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SHORT COMMUNICATION

The metalloprotease ADAM8 is associated with and regulates the function of the adhesion receptor PSGL-1 through ERM proteins

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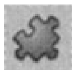
The P-selectin glycoprotein ligand-1 (PSGL-1) is involved in the initial contact of leukocytes with activated endothelium, and its adhesive function is regulated through its proteolytic processing. We have found that the metalloprotease ADAM8 is both associated with PSGL-1 through the ezrin–radixin–moesin actin-binding proteins and able to cause the proteolytic cleavage of this adhesion receptor. Accordingly, ADAM8 knockdown increases PSGL-1 expression, and functional assays show that ADAM8 is able to reduce leukocyte rolling on P-selectin and hence on activated endothelial cells. We conclude that ADAM8 modulates the expression and function of PSGL-1.

Key words: ADAM8 · Adhesion · Cell migration · ERM · Neutrophil · PSGL-1



See accompanying Commentary by Zarbock and Rossaint

Introduction

ADAM family is a group of transmembrane glycoproteins that possess proteolytic and signaling properties and are implicated in both cell adhesion and cell fusion processes [1]. ADAMs are usually activated by furin or other convertases as well as autocatalytically, in the case of ADAM8 [2]. It has been described that ADAM8 cleaves important cell surface proteins [3, 4], cytokines and growth factors [5]. ADAM8 is overexpressed under several pathological conditions involving inflammation and remodeling of the extracellular matrix, including malignant diseases and asthma [6–8].

P-selectin glycoprotein ligand-1 (PSGL-1), through its interaction with P-, E- and L-selectins, mediates the tethering and rolling of leukocytes on endothelial cells prior to their extravasation [9, 10], triggers the activation of transcription factors like cFos [11] in leukocytes and induces the generation of tolerogenic DCs which promote the differentiation of Treg cells [12]. Although it was described that PSGL-1 was a substrate of the proteases BACE1 and ADAM10 [13], neither the physiological context of cleavage nor the mechanism responsible for its shedding have been identified so far. In this work, we demonstrate that in leukocytes

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ADAM8 associates with PSGL-1 through ezrin–radixin–moesin (ERM) proteins, and that this interaction modulates the expression and function of this adhesion receptor.

Results and discussion

Association of PSGL-1 with ADAM8

To identify intracellular molecules able to associate with PSGL-1, we performed a proteomic analysis of HL-60 cell lysates pulled down with the cytoplasmic tail of PSGL-1 fused to GST (GST-PSGL-1cyt) [11]. This analysis revealed the presence of a 98-kDa protein that corresponded to ADAM8 (data not shown). Additional pull-down experiments performed with fragments of the cytoplasmic tail of PSGL-1 fused to GST, detected an additional protein of 75–80 kDa, which likely corresponded to a cleaved form of ADAM8 (Fig. 1A, left panel). To map the region of PSGL-1 involved in this interaction, pull-down experiments from lysates of HL-60 cells were performed with different fragments of the cytoplasmic tail of PSGL-1 fused to GST. We found that the 18 juxtamembrane amino acids of PSGL-1 were sufficient to mediate its interaction with ADAM8 (Fig. 1A, right panel).

We next assessed whether endogenous ADAM8 and PSGL-1 were associated. ADAM8 was first immunoprecipitated from HL-60 cell lysates, and WB analysis of the immunoprecipitated molecules showed that PSGL-1 was present in the ADAM8 immunoprecipitate (Fig. 1B). Likewise, reciprocal analysis revealed the presence of ADAM8 in the PSGL-1 immunoprecipitate (Fig. 1C), thus confirming the association of these two endogenous molecules in the HL-60 cell line. To assess whether the PSGL-1/ADAM8 association also occurred in primary myeloid cells, we performed experiments using human neutrophils. PSGL-1 was found in the ADAM8 immunoprecipitate from resting neutrophils (Fig. 1D, left panel) and the PSGL-1/ADAM8 association increased after neutrophil activation with PMA (Fig. 1D, right panel), indicating that PSGL-1 and ADAM8 were also associated in neutrophils.

ERM proteins link PSGL-1 and ADAM8

Since we had observed the presence of a cluster of basic residues in the juxtamembrane cytoplasmic tail of ADAM8 (Fig. 1E, upper panel underlined residues) that has been reported to act as binding sites for ERM proteins [11], we performed immunoprecipitation (IP)/WB experiments to assess whether ADAM8 could also associate with the ERMs and found the presence of ERM proteins in the ADAM8 immunoprecipitates (Fig. 1E, lower panel). Additional assays showed that in ERM-knocked down cells PSGL-1 failed to associate with ADAM8 (Fig. 1F). Therefore, these results strongly suggest that ERM proteins link PSGL-1 and ADAM8. In the activated state, the ERM molecules can interact with other ERM molecules. Our data suggest that two or more molecules of ezrin or moesin, that are interacting with each other, could also be interacting with different membrane receptors, acting as connectors between membrane proteins, as it could be the case for ADAM8 and PSGL-1. This interaction would allow their localization at specific protrusive membrane regions, favoring their binding to ligands [14] and facilitating the shedding of adhesion receptors during the rolling phase.

Proteolytic cleavage of PSGL-1 by ADAM8

The reports that PSGL-1 can be processed by a protease [15, 16] and that PSGL-1 could be a candidate substrate for ADAM8 [17], in addition to our finding of PSGL-1/ADAM8 association, prompted us to investigate the potential involvement of ADAM8 in the cleavage of PSGL-1. Experiments with neutrophils incubated with activated sADAM8 showed a significant diminution in the membrane expression of PSGL-1 (Fig. 2A, left panel), while the expression of CD11a and CD44 did not change (Fig. 2A, right panel). Accordingly, when PSGL-1 and ADAM8 were co-expressed by J77 T cells, a significant reduction of PSGL-1 expression was detected (Fig. 2B). In contrast, a mutated form of ADAM8, which lacks catalytic activity [3], did not affect the amount of PSGL-1 in the cell surface. Accordingly, higher amount of soluble PSGL-1 (sPSGL-1) was detected in the supernatant of the cells transfected with active ADAM8, whereas transfection with the inactive form of ADAM8 did not change the amount of sPSGL-1 released by cells transfected with the empty plasmid (Fig. 2C). Furthermore, the silencing of ADAM8 in HL-60 cells with siRNA increased the amount of PSGL-1 and L-selectin on these cells (Fig. 2D). To further demonstrate that ADAM8 is able to cleave PSGL-1, we incubated human recombinant PSGL-1 with thermolysin-activated sADAM8 and found that PSGL-1 is processed by ADAM8 in a cell-free system (Fig. 2E). Altogether, these data demonstrate not only that ADAM8 is associated with PSGL-1 but also that ADAM8 is able to proteolytically modulate the expression of this adhesion receptor.

ADAM8 can regulate PSGL-1 rolling function

We next determined the functional consequences of PSGL-1 processing by ADAM8, by testing the effect of sADAM8 on the rolling of CEM cells on P-selectin and on the rolling of THP1 on HUVEC. As expected, the pre-incubation with activated sADAM8 significantly reduced the ability of the CEM cells to roll on P-selectin (Fig. 3A) and the ability of THP1 cells to roll on TNF-α-HUVEC activated (Fig. 3B). Moreover, the number of rolling HL-60 cells on histamine-activated HUVEC increased two-fold upon knockdown of ADAM8 expression (Fig. 3C). This result strongly indicates that ADAM8 controls the function of this adhesion receptor.

It has been reported that ADAM8 is involved in the pathogenesis of inflammatory diseases such as experimental autoimmune arthritis or airway hypersensitivity reactions [6, 7, 18]. Our previous work showed that ADAM8 promoted the shedding of

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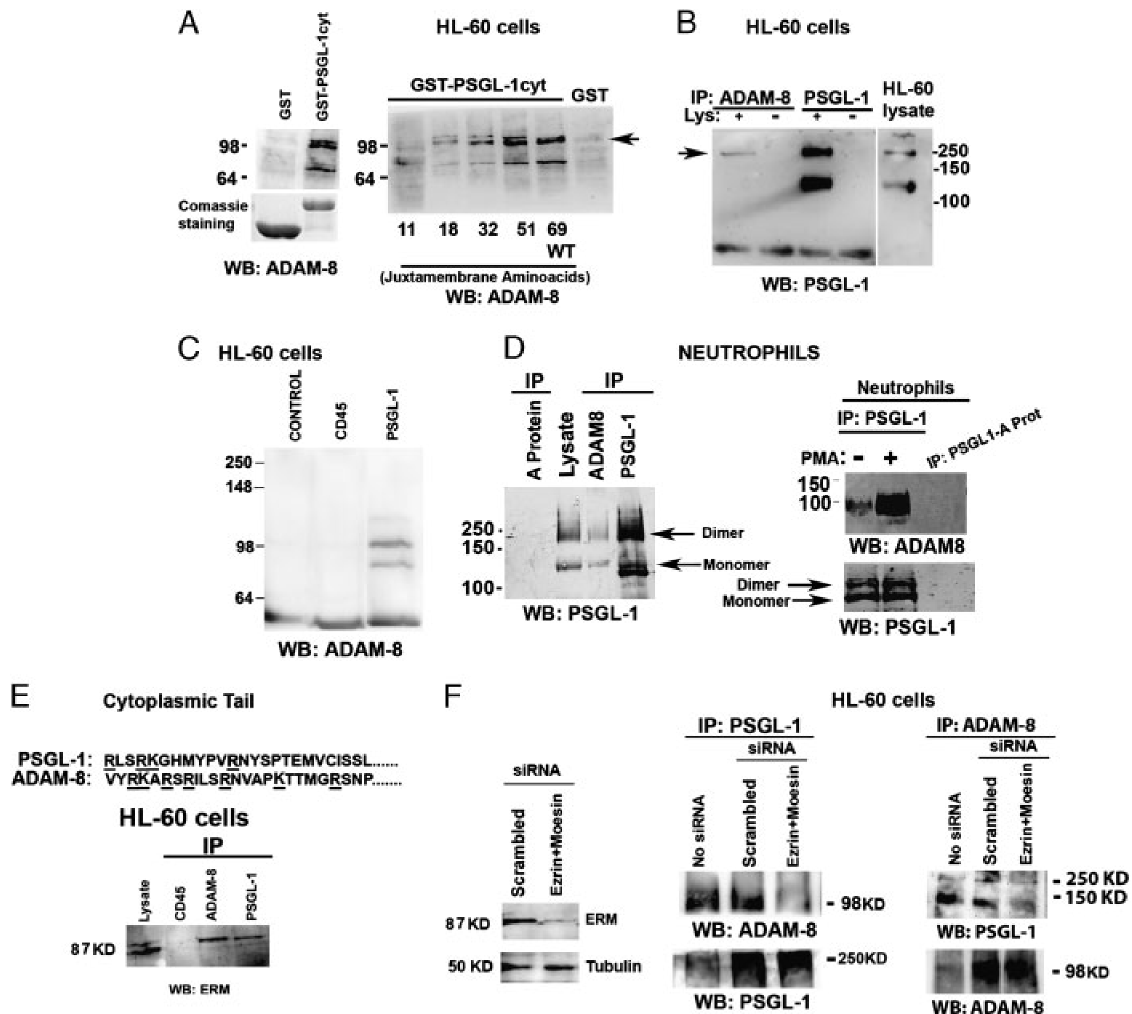

Figure 1. Association of PSGL-1 with ADAM8. (A) Left panel: lysates from HL-60 cells were incubated with GST protein and GST fused to the cytoplasmic tail of PSGL-1 (GST-PSGL-1cyt). Right panel: HL-60 cell lysates were incubated with GST and GST fused with constructs of PSGL-1 cytoplasmic tail containing 11, 18, 32, 51 or 69 (full-length cytoplasmic tail) amino acids of the juxtamembrane domain. Proteins pulled down were analyzed for the presence of ADAM8 by western blot (WB). (B) PSGL-1 WB in the ADAM8 and PSGL-1 immunoprecipitates from HL-60 lysates. (Arrow in (A) indicates the 98-kDa band corresponding to ADAM8 full length and in (B) indicates the 250-kDa band corresponding to the dimer form of PSGL-1.) (C) ADAM8 WB in the control, CD45 or PSGL-1 immunoprecipitates from HL-60 lysate. (D) Left panel: PSGL-1 WB in the ADAM8 and PSGL-1 immunoprecipitates from neutrophil lysates. Right panel: ADAM8 and PSGL-1 WB from PSGL-1 immunoprecipitates from resting or PMA-activated neutrophils. (E) Upper panel: juxtamembrane cytoplasmic sequences of PSGL-1 and ADAM8. The basic residues are underlined. Lower panel: ezrin and moesin WB in the immunoprecipitates of ADAM8 from HL-60 lysates. Immunoprecipitates of CD45 and PSGL-1 were included as negative and positive controls, respectively. (F) Ezrin and moesin RNA were silenced in HL-60 cells. In the left panel, the expression of ezrin plus moesin (ERMs) was analyzed by WB in the lysates of ezrin+moesin-silenced HL-60 cells. In the middle panel, the presence of ADAM8 and PSGL-1 was analyzed by WB in the PSGL-1 immunoprecipitates. In the right panel, the presence of PSGL-1 and ADAM8 was analyzed by WB in the ADAM8 immunoprecipitate. The results shown are representative of three independent experiments.

L-selectin [4]. In this report, we describe a role of ADAM8 in the shedding of PSGL-1 and its effect on leukocyte rolling on P-selectin and on activated endothelial cells. PSGL-1 mediates the initial contact of leukocytes with the activated endothelium through P-selectin. In addition, L-selectin mediates their secondary rolling on the extravasating leukocytes. Therefore, it is tempting to postulate that during the inflammatory phenomenon ADAM8 could modulate the extravasation of leukocytes through the shedding of both L-selectin and PSGL-1. This effect on leukocyte extravasation could account for the role of ADAM8 in the pathogenesis of inflammatory diseases. Hence, we propose that ADAM8 could be a potential therapeutic target in inflammatory conditions through the modulation of its expression and/or function.

Concluding remarks

In this work, we show that PSGL-1 and ADAM8 associate in leukocytes and that the ERMs act as connector proteins. We propose that the activation triggered during leukocyte contact with the activated endothelium would activate the proteolytic activity of ADAM8 which then would be able to process both PSGL-1 and

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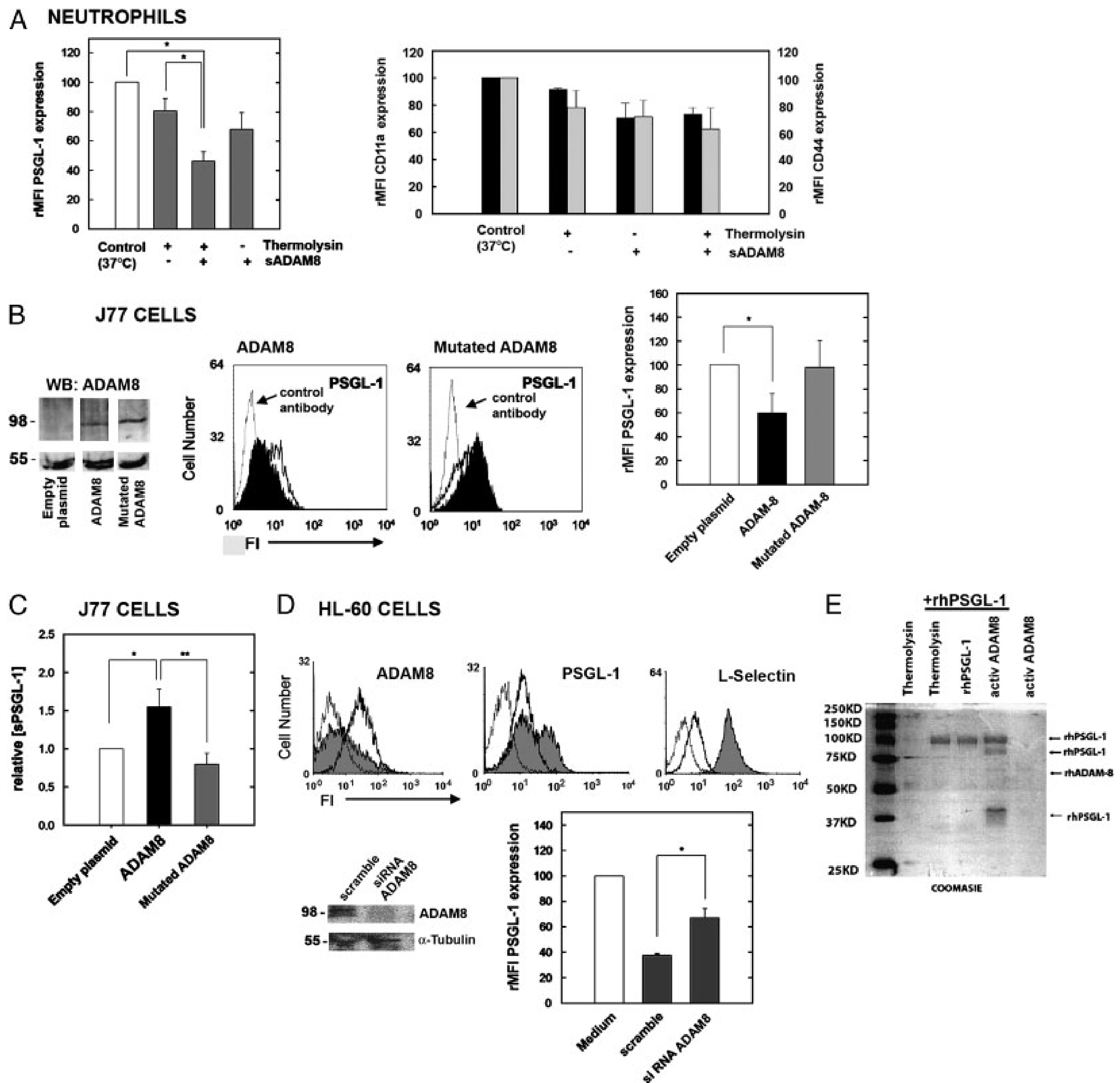

Figure 2. Proteolytic cleavage of PSGL-1 by ADAM8. (A) Human neutrophils were untreated or incubated for 20 min with non-active or thermolysin-activated sADAM8. The expression of PSGL-1 (left panel) and CD11a and CD44 (right panel, black and gray bars, respectively) was analyzed by flow cytometry in control and treated cells. Data are presented as mean+SE of rMFI (MFI relative to MFI of cells maintained in medium alone considered 100%) from five independent experiments. *$p<0.05$ by Wilcoxon signed rank test. (B) J77 cells were nucleofected with full-length or mutated ADAM8. Left panel: Western blot analysis of ADAM8 and α-tubulin expression in cell lysates from transfected cells. Middle panels: flow cytometric analysis of PSGL-1 surface expression in J77 24 h after transfection with both cDNAs: Shaded histogram: PSGL-1 expression in ADAM8 transfected cells; unshaded histogram with dark line: basal surface expression in cells transfected with the empty plasmid; dotted histograms (light line): negative control antibody (PX63). One representative experiment of five is shown. Right panel: surface expression of PSGL-1 in transfected J77 cells. Data are presented as mean+SE rMFI (MFI relative to MFI of cells maintained in medium alone considered 100%) from four independent experiments. *$p<0.05$ by Wilcoxon signed rank test. (C) Release of PSGL-1 by J77 transfected cells. Soluble PSGL-1 was measured by ELISA in the cell-free supernatants collected 24 h after cell transfection with either ADAM8 WT or mutated ADAM8 cDNAs. Data are expressed as mean+SD of PSGL-1 concentration (relative to the concentration in the supernatant of cells transfected with the empty vector) from seven independent experiments. *$p<0.05$ by Wilcoxon signed rank test and **$p<0.01$. (D) HL-60 cells were nucleofected with ADAM8 or scrambled siRNAs. Lower left panel: Western blot analysis of ADAM8 and α-tubulin expression in cell lysates 24 h after transfection from one representative experiment. Upper panels: flow cytometry analysis of ADAM8, PSGL-1 and L-selectin surface expression in cells transfected with ADAM8 siRNA (shaded histograms) or scrambled siRNA (unshaded histogram, dark line) 24 h after transfection. Dotted histograms (light line) represent the negative control antibody (PX63). One representative experiment of five is shown. Lower right panel: surface PSGL-1 expression in ADAM8-silenced HL-60 cells. Basal surface expression of PSGL-1 in HL-60 cells maintained in medium alone (medium) was considered 100%. Data are presented as mean+SE rMFI of four independent experiments. *$p<0.05$ by Wilcoxon signed rank test. (E) ADAM8 cleaves PSGL-1 in a cell-free system. Human recombinant PSGL-1 was incubated with human recombinant ADAM8 activated with thermolysin and analyzed by Coomassie staining.

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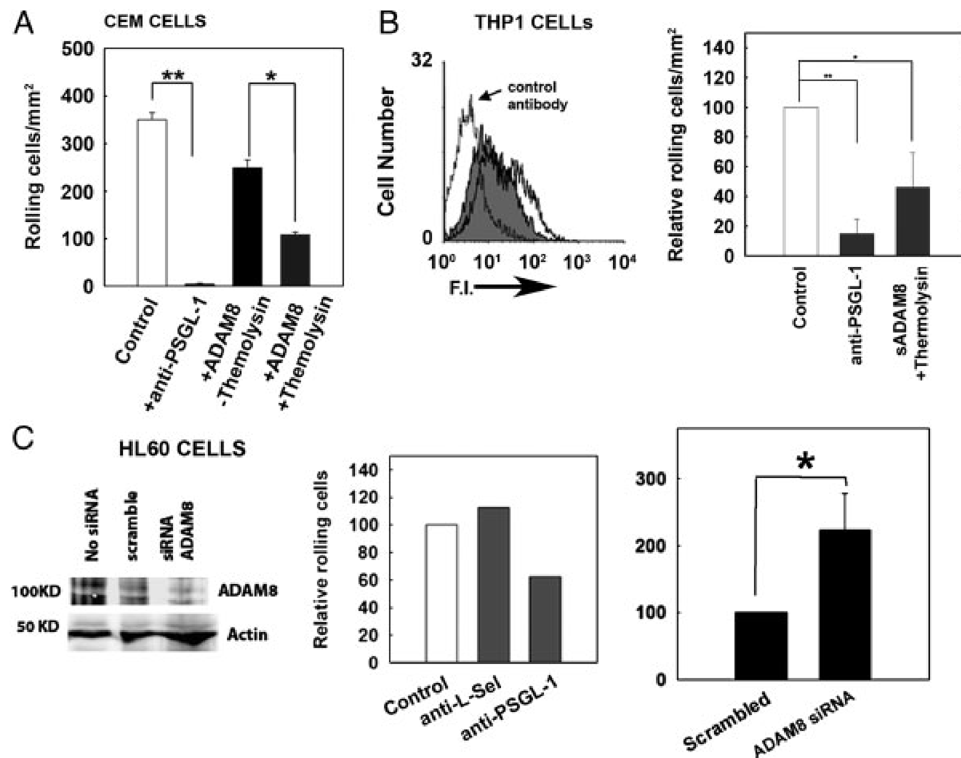

Figure 3. Pretreatment with sADAM8 reduces leukocyte rolling. (A) CEM cells were pre-incubated with non-active sADAM8, thermolysin-activated sADAM8, anti-PSGL-1 blocking mAb KPL-1 or in medium alone, and their rolling on recombinant P-selectin-coated surfaces at 2 dynes/cm^2 over a 9 min time span was determined as described in the *Materials and Methods*. (B) Flow cytometry histogram of PSGL-1 surface expression in THP1 cell incubated for 2 h at 37°C in the presence of thermolysin (unshaded histogram) or sADAM8+thermolysisn (shaded histogram). Dotted histogram represents the fluorescence of the negative control antibody (PX63). Graph showing the rolling of THP1 cells on TNF-activated HUVEC, at 2 dynes/cm^2. THP1 cells were pre-incubated with thermolysin alone (control), themolysin-activated sADAM8 or with anti-PSGL-1 blocking mAb KPL-1. (A) and (B) represent the mean+SE of three independent experiments. *$p<0.05$ and **$p<0.01$ by Wilcoxon signed-rank test. (C) WB of HL-60 cells transfected with scramble or ADAM8 siRNA showing the ADAM8 expression. Graph showing the rolling of HL-60 cells on histamine-activated HUVEC at 0.75 dynes/cm^2. HL-60 cells were previously incubated with KPL1 or anti-L-selectin antibodies or transfected with scramble or ADAM8 siRNAs (a representative experiment is shown). (Error bar indicates the mean of three independent experiments). Data are presented as the absolute cell number of cells rolling per mm^2 per min (A) or the relative number of cells with respect to controls which were considered 100% (B and C).

L-selectin, and hence influence the leukocyte infiltration during inflammation. We also propose that the observed consequences of a deregulated ADAM8 in inflammatory diseases, as allergic asthma, would be explained through its association with PSGL-1, which probably regulates the expression and activation of this adhesion receptor during leukocyte extravasation.

Materials and methods

Antibodies, reagents, plasmids and cells

ADAM8 mAb (clone 143338), human recombinant ADAM8 ectodomain, human recombinant P-selectin and human recombinant chimeric Fc-PSGL-1 were from R&D Systems (Minneapolis, MN, USA). Rabbit anti-human ADAM8 polyclonal antibody (clone H-50) and ADAM8 siRNA were from Santa Cruz Biotechnology (CA, USA). Anti-PSGL-1 KPL-1 mAbs were from BD Pharmingen (San José, CA, USA). The rabbit anti-ezrin 90/3 polyclonal antibody was generously provided by Dr. Heinz Furthmayr. The mAbs CD44 and CD11a were generated in our group. The expression vector pcDNA3 containing full-length ADAM8 and its catalytically inactive form (H604A, H608A) have been previously described [3]. GST-PSGL-1 constructs were described elsewhere [11]. Ezrin and moesin siRNA primers: EZRIN sense: 5′-UCCACUAUGUGGAUAAUAA-3′, ezrin antisense: 5′-UUAUUAUCCACAUAGUGGA-3′; moesin sense: 5′-AGAUC GAGGAACAGACUAA-3′, moesin antisense: 5′-UUAGUCUGUUC CUCGAUC-3′ were purchased from Sigma-Aldrich Chemical (St. Louis, MO, USA). sPSGL-1 7 ELISA kit was from Bender Medsystem (Vienna, Austria).

Human cell lines and HUVEC were cultured as described elsewhere. Human neutrophils were isolated and cultured as described [4].

Immunoprecipitation, western blot and pull down of proteins with GST-fused proteins

HL-60 and neutrophils, resting and activated with PMA (15 min at 37°C), were processed for immunoprecipitation

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and western blot as described elsewhere. Pull down of proteins from HL-60 cell extracts was performed as previously described [11].

Transfection assays

All nucleofection assays were performed following Amaxa nucleofector instructions. J77 cells were nucleofected with 5 μg of either full-length or mutated pcDNA3-ADAM8 with Amaxa nucleofector. For RNA silencing, HL-60 cells (2×10^6 cells) were nucleofected with 1.6 μg of ADAM8 siRNA, ERM siRNA [19] or scramble siRNA. ADAM8 and ezrin/moesin expression was checked by immunoprecipitation and WB. PSGL-1 expression was analyzed by ELISA, WB and flow cytometry 24 h after transfection with ADAM-8 siRNA and 72 h after transfection with ERM siRNA.

Soluble ADAM8 proteolytic activity

Human neutrophils suspended in HBSS were pre-incubated with either thermolysin (0.75 μg/mL), recombinant non-activated sADAM8 or thermolysin-activated sADAM8 (20 min at 37°C). Surface expression of PSGL-1, CD11a and CD44 was analyzed by flow cytometry. ELISA assay was performed following manufacturer' instructions. PSGL-1-Fc chimera was incubated for 2 h 30 min a 37°C with thermolysin-activated ADAM-8 (1:1 proportion) and analyzed by Coomassie staining.

Rolling experiments

Plates were incubated overnight at 4°C with 10 μg/mL human rP-selectin and blocked for 1 h at 37°C with 2% BSA. HUVEC were seeded in plates previously incubated with 20 μg/mL fibronectin (Sigma-Aldrich Chemical) for 1 h at 37°C, grown to confluence and stimulated for 6 h with 20 ng/mL TNF-α or for 10 min with 100 μM histamine. THP1 and CEM cells were pre-incubated 2 h at 37°C with 0.75 μg/mL thermolysin, 2.8 mg of non-activated ADAM8 or thermolysin-activated (30 min at 37°C) ADAM8 and then incubated for 10 min at 4°C in the presence or absence of 50 μg/mL of anti-PSGL-1 Ab. HL-60 was previously nucleofected with siRNA ADAM-8 and incubated for 24 h at 37°C. Treated cells were injected at 2 (CEM and THP1 cells) and 0.75 (HL60 cells) dynes/cm^2, in the flow chamber (Glycotech, MD, USA) and assayed for rolling experiment as described elsewhere.

Statistical analysis

Results were expressed as arithmetic mean ± standard deviation (SD) or standard error (SE) of the mean. Wilcoxon signed rank test was used to determine significant differences between means.

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Conflict of interest: The authors declare no financial or commercial conflict of interest.

References

1 **White, J. M.,** ADAMs: modulators of cell–cell and cell–matrix interactions. *Curr. Opin. Cell Biol.* 2003. **15**: 598–606.

2 **Schlomann, U., Wildeboer, D., Webster, A., Antropova, O., Zeuschner, D., Knight, C. G., Docherty, A. J. et al.,** The metalloprotease disintegrin ADAM8. Processing by autocatalysis is required for proteolytic activity and cell adhesion. *J. Biol. Chem.* 2002. **277**: 48210–48219.

3 **Fourie, A. M., Coles, F., Moreno, V. and Karlsson, L.,** Catalytic activity of ADAM8, ADAM15, and MDC-L (ADAM28) on synthetic peptide substrates and in ectodomain cleavage of CD23. *J. Biol. Chem.* 2003. **278**: 30469–30477.

4 **Gómez-Gaviro, M., Domínguez-Luis, M., Canchado, J., Calafat, J., Janssen, H., Lara-Pezzi, E., Fourie, A. et al.,** Expression and regulation of the metalloproteinase ADAM-8 during human neutrophil pathophysiological activation and its catalytic activity on L-selectin shedding. *J. Immunol.* 2007. **178**: 8053–8063.

5 **Blobel, C.,** ADAMs: key components in EGFR signalling and development. *Nat. Rev. Mol. Cell. Biol.* 2005. **6**: 32–43.

6 **Koller, G., Schlomann, U., Golfi, P., Ferdous, T., Naus, S. and Bartsch, J. W.,** ADAM8/MS2/CD156, an emerging drug target in the treatment of inflammatory and invasive pathologies. *Curr. Pharm. Des.* 2009. **15**: 2272–2281.

7 **Matsuno, O., Kumamoto, T. and Higuchi, Y.,** ADAM8 in allergy. *Inflamm. Allergy Drug Targets* 2008. **7**: 108–112.

8 **Valkovskaya, N., Kayed, H., Felix, K., Hartmann, D., Giese, N., Osinsky, S., Friess, H. and Kleeff, J.,** ADAM8 expression is associated with increased invasiveness and reduced patient survival in pancreatic cancer. *J. Cell Mol. Med.* 2007. **11**: 1162–1174.

9 **Harakawa, N., Shigeta, A., Wato, M., Merrill-Skoloff, G., Furie, B., Furie, B., Okazaki, T. et al.,** P-selectin glycoprotein ligand-1 mediates L-selectin-independent leukocyte rolling in high endothelial venules of peripheral lymph nodes. *Int. Immunol.* 2007. **19**: 321–329.

10 **Sperandio, M., Smith, M., Forlow, S., Olson, T., Xia, L., McEver, R. and Ley, K.,** P-selectin glycoprotein ligand-1 mediates L-selectin-dependent leukocyte rolling in venules. *J. Exp. Med.* 2003. **197**: 1355–1363.

11 **Urzainqui, A., Serrador, J. M., Viedma, F., Yanez-Mo, M., Rodriguez, A., Corbi, A. L., Alonso-Lebrero, J. L. et al.,** ITAM-based interaction of ERM proteins with Syk mediates signaling by the leukocyte adhesion receptor PSGL-1. *Immunity* 2002. **17**: 401–412.

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12 **Urzainqui, A., Martínez del Hoyo, G., Lamana, A., de la Fuente, H., Barreiro, O., Olazabal, I., Martin, P. et al.,** Functional role of P-selectin glycoprotein ligand 1/P-selectin interaction in the generation of tolerogenic dendritic cells. *J. Immunol.* 2007. **179**: 7457–7465.

13 **Lichtenthaler, S., Dominguez, D., Westmeyer, G., Reiss, K., Haass, C., Saftig, P., De Strooper, B. and Seed, B.,** The cell adhesion protein P-selectin glycoprotein ligand-1 is a substrate for the aspartyl protease BACE1. *J. Biol. Chem.* 2003. **278**: 48713–48719.

14 **Ivetic, A. and Ridley, A.,** Ezrin/radixin/moesin proteins and Rho GTPase signalling in leucocytes. *Immunology* 2004. **112**: 165–176.

15 **Davenpeck, K. L., Brummet, M. E., Hudson, S. A., Mayer, R. J. and Bochner, B. S.,** Activation of human leukocytes reduces surface P-selectin glycoprotein ligand-1 (PSGL-1, CD162) and adhesion to P-selectin in vitro. *J. Immunol.* 2000. **165**: 2764–2772.

16 **Stampfuss, J.-J., Censarek, P., Fischer, J. W., Kaber, G., Rauch, B. H., Freidel, K., Fischer, U. et al.,** Complete downmodulation of P-selectin glycoprotein ligand in monocytes undergoing apoptosis. *Arterioscler. Thromb. Vasc. Biol.* 2008. **28**: 1375–1378.

17 **Naus, S., Reipschläger, S., Wildeboer, D., Lichtenthaler, S., Mitterreite, S., Guan, Z., Moss, M. and Bartsch, J.,** Identification of candidate substrates for ectodomain shedding by the metalloprotease-disintegrin ADAM8. *Biol. Chem.* 2006. **387**: 337–346.

18 **Zack, M. D., Melton, M. A., Stock, J. L., Storer, C. E., Barve, R. A., Minnerly, J. C., Weiss, D. J. et al.,** Reduced incidence and severity of experimental autoimmune arthritis in mice expressing catalytically inactive A disintegrin and metalloproteinase 8 (ADAM8). *Clin. Exp. Immunol.* 2009. **158**: 246–256.

19 **Barrero-Villar, M., Cabrero, J., Gordón-Alonso, M., Barroso-González, J., Alvarez-Losada, S., Muñoz-Fernández, M., Sánchez-Madrid, F. and Valenzuela-Fernández, A.,** Moesin is required for HIV-1-induced CD4-CXCR4 interaction, F-actin redistribution, membrane fusion and viral infection in lymphocytes. *J. Cell Sci.* 2009. **122**: 103–113.

Abbreviations: **ERM:** ezrin–radixin–moesin · **PSGL-1:** P-selectin glycoprotein ligand-1 · **sPSGL-1:** soluble PSGL-1 · **WB:** western blot

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