD89. Crosslinking of CD89, either via its ligand IgA or with anti-CD89 mAb's similarly resulted in sCD89 release. Using CD89 transfected cells, we showed ligand-induced shedding to be dependent on co-expression of the FcR γ-chain subunit. Shedding of sCD89 was dependent on signaling via the γ-chain and prevented by addition of inhibitors of protein kinase C (staurosporine) or protein tyrosine kinases (genistein). Western blotting revealed sCD89 to have an apparent molecular mass of 30 kD, and to bind IgA in a dose-dependent fashion. In conclusion, the present data document a ligand-binding soluble form of CD89 that is released upon activation of CD89-expressing cells. Shedding of CD89 may play a role in fine-tuning CD89 immune effector functions.

[#] ***Journal of Immunology 163: 5806-5812, 1999***

INTRODUCTION

Immunoglobulin A (IgA) plays a critical role in protecting the host against environmental pathogens and antigens encountered at mucosal surfaces. In humans IgA is the predominant isotype produced (±66mg/kg/day), with 80 % of all B cells committed to IgA production (1). Receptors for the Fc portion of IgA (FcαR) have been identified on a variety of cell types within the immune system and provide a crucial link between the humoral and cellular branches of the immune system *(2)*. Compared to the Fc receptors for IgE (FcεRI and FcεRII) and IgG (FcγRI, FcγRII and FcγRIII), relatively little is known about the nature and function of Fcα receptors. The best characterized human FcαR described until now, FcαRI/CD89, is a type I transmembrane glycoprotein that binds both IgA1 and IgA2 subclasses with similar affinity ($K_a \sim 10^6$ M^{-1})(3). Molecular cloning demonstrated CD89 to be a member of the Ig superfamily (4). The site of interaction between IgA and CD89 was identified on the junction of Cα2 and Cα3 of the IgA molecule (5), and in the membrane distal EC-1 domain of CD89 as shown by both mutagenesis (3) and domain swapping (6). Comparison of the primary amino acid sequence showed CD89 to be more closely related to killer cell inhibitory receptors (KIR) than to human FcγR (7).

CD89 is constitutively expressed as a 50-70 kD protein on neutrophils and monocytes/macrophages, or as a 70-100 kD glycoprotein on eosinophils due to increased glycosylation (2,8). The CD89 molecule is associated through a charge-based mechanism with the common FcR γ-chain, which connects CD89 to intracellular signaling pathways via ITAM signal motifs located within the cytoplasmic tail of the FcR γ-chain (9,10). Crosslinking of CD89 on myeloid cells can trigger diverse processes including phagocytosis, superoxide generation, ADCC and release of inflammatory mediators (2).

Several signals have been shown to modulate surface expression of CD89. Cytokines (TNF-α, GM-CSF, IL-1β, IL-8), LPS, PMA and aggregated IgA can induce increased CD89 expression on cells (8,11,12). In contrast, TGFβ (13) and suramin (14) were shown to downregulate its expression. Altered CD89 expression may directly affect the effector function of CD89-expressing cells. Soluble forms have been identified for various Fc receptors for IgG (FcγRII/CD32, and FcγRIII/CD16) and IgE (FcεRII/CD23) (15-17). Furthermore, it has been proposed that these soluble Fc receptors have a pathophysiological role in several diseases (18-20). No information is available on the existence of soluble forms of FcαR. In the present study we demonstrate the existence of a soluble CD89 protein (sCD89), a 30 kD glycosylated protein with retained ability to bind human IgA.

MATERIALS AND METHODS

Production of recombinant CD89 protein and anti-CD89 reagents

A recombinant soluble form of CD89 was produced by expressing the cDNA encoding the extracellular part of CD89 (21) in CHO-K1 cells using the pEE14 expression system (Celltech, Slough, UK). The stable CHO-K1 transfectant produced approximately 15 μg/ml of recombinant soluble CD89. Using columns of Immobilized IgA, more than 99% of the recombinant soluble protein was recovered from culture supernatants (22). The purity of the preparations was checked by SDS-page and a single band by Coomassie-brilliant-blue-staining was detected. The recombinant sCD89 protein was used to immunize mice, a rabbit and a goat. CD89-reactive rabbit (Rb) and goat (Gt) antisera were raised and used as purified IgG fractions. Using standard hybridoma technology we raised novel mouse mAb's specific for CD89. The specificity of these reagents was confirmed by FACS analysis on CD89-transfected cells and by immunoprecipitation and Western blotting (6,23).

ELISA for sCD89

Rabbit anti-CD89 IgG (2 μg/ml) was coated to ELISA plates (NUNC Maxisorb, Life Technologies, Gaithersburg, MD) by overnight incubation at room temperature in coating buffer (0.1M $NaHCO_3/Na_2CO_3$, pH9.6). The wells were washed 3 times using washing buffer (PBS, 0.02% Tween20) and subsequently varying concentrations of the recombinant sCD89 protein or BSA (as a control) were added. All samples were diluted in ELISA buffer (PBS, 0.02% Tween20, 1% FCS) and incubated for 1 hour at 37 °C. Following incubation, wells were washed as above and incubated first with digoxigenin (DIG)-conjugated rabbit $F(ab`)_2$ anti-CD89 (1$\mu$g/ml), followed by Horseradish peroxidase (HRP)-conjugated $F(ab`)_2$ anti-DIG (1/5000, Boehringer Mannheim; both for 1 hour at 37 °C and washed in between as above). OD415 was measured after addition of $ABTS/H_2O_2$ as substrate. The optical density at 415 nm was assessed using a Microplate Biokinetics Reader EL 312e (Bio-tek).

We also used a sandwich ELISA of monoclonal and polyclonal antibodies for the quantification of CD89 in supernatants of PMA/ionomycin activated cells and found similar values as measured in an ELISA with polyclonal antibody coating. However, since 4 out of 5 mAb's recognize the IgA binding site on CD89 (6), this hampers the study of IgA- or anti-CD89 induced shedding. Therefore, for consistency in our work, we have chosen to present all data from one type of ELISA were we used the polyclonal antibody as a coating.

Soluble CD16 was measured by ELISA (24).

Cell culture and activation

PMN's and monocytes were isolated from whole blood of healthy donors by Ficoll density centrifugation. The following CD89-expressing cell lines were used: U937 (ATCC nr CRL-1593.2)(25), THP-1 (ATCC nr TIB-202)(26) and MonoMac-6 (kindly provided by Dr. H.W.L. Ziegler-Heitbrock, Institut fur Immunologie, Universitat Munchen, Germany)(27). All cells were cultured at 37 °C with 5% CO2 in a humidified atmosphere in RPMI 1640 supplemented with 10% heat-inactivated FCS, 100 U/ml penicillin and 100 μg/ml streptomycin (all from Life Technologies). IIA1.6 cells were grown in the same medium supplemented with geneticin (G418, 0.8 mg/ml; Life Technologies) for CD89-transfected cells, or geneticin and methotrexate (MTX, 10 mM; Pharmachemie, Haarlem , The Netherlands) for cells co-transfected with CD89 and FcR γ-chain (10). Cell viability was greater than 95% for all cell preparations used.

For activation PMN's were cultured at a concentration of $1.0x10^7$ cells/ml (24). Monocytes and myeloid cell lines were activated at a concentration of $2.0x10^6$ cells/ml. All activation experiments were performed in triplicate. After the indicated times, cells were harvested and tested by FACS analysis or supernatants were harvested and tested by ELISA. The following stimuli were used: PMA (10 ng/ml), Ionomycin (1 μg/ml) and LPS (*Salmonella thyphosa*, 100 ng/ml) (all from Sigma, St Louis, MO). In addition various purified IgA preparations isolated from normal human serum, and sera from myeloma patients and anti-CD89 monoclonal antibodies and goat anti-mouse-Ig antibodies were used (all prepared in our laboratory) (6,22,23,28).

For inhibition of γ-chain-induced signal transduction, we have used inhibitors of protein kinase C (staurosporine; 50 ng/ml) or protein tyrosine kinases (genistein; 100μM)(both from Sigma). These concentrations were non-toxic for the cells as determined by trypan-blue exclusion.

FACS analysis

For FACS analysis, cells ($5x10^5$) were incubated with the CD89 mAb 2D11 (IgG1) or an isotype matched control, diluted in FACS buffer (PBS/0.5% BSA/0.02% Azide). Following incubation for 1 hour at 4 °C, cells were washed with FACS buffer and incubated for 1 hour with PE-conjugated goat anti-mouse IgG1 polyclonal IgG (Southern Biotechnology, Birmingham, AL). After washing, cells were fixed with 1% paraformaldehyde in PBS and analyzed on a FACScan

(Becton Dickinson, San Jose, CA). Data acquisition and analysis were conducted with Lysis II (Becton Dickinson).

Immuno-precipitation and Western blotting

As a positive control for intact CD89, U937 cells ($1x10^8$) were lysed, using PBS/ 1% NP40. Cell lysates and culture supernatants were separated on 10% SDS polyacrylamide gels under reducing conditions, and blotted onto PVDF membrane (Millipore, Bedford, MA). Using standard Western blotting protocols, different forms of CD89 were detected with a mixture of Rb- and Gt IgG anti-CD89 (both 10 μg/ml). After incubation and washing, followed by subsequent incubation with HRP-conjugated Swine anti-Rabbit-IgG (1/50,000; Dako, Denmark) and HRP-conjugated Rabbit anti-Goat-IgG (1/50,000; Dako). Signals were visualized using Super Signal Chemiluminescence substrate, according to manufacturers' instructions (PIERCE, Rockford, IL).

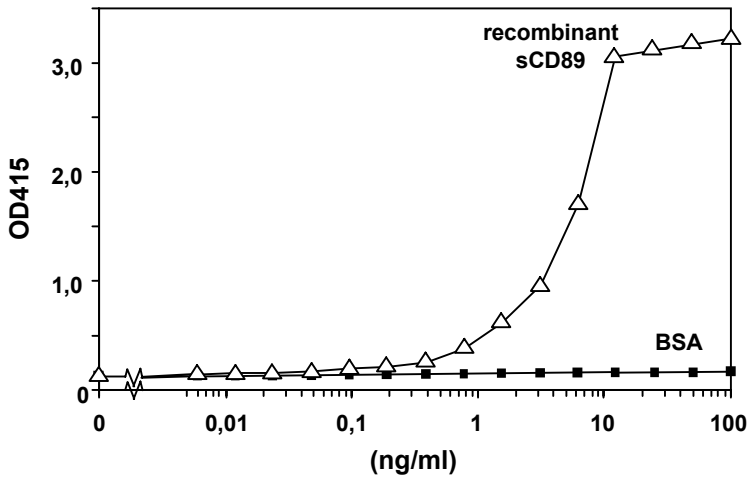

Figure 1. Detection of soluble CD89 protein by ELISA. Rabbit anti-CD89 IgG (2μg/ml) was coated to ELISA plates and samples, containing different concentrations of either recombinant soluble CD89 (open triangles) or BSA (closed squares), were added and after incubation wells were washed and bound CD89 detected with digoxigenin (DIG)-conjugated rabbit F(ab`)$_2$ anti-CD89 (1$\mu$g/ml), followed by Horseradish peroxidase (HRP)-conjugated F(ab`)$_2$ anti-DIG. OD415 was measured after addition of ABTS/H_2O_2 as substrate. The detection limit of soluble CD89 ELISA was reproducibly found to be 50 pg/ml (extinction background +2x standard deviation in 6 independent experiments).

Isolation of sCD89 and IgA binding ELISA

The sCD89 protein was isolated from culture supernatant of PMA/ionomycin stimulated U937 cells using an affinity column of human IgA isolated from normal serum (22). Preparations of purified IgA from normal human serum (2μg/ml) were coated to an ELISA plate and binding of sCD89 was detected using DIG-conjugated Rb F(ab`)$_2$ anti-CD89, similar to the CD89 ELISA described above.

RESULTS

Establishment of an ELISA for sCD89

To study the presence of a soluble form of CD89, we developed a CD89-specific ELISA, using rabbit polyclonal antibodies. As a positive control a recombinant sCD89 protein produced in CHO cells was employed.

Increasing concentrations of recombinant sCD89 resulted in a dose-dependent signal in this ELISA. The detection limit of this ELISA was reproducibly found to be ~ 50 pg/ml (Figure 1).

Monocytic cells release sCD89 upon activation

We investigated the release of soluble CD89 in supernatants of activated human cells. First, the release of CD89 was studied in the pro-monocytic cell line U937, a cell type expressing high levels of CD89. Stimulation of U937 with PMA and ionomycin consistently induced release of a soluble form of CD89 (Figure 2A).

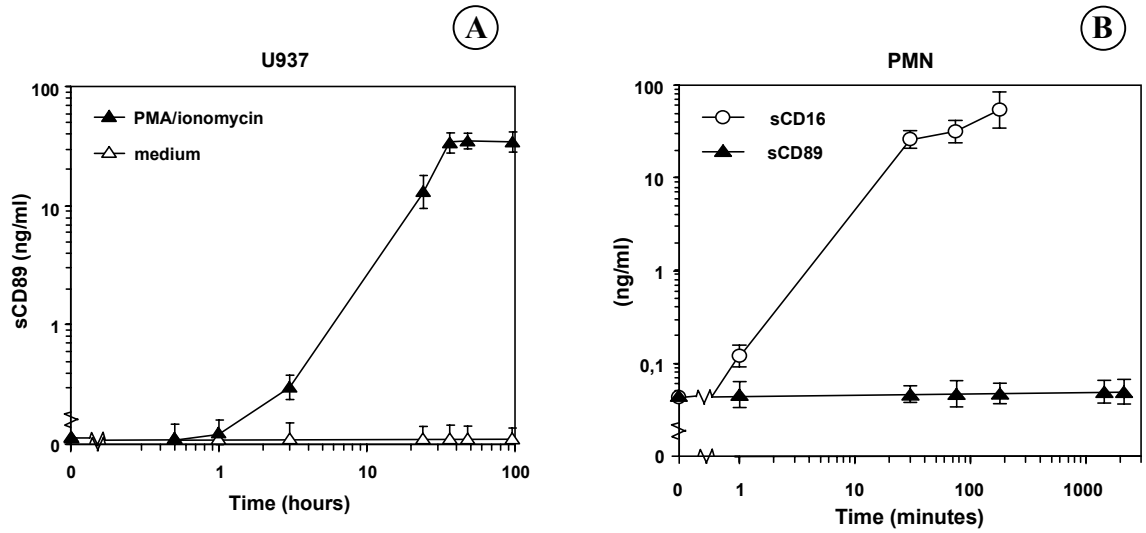

Figure 2. Detection of sCD89 in supernatants of PMA/ionomycin-stimulated cells. A. U937 cells were cultured in medium alone (open triangles) or medium supplemented with PMA/ionomycin (closed triangles). At indicated time points supernatants were tested for sCD89. Results are expressed as the mean ± SEM of 3 independent experiments. **B.** Freshly isolated PMN were cultured in medium with PMA/ionomycin. At the indicated time points supernatants were harvested and tested for sCD89 (closed triangles) and sCD16 (open circles). Results are expressed as a mean ± SEM of 3 independent experiments, using cells from 3 different donors

Soluble CD89 was first detectable after 3 hours, and reached a maximum at 36 hours. In 5 independent experiments the amount of sCD89 detected ranged from 25-43 ng/ml. When the same number of U937 cells were cultured in the absence of PMA/ionomycin, no sCD89 could be detected (Figure 2A). Next PMN, a cell that expresses similar amounts of CD89 to U937, were stimulated with PMA and ionomycin. Irrespective of the time points tested, varying from 1 minute to 36 hours, soluble CD89 could not be detected in the supernatants (Figure 2B). As a positive control for the activation conditions, the same supernatants were tested for the release of CD16. In accordance with the literature (16,24), after 1 minute soluble CD16 was detectable in supernatants of activated PMN, and this production further increased in time (Figure 2B).

Regulation of CD89 surface expression

Since modulation of surface expression might contribute to the release of CD89, we investigated the effect of PMA/ionomycin on CD89 membrane expression as detected with monoclonal antibodies. FACS analysis showed that PMA/ionomycin induced a 3-10 fold (range of 4 independent experiments) increase in CD89 surface expression on U937 cells compared with cells cultured in medium alone (Figure 3).

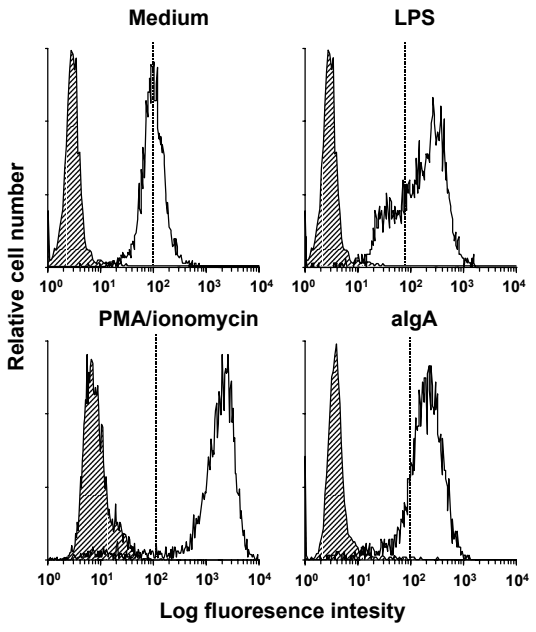

Figure 3. FACS analysis of CD89 surface expression. U937 cells were cultured in medium alone or in medium supplemented with PMA/ionomycin, LPS or heat aggregated IgA. After a 36-hour culture, CD89 surface expression was assessed with CD89 mAb 2D11 (1/1,000) followed by PE-conjugated goat anti-mouse-IgG1. Hatched histograms represent conjugate controls; open histograms represent CD89 expression. Out of 3 independent experiments one histogram is shown per condition. The dotted line represents the median fluorescence for CD89 expression of cells cultured in medium alone.

This upregulation was not unique for PMA/ionomycin and could also be observed with other stimuli, which have previously been found to affect CD89 expression (12). Both LPS and heat-aggregated human IgA (aIgA) enhanced CD89 surface expression on U937 cells.

Crosslinking of CD89 induces release of a soluble form

To verify whether the release of sCD89 is correlated with upregulated surface expression, supernatants of stimulated U937 were tested. Next to PMA/ionomycin, also high molecular weight forms of both IgA1 and IgA2 (polymeric or heat aggregated) consistently induced release of sCD89 from U937 cells (Figure 4A). In contrast, little activation was observed with preparations containing monomeric IgA. No sCD89 was detected after LPS stimulation of U937 (Figure 4A). We then tested FcαRI-crosslinking using different concentrations of the anti-CD89 mAb 2D11 and found a dose-dependent induction of sCD89 release (Figure 4B). An isotype matched control antibody did not induce the release of CD89, whereas crosslinking 2D11 using goat anti-mouse IgG antibodies increased CD89 shedding.

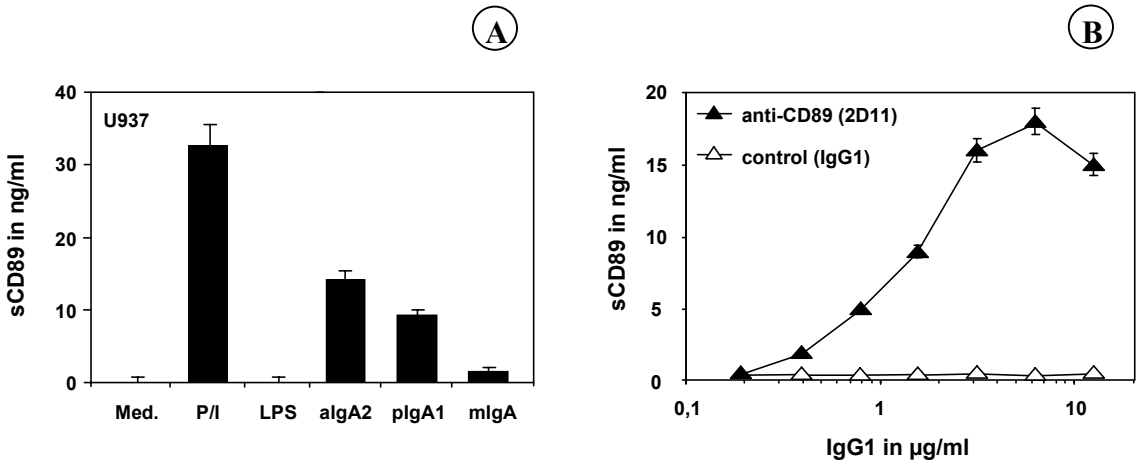

Figure 4. CD89-specific crosslinking triggers sCD89 release from U937. A. U937 cells were cultured in medium alone or in medium supplemented with PMA/ionomycin, LPS or IgA preparations consisting of heat aggregated IgA2 (aIgA2) from a patient with an IgA2 myeloma, polymeric IgA1 (pIgA1) from a patient with an IgA1 myeloma or monomeric serum IgA (mIgA) as indicated. After 36 hours of culture, supernatants were collected and tested for sCD89. Results are expressed as the mean ± SEM of 3 independent experiments. **B.** U937 cells were cultured in medium supplemented with different concentrations of the anti-CD89 mAb 2D11 (closed triangles) or an isotype matched control (open triangles). After 36 hours supernatants were collected and tested for sCD89. Results are expressed as the mean ± SEM of 3 independent experiments.

Similar activation conditions were applied to freshly isolated peripheral blood monocytes and two other myeloid cell lines, MonoMac-6 and THP-1. Activation with PMA/ionomycin or IgA stimulated an increased surface expression on all three cell types (data not shown). In addition, both PMA/ionomycin and aIgA, as well as anti-CD89 antibodies, induced the release of soluble CD89 (Figure 5).

The FcR γ-chain is essential for CD89-triggered release of sCD89

To study the mechanism of CD89-triggered release of sCD89 in more detail, we used murine IIA1.6 cells transfected with human CD89 alone, or with human CD89 in combination with the FcR γ-chain subunit. Both transfectants were previously shown to have a comparable CD89 expression and both cell lines displayed a similar IgA binding (29). Activation of CD89/γ-chain transfected cells with increasing amounts of heat aggregated human IgA (aIgA) triggered a dose-dependent release of sCD89. No sCD89 could be detected in supernatants of aIgA-stimulated cells transfected with CD89 alone (Figure 6A).

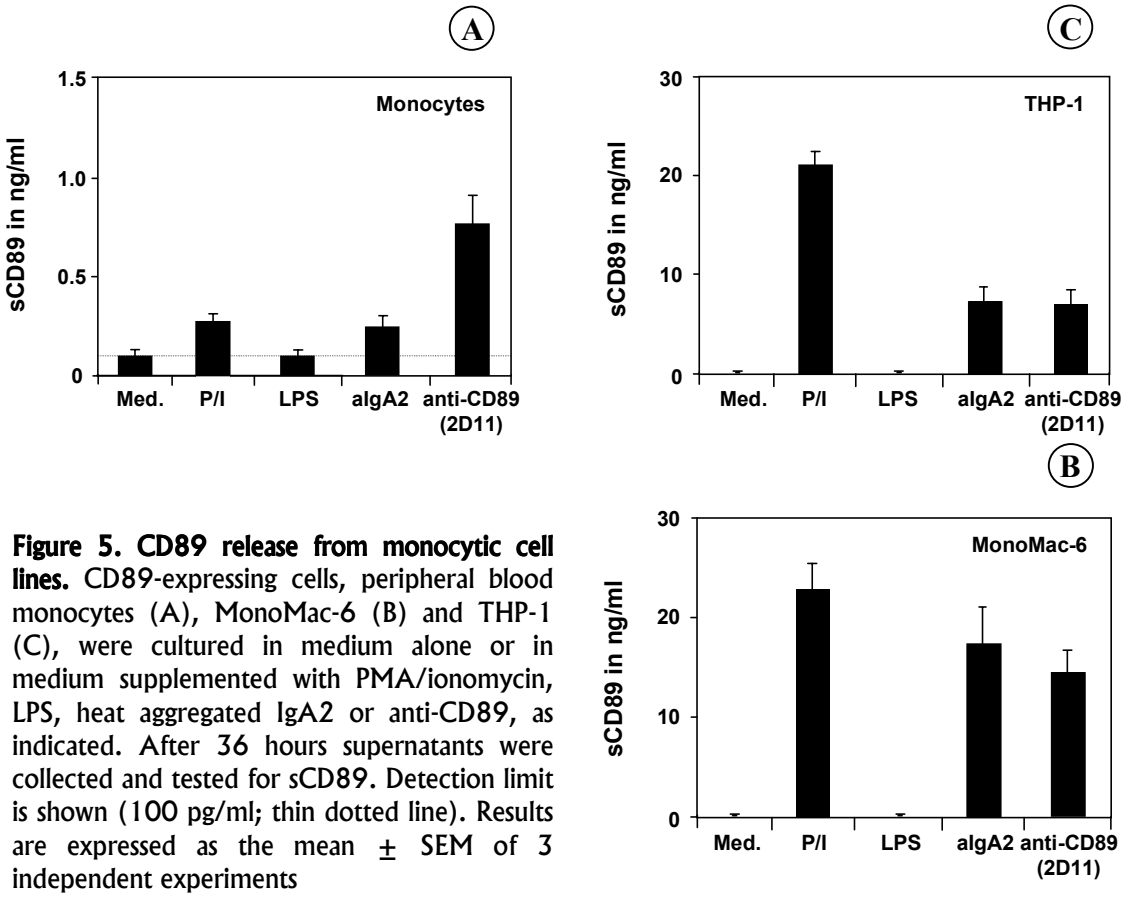

Figure 5. CD89 release from monocytic cell lines. CD89-expressing cells, peripheral blood monocytes (A), MonoMac-6 (B) and THP-1 (C), were cultured in medium alone or in medium supplemented with PMA/ionomycin, LPS, heat aggregated IgA2 or anti-CD89, as indicated. After 36 hours supernatants were collected and tested for sCD89. Detection limit is shown (100 pg/ml; thin dotted line). Results are expressed as the mean ± SEM of 3 independent experiments

Similarly, anti-CD89 antibodies induced release of sCD89 only in cells co-expressing the γ-chain (Figure 6B). PMA/ionomycin stimulation led to release of sCD89 in both cell types (Figure 6B).

To investigate whether signaling via the γ-chain is important for the release of sCD89, two specific inhibitors were used. Addition of either an inhibitor of protein kinase C (staurosporine) or an inhibitor of protein tyrosine kinases (genistein) prevented the shedding of sCD89 from the surface of aIgA-stimulated IIA1.6 CD89/γ chain transfectants or U937 cells (Figure 7).

Biochemical characterization of soluble CD89

To further analyze the nature of soluble CD89, Western blotting was performed on cell lysates and supernatants of myeloid cells. Utilizing rabbit and goat polyclonal anti-CD89 antibodies, a broad band ranging from 55-75 kD was detected on U937 cell lysates (Figure 8A, lane 2).

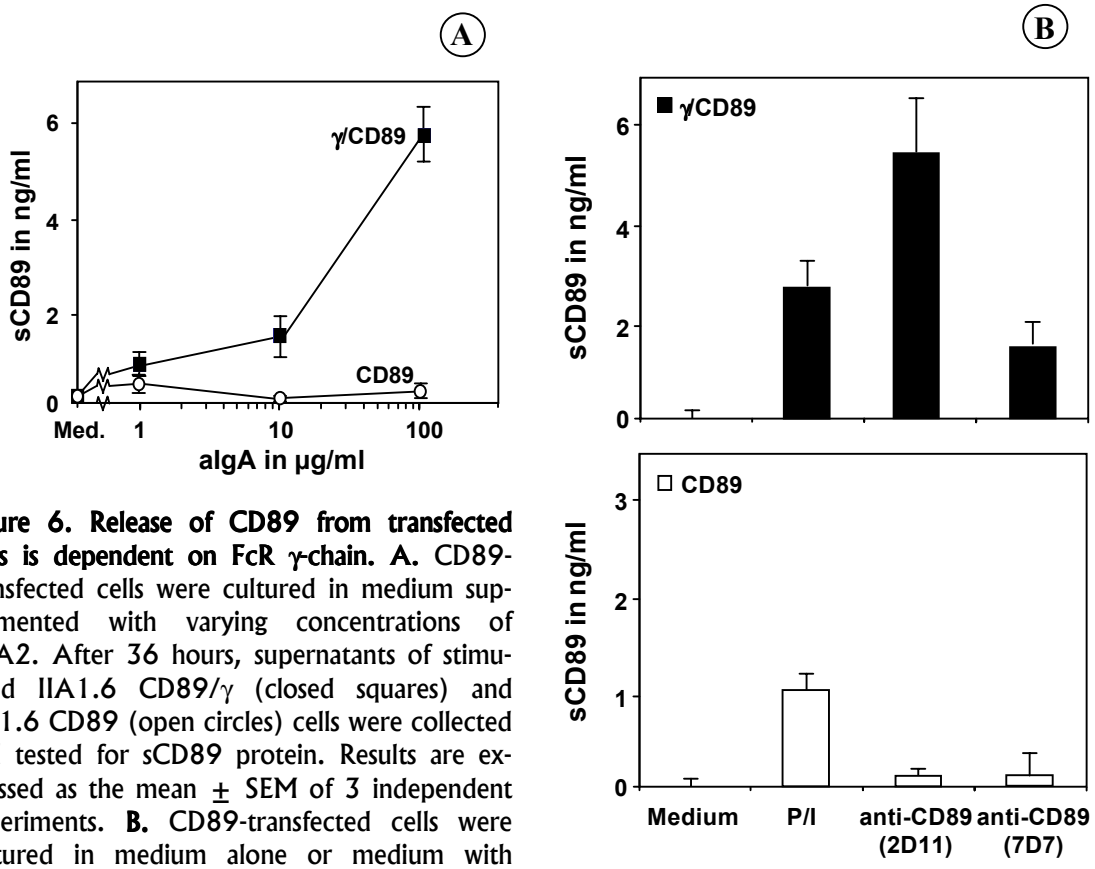

Figure 6. Release of CD89 from transfected cells is dependent on FcR γ-chain. A. CD89-transfected cells were cultured in medium supplemented with varying concentrations of algA2. After 36 hours, supernatants of stimulated IIA1.6 CD89/γ (closed squares) and IIA1.6 CD89 (open circles) cells were collected and tested for sCD89 protein. Results are expressed as the mean ± SEM of 3 independent experiments. **B.** CD89-transfected cells were cultured in medium alone or medium with PMA/ionomycin, or anti-CD89 mAb's 2D11 and 7D7. After 36 hours supernatants of the stimulated IIA1.6 CD89/γ (closed bars) and IIA1.6 CD89 (open bars) cells were collected and tested for sCD89 protein. Results are expressed as a mean ± SEM of 3 independent experiments.

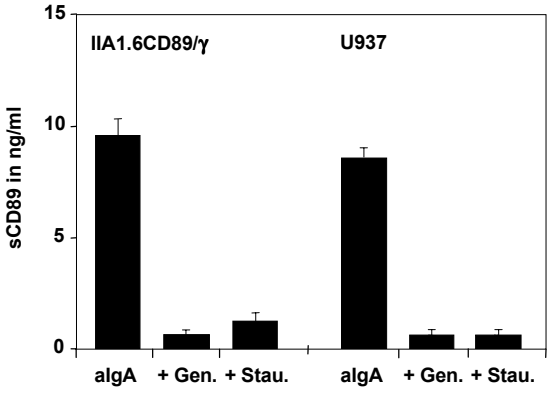

Figure 7. Shedding of sCD89 is prevented by genistein and staurosporine. Both the CD89/γ transfectants (left) and U937 cells (right) were cultured stimulated with algA2 in the absence or presence of an inhibitor of protein tyrosine kinases (gen. = genistein; 100µM) or an inhibitor of protein kinase C (stau. = staurosporine; 50 ng/ml). After 36 hours supernatants were collected and tested for sCD89 protein. Of three independent experiments, one representative example is shown.

When analyzing the supernatants of U937 cells, a specific product of approximately 30 kD was found after PMA/ionomycin stimulation, which was not observed under non-stimulated conditions (lanes 3,4). Reactivity against the 30 kD protein was completely blocked by preincubating the antisera with recombinant sCD89 (lane 5). A similar 30 kD protein was found in the supernatant of PMA/ionomycin stimulated THP-1 cells (lanes 6,7) and in supernatant of aIgA stimulated CD89/γ transfected IIA1.6 cells (lanes 8,9).

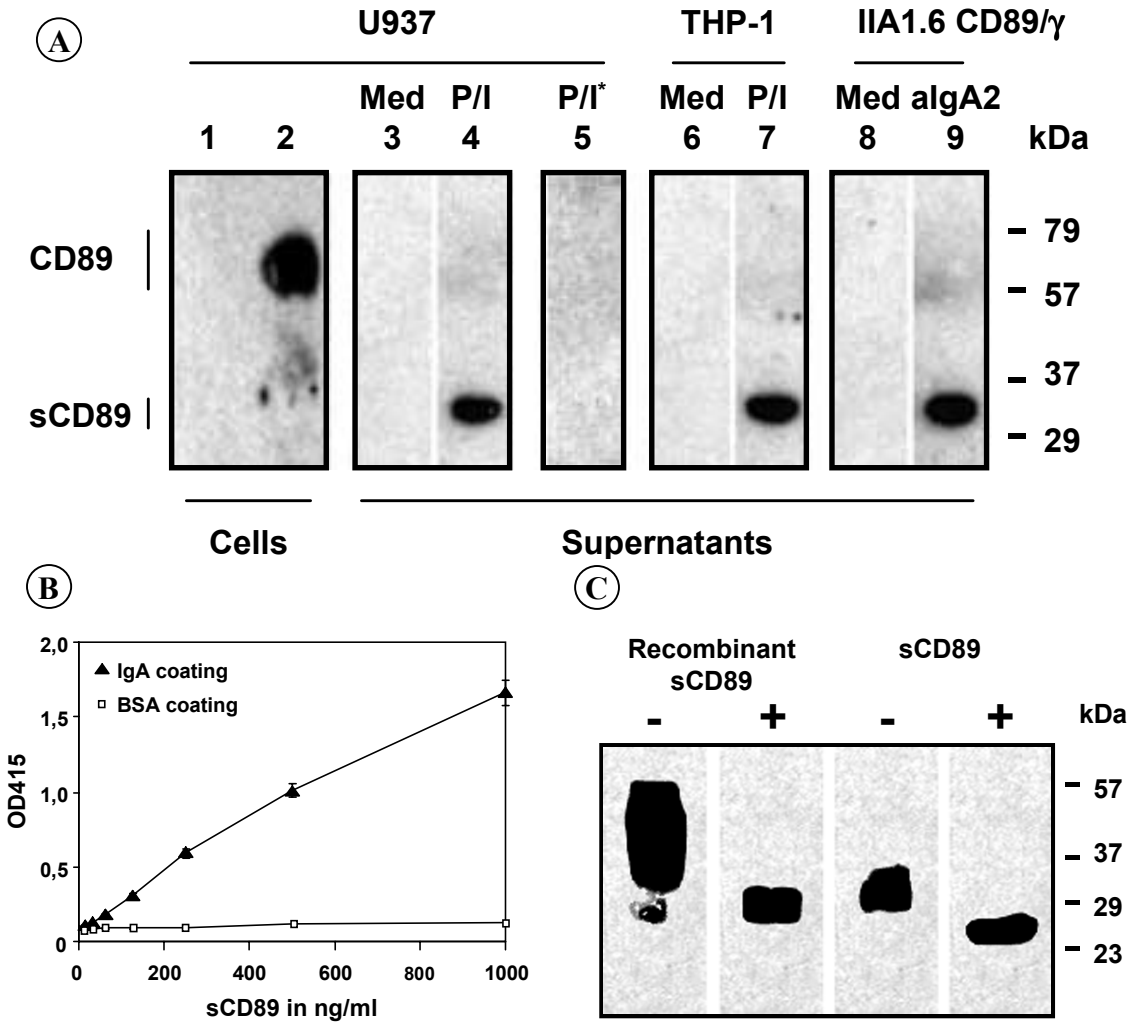

Figure 8. Immuno-chemical analysis of sCD89 protein. A. Cell lysates from U937 cells were blotted with normal rabbit and goat sera as a control (lane 1), or CD89 specific antisera (lane 2). Supernatants of U937 and THP-1 cells cultured in medium alone or stimulated for 36 hours with PMA/ionomycin (as indicated) were blotted with CD89 specific sera (resp. lane 3, 4 and 6, 7). As a specificity control (lane 5), rabbit and goat polyclonal anti-CD89 antibodies, were preincubated with 25 μg recombinant soluble CD89 and used for blotting of supernatant from PMA/ionomycin stimulated U937 (P/I*). Similar as for U937 and THP-1 supernatants of IIA1.6 CD89/γ cultured in medium alone or stimulated with aIgA2 were blotted (resp. lane 8, 9). **B.** Purified sCD89 was tested at the indicated concentrations for binding to IgA (coated 2 μg/ml, closed triangles) or BSA (coated 2 μg/ml, open squares)(as a specificity control). Results are expressed as the mean ± SEM of 3 independent experiments. **C.** Recombinant soluble CD89 and purified sCD89 were treated with N-Glycosidase F for removal of N-linked sugars. Deglycosylated or untreated samples, as indicated, were separated on 10 % SDS-PAGE and blotted specific for CD89.

In order to obtain more detailed information on the structure of sCD89, the protein was purified from supernatant of PMA/ionomycin activated U937 cells. Soluble CD89 could be purified using an IgA-affinity absorbent, which showed the natural form of sCD89 to be capable of binding IgA. This was confirmed in an ELISA system, with IgA coated to ELISA wells, where a dose-dependent binding of sCD89 to IgA was observed (Figure 8B). When the purified sCD89 was analyzed by Western blotting, we observed a 30 kD molecule. Treatment with N-glycanase reduced the MW to 25 kD. For comparison, the recombinant soluble form (37-55 kD) was treated in the same way, and predictably showed a 27 kD backbone after removal of the N-linked glycans (Figure 8C) (21).

DISCUSSION

In the present study we demonstrate that upon activation, myeloid cells can release a soluble form of the FcαRI/CD89. Biochemical analysis showed this soluble receptor to represent a 30 kD glycosylated protein, which is capable of binding IgA. Both aIgA and anti-CD89 antibodies induced the release of sCD89, which suggests that IgA antibodies produced during a mucosal immune response might have a regulatory effect on the CD89 effector functions.

In our experiments we concentrated on the myeloid cell line U937, although similar effects were found with other monocytic cell lines (THP-1 and MonoMac-6). Activation of PMN with PMA/ionomycin results in a strong and fast release of sCD16, but does not result in release of sCD89, suggesting that regulation of receptor shedding is different between CD16/FcγRIII and CD89/FcαRI. Comparing peripheral blood monocytes and myeloid cell lines, we found that the regulation of sCD89 shedding was qualitatively similar. However in peripheral blood monocytes, we detected lower amounts of sCD89 in our ELISA. This might be partially explained by a low-level expression of CD89 on monocytes, or by differences in regulation between these myeloid cell lines and monocytes. We showed that the release of sCD89 is dependent on an active signaling event, which might be quantitatively different in cell lines. It was demonstrated that release of CD89 after CD89 crosslinking is dependent on the presence of the common γ-chain, which is also associated with FcεRI, FcγRIIIa/CD16 and FcγRIIa/CD32 (2) (Figure 6A). Signaling via this subunit, induces PKC activation (9), as well as tyrosine phosphorylation of the γ-chain by members of the Src family (*Lyn*, *Syk*), PI-3 kinase activation and Bruton tyrosine kinase (Btk) activation (30,31). In accordance, we were able to block the release of sCD89 by inhibition of protein kinase C or protein tyrosine kinases.

In contrast to sCD16 shedding (16), release of sCD89 was rather slow, suggesting the involvement of secondary processes. Induction of sCD89 release was accompanied by upregulation of surface expression, although increased surface expression not always resulted in sCD89 shedding. Previous experiments have shown that the IIA1.6 transfectants we have used in our experiments have a comparable CD89 expression and display a similar IgA binding (32). Therefore, the presence of the γ-chain seems to have no effect on the affinity for IgA. These findings are different from data published for FcγR (33) and require further investigation.

The molecular weight of 30 kD of CD89 rules out the possibility that the products measured in ELISA are released membrane vesicles containing full length CD89. Recently, at least 11 different splice variants of CD89 have been identified (34-38). It seems unlikely that they are responsible for the sCD89 molecule, as most of them showed partial or complete deletions of EC1 or EC2, while still containing the predicted transmembrane region. Finally, we found that IIA1.6 cells transfected with full length CD89 cDNA, excluding alternative splicing, also release a similar 30 kD molecule upon activation (Figure 7A, lane 8). These data suggest a role for proteolytic cleavage, as demonstrated for various molecules, including cytokines (TNF-α), cytokine receptors, adhesion molecules and Fc receptors (18,24,39).

The cleavage site of CD16/FcγRIII was identified between Val196 and Ser197 after C-terminal sequencing large amounts of sCD16 purified from human serum (40). In preliminary experiments we found that both EDTA and 1,10 phenantriolin, which are inhibitors of metalloproteinases, prevented the release of sCD89 (data not shown), suggesting the involvement of metalloproteinases in cleavage of CD89. The difference in core size between recombinant sCD89 and sCD89 cleaved from U937 shows that the cleavage site is N-terminal from Tyr^{207}, the C-terminal amino acid of the recombinant product.

An important question concerns the (patho-) physiological role of soluble CD89. Release of soluble receptors has been suggested to represent a universal mechanism of receptor regulation, which might be dysregulated in various human diseases (18). Shedding of CD89 will uncouple the receptor from its signaling transduction pathways and therefore represents a means of effector function downregulation. Quantification of CD89 in cell lysates of U937 as compared to their supernatants suggested that upto 5% of the receptor might appear in soluble form after stimulation with PMA/ionomycin (data not shown). It is likely that soluble CD89 might immediately interact with circulating IgA and influences the function of IgA. We have obtained preliminary evidence that sCD89 is present in

the circulation. It is possible that IgA-CD89 complexes might have "nephritogenic" activities as has been suggested recently (41).

Levels of sCD16 (FcγRIII) have been proposed to be a measure for the number of neutrophils (16). Our *in vitro* data suggest that PMN do not release CD89 and that monocytes might be the most important source of soluble CD89. Therefore sCD89 levels might represent a measure for monocyte numbers and/or activation. Recently, monocytes (but not neutrophils) of patients with primary IgA nephropathy, were found to display a marked reduction of surface CD89 expression that correlated with the increased levels of serum IgA (42). At present it is unclear whether the negative regulation of monocytic CD89 expression might be associated with an increased release of soluble CD89.

In conclusion, in the present study we have shown that the myeloid FcαRI/CD89 can be released as a 30 kD soluble molecule. The release of sCD89, which can bind IgA, is induced upon activation of myeloid cells. This may provide a mode of "fine-tuning" effector functions of CD89 expressing cells. In recent years the CD89 molecule has evolved as a candidate target for bispecific antibody therapy (43,44). It will be important to unravel the mechanisms of CD89 shedding, not only to potentially improve the efficacy of therapy, but also to monitor immune activation.

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