Species Specificity of ADAM10 and ADAM17 Proteins in Interleukin-6 (IL-6) Trans-signaling and Novel Role of ADAM10 in Inducible IL-6 Receptor Shedding*\[5\

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Christoph Garbers\[1\], Nathalie Jänner\[1\], Athena Chalaris\[1\], Marcia L. Moss\[5\], Doreen M. Floss\[5\], Dörte Meyer\[1\], Friedrich Koch-Nolte\[1\], Stefan Rose-John\[5\], and Jürgen Scheller\[5\]
From the\[4\] Institute of Biochemistry, Christian-Albrechts-University, Olshausenstrasse 40, Kiel, Germany, \[5\] BioZyme, Inc., Apex, North Carolina 27523, the\[4\] Institute of Biochemistry and Molecular Biology II, Medical Faculty, Heinrich-Heine-University, Universitätsstrasse 1, Düsseldorf, Germany, and the\[3\] Institute of Immunology, University Medical Center Hamburg-Eppendorf, Martinistrasse 52, Hamburg, Germany

Hypomorphic ADAM17ex/ex mice showed defects in mucosal regeneration due to inefficient enhanced GFR shedding. ADAM17 is the main sheddase of interleukin-6 receptor (IL-6R) to induce IL-6 trans-signaling. However, serum levels of soluble murine IL-6R were not reduced in ADAM17ex/ex mice, and murine ADAM17 was not the major sheddase of murine IL-6R. Shedding of murine IL-6R by murine ADAM17 was rescued in chimeric murine IL-6R proteins containing any extracellular domain but not the transmembrane and intracellular domain of human IL-6R. Apoptosis is a physiological stimulus of ADAM17-mediated shedding of human IL-6R. Even though apoptosis induced IL-6R shedding in mice, the responsible protease was identified as ADAM10. ADAM10 also was identified as protease responsible for ionomycin-induced shedding of murine and human IL-6R. However, in ADAM10-deficient murine embryonic fibroblasts, compensatory shedding of human IL-6R was mediated by ADAM17, but loss of ADAM10-mediated shedding of murine IL-6R was compensated by an as-yet-unidentified protease. Finally, we identified physiological purinergic P2X7 receptor stimulation as a novel inducer of murine and human IL-6R shedding solely mediated by ADAM10. In conclusion, we describe an unexpected species specificity of ADAM10 and ADAM17 and identified ADAM10 as novel inducible sheddase of IL-6R in mice and humans, which might have consequences for the interpretation of phenotypes from ADAM17- and ADAM10-deficient mice.

IL-6 cytokine signaling plays a critical role in the coordination of the immune system and IL-6 dysregulation leads to chronic inflammation and cancer development (1, 2) The IL-6 receptor (IL-6R)\[4\] complex consists of the transmembrane proteins gp130 and IL-6R. However, a soluble form of IL-6R (sIL-6R) acts as an agonist and forms complexes with IL-6. The resulting IL-6/sIL-6R complex can stimulate cells only containing gp130 on their surface and not the IL-6R in a process called IL-6 trans-signaling (3). Because most cells of the body do not express the membrane bound IL-6R, these cells are normally not responsive to IL-6 alone. IL-6 trans-signaling regulates cellular differentiation and apoptosis and is critically involved in acute and chronic inflammation as well as tumor development (4, 5).

In humans, shedding of the hIL-6R is the predominant mechanism for the generation of the shIL-6R protein. Proteases (“sheddases”), including members of the ADAM (a disintegrin and metalloprotease) family, such as ADAM10 and ADAM17, are responsible for ectodomain shedding of >100 transmembrane proteins, resulting in the release of their extracellular domains from the cell membrane (6). Different stimuli, including phorbol ester (phorbol-12-myristate-13-acetate (PMA)), bacterial toxins, bacterial metalloproteinases, and apoptosis, activate ADAM17-mediated shedding of the hIL-6R in a process called “induced ectodomain shedding” (7–10). Induced ectodomain shedding of the hIL-6R is mediated by ADAM17 and constitutive shedding by ADAM10 (11). However, overexpression of ADAM10 resulted in inducible shedding of the hIL-6R after PMA stimulation or cholesterol depletion (11). We now know that the proinflammatory activities of IL-6 rely on the sIL-6R derived from ADAM17-mediated shedding of IL-6R (7, 12, 13).

ADAM17 is the main sheddase of various membrane proteins such as TNFα, L-selectin, and some EGF-R ligands. ADAM17-deficient mice were not viable, and the phenotype was reminiscent of mice lacking TGFα (14). Recently, hypomorphic ADAM17ex/ex mice, which expressed only ~5% of the ADAM17 wild-type level, and conditional ADAM17 knock-out
animals were generated (15, 16). Hypomorphic ADAM17<sup>ex/ex</sup> mice had reduced serum level of sTNFRI and revealed an increased susceptibility to inflammation in dextran sulfate sodium-induced colitis due to reduced shedding of EGF-R ligands and reduced mucosal regeneration (17). However, the role of mIL-6R shedding and IL-6 trans-signaling in ADAM17<sup>ex/ex</sup> mice was not investigated. Here, we present data that in mice, the mIL-6R is not a target of ADAM17 but instead, a substrate of its closest homologue ADAM10. These results have major implications for the translation of studies with ADAM17- and ADAM10-deficient mice to the human situation.

**EXPERIMENTAL PROCEDURES**

**Plasmid Construction**—For creation of the IL-6R chimeras, sequences were aligned, and borders between the domains were determined accordingly (supplemental Fig. S1). All constructs were cloned with a 5′ KpnI site and a 3′ NotI site into pcDNA3.1. For the generation of the murine pre-B cell line Ba/F3-gp130 expressing murine IL-6R the plasmid pcDNA3.1-mIL-6R-GFP was digested with Pmel and EcoRI and subcloned into the pMOWS plasmid (18).

**Reagents**—The metalloprotease inhibitors GI254023X (GI, ADAM10-selective) and GW280264X (GW, ADAM10- and ADAM17-selective) were a generous gift from Glaxo Smith Kline (Stevenage, UK) (19). The recombinant murine ADAM10 prodomain was described previously (20). PMA was purchased from Calbiochem (Schwalbach am Taunus, Germany). Ionomycin, 2′(3′)-O-(4-benzoylbenzoyl)adenosine 5′-triphosphate (BzATP), doxorubicin, the P2X7 receptor inhibitor 1-(N,O-bis-(5-isoquinolinesulfonyl)-N-methyl-l-tyrosyl)-4-phenylpiperazine (KN62), and concanavalin A-Sepharose were purchased from Sigma-Aldrich (Deisenhofen, Germany). siRNAs were from Invitrogen. The mIL-6R-specific mAbs A1F830 and the biotinylated BAF1830 were from R&D Systems (Minneapolis, MN); the monoclonal β-actin mAb (catalog no. sc-47778) was purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Human IL-6R-specific mAbs 4–11 and 14–18 were described elsewhere (7), the polyclonal biotinylated anti-hIL-6R mAb (BAF227) was from R&D Systems. Annexin V-APC and the FITC-coupled rat anti-mCD62L mAbs and anti-CD16/CD32 mAbs were obtained from BD Biosciences. PE-coupled anti-mouse/rat CD126 (IL-6R) and PerCP-coupled anti-mCD3e mAbs were from BioLegend (San Diego, CA). The plasmids pcDNA6-hP2X7R and pcDNA6-mP2X7R were described previously (21).

**Cell Culture**—NIH3T3 and HEK293 cells were from the American Type Culture Collection (Manassas, VA). The murine pre-B cells line Ba/F3-gp130, ADAM17<sup>−/−</sup>, ADAM10<sup>−/−</sup> and ADAM10/ADAM17<sup>−/−</sup> mouse embryonic fibroblasts (MEFs) and the packaging cell line Phoenix-Eco were described previously (7, 18, 22, 23). For cultivation of Ba/F3-gp130 cells, DMEM containing FCS, penicillin, and streptomycin was supplemented with 10 ng/ml hyper-IL-6 (24). After selection, Ba/F3-gp130 cells expressing human or murine IL-6R were cultured in the respective medium as described above containing 10 ng/ml hIL-6 instead of hyper-IL-6.

**Transfection, Transduction, and Selection of Cells**—NIH3T3, HEK293, and MEF cells were transiently transfected using TurboFect (Fermentas, St. Leon-Rot, Germany). Ba/F3-gp130 cells were transduced retrovirally with plasmid derivatives of pMOWS as described previously (18).

**Preparation of Mouse Spleen Cells and Isolation of T Cells**—Cell suspensions from mouse spleens were prepared using an iron mesh sieve. Erythrocytes were lysed, and cells were washed in PBS supplemented with 0.5% BSA. T cells were purified by negative selection with magnetic beads using the Pan T cell isolation kit from Miltenyi Biotec (Bergisch Gladbach, Germany) according to the manufacturer’s protocol. Immediately before stimulation, whole spleen cells or purified T cells were diluted in NaCl buffer (0.14 M NaCl, 5 mM KCl, 0.01 M HEPES, and 0.01 M glucose) to a final concentration of 2 × 10<sup>6</sup> cells/ml.

**Stimulation, Staining, and Flow Cytometry Analysis of Primary Mouse Cells**—Inhibitors were added for 30 min before stimulation with BzATP for 15 min and with PMA for 1 h at 37 °C. Cells were washed twice, blocked with rat serum and anti-CD16/CD32 antibody, stained with antibodies (CD3ε-PerCP, CD62L-FITC, mIL-6R-PE) and annexin V-APC and analyzed by flow cytometry, using a FACS Canto and DIVA software (Becton-Dickinson).

**Induction of Apoptosis in T Cells and Murine Pre-B Cells**—Aptosis of Ba/F3 cells lines and primary T cells was induced by doxorubicin (500 ng/ml and 100 ng/ml, respectively). Cells were stained with annexin V-APC and analyzed by flow cytometry.

**Shedding Experiments and Analysis by FACS, Western Blotting, and ELISA**—To determine shedding of the IL-6R by human ADAM10 mediated via P2X7R, HEK293, and NIH3T3, cells were transiently transfected with plasmids containing hP2X7R and human or murine IL-6R (2.5 μg each). Cells were stained with anti-hIL-6R (clone 4-11) and anti-mouse APC or with mIL-6R-PE and analyzed with flow cytometry. Precipitation of soluble IL-6R, immunoblotting, ECL detection, and ELISA were performed as described previously (7).

**RESULTS**

**Murine IL-6R Is Not a Major Substrate of Murine ADAM17**—Serum level of soluble murine IL-6R was shown to be dependent on IL-6R shedding from immune (~60%) and hepatic cells (~30%) (26). ADAM17 as major sheddase of hIL-6R was a promising candidate, but analysis of sIL-6R plasma levels in wild-type and hypomorphic ADAM17<sup>ex/ex</sup> mice revealed no significant differences (17.6 ng/ml versus 17.2 ng/ml) (Fig. 1A). PMA is the strongest known activator of ADAM17-mediated shedding of hIL-6R. However, PMA induced only weak shedding of endogenous mIL-6R from murine CD3<sup>+</sup> spleen cells of wild-type (79.3 ± 2.5% versus 56.7 ± 0.6%) mice, which was partially inhibited in ADAM17<sup>ex/ex</sup> mice (80.7 ± 2.8% versus 72.4 ± 2.7%), indicating that ADAM17 is a functional sheddase of mIL-6R, but ADAM17-induced shedding of IL-6R was much lower than expected (Fig. 1). In contrast, PMA induced almost complete ADAM17-mediated shedding of endogenous L-selectin...
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FIGURE 1. ADAM17 is not involved in IL-6R shedding in mice. A, serum from both WT and ADAM17<sup>+/−</sup> (A17<sup>+/−</sup>) mice was prepared from full blood (n = 3), and sml-6R was quantified by ELISA. Values shown are mean of ± S.D., p = 0.31. B and C, spleen cells from WT and ADAM17<sup>+/−</sup> were stimulated with PMA for 2 h. The percentage of IL-6R-expressing CD<sup>3</sup> spleen cells (B) or CD62L-expressing CD<sup>3</sup> spleen cells (C) was quantified by flow cytometry. Data shown are mean of ± S.D. of four or two individually analyzed mice per group (WT and ADAM17<sup>+/−</sup>). D, murine NIH3T3 cells were transfected with expression plasmids encoding either human or murine IL-6R and treated for 2 h with PMA (100 nm). GI254023X (3 μM) and GW280264X (3 μM) were added 30 min prior to stimulation. Soluble IL-6R was measured by ELISA, the amount of sIL-6R without stimulation (constitutive shedding) was set to 1 and used for calculation of sIL-6R increase after stimulation. E, NIH3T3 cells were treated as described in A, the glycoprotein sIL-6R was precipitated from conditioned media using the glycoprotein-binding concanavalin A-Sepharose and subsequently visualized by Western blotting using mAb 14–18 for detection of sIL-6R and β-actin loading control. F, NIH3T3 human IL-6R or murine IL-6R was determined as described in A. Inhibitory recombinant ADAM10 prodomain (A10 pro, 1 and 10 μM) was added 30 min prior to stimulation with PMA. The amount of sIL-6R was quantified by ELISA. All ELISA data are the mean (± S.D.) from three independent experiments (n = 9), Western blotting showed one representative experiment.

This prompted us to analyze whether ADAM17-mediated shedding of mIL-6R is generally reduced in murine cells after PMA-stimulation. Interestingly, human but not murine IL-6R was released by murine ADAM17 after PMA stimulation in murine NIH3T3 cells. Shedding of hIL-6R after PMA stimulation was inhibited in presence of the combined ADAM10/ADAM17-selective inhibitor GW280264X (GW) but not by the ADAM10-selective inhibitor GI254023X (GI) (Fig. 1, D and E) (7, 19). Comparable results were obtained using murine pro-B-cells stably transfected with murine or human IL-6R cDNAs (supplemental Fig. S2A). Surprisingly, murine IL-6R was shed by human ADAM17 after PMA stimulation in HEK293 cells to almost the same extent as human IL-6R, which resulted in a 3.5- and 3-fold increase in sml-6R and shIL-6R 2 h after PMA stimulation, respectively (supplemental Fig. S2, B and C). Moreover, we specifically inhibited ADAM10 with the recombinant prodomain of ADAM10 in PMA-stimulated murine NIH3T3 cells transiently transfected with hIL-6R or mIL-6R cDNAs (20). As expected, no inhibition of human IL-6R shedding was observed in murine NIH3T3 cells after incubation with the prodomain of ADAM10 (Fig. 1F). However, ADAM10 was in part responsible for generation of background level of sml-6R because basal shedding activity was inhibited partially by addition of the ADAM10-selective inhibitor GI (Fig. 1, D and E) and by the recombinant prodomain of ADAM10 (Fig. 1F, A10 pro).

Extracellular Domains of hIL-6R Are Responsible for ADAM17-mediated Shedding—We hypothesized that structural differences between human and murine IL-6R might be responsible for the discrepancy of mADAM17-mediated shedding of human and murine IL-6R. To identify structural prerequisites in hIL-6R, which are responsible for ADAM17-mediated shedding, we conducted gain-of-function PMA-induced shedding experiments with chimeric murine/human IL-6R proteins (Fig. 2). These experiments were designed to analyze whether a single domain or the general overall structure of hIL-6R is responsible for ADAM17 substrate processing. The alignment of murine and human IL-6R showed that amino acids surrounding the cleavage site of ADAM17 in hIL-6R between Gln<sup>357</sup> and Asp<sup>358</sup> were conserved except for one amino acid exchange at position 358 (D358E) (supplemental Fig. S1). Mutagenesis of position 358 in hIL-6R resulted in reduced shedding of the hIL-6R by a factor of 2 to 5 (8). However, the single amino acid exchange (D358E) to mimic the perfect cleavage site of hIL-6R in mIL-6R was not sufficient to achieve PMA-induced shedding of the mIL-6R (D358E) mutant in murine NIH3T3 cells, even though shedding of mIL-6R (D358E) chimera in human HEK293 cells by ADAM17 was not affected (Fig. 2 and supplemental Fig. S3, A and B). The intracellular
domain of hIL-6R was not important to induce PMA-mediated shedding (8). Exchange of the intracellular or the transmembrane domain of the mIL-6R with its human counterparts did not enable PMA-induced shedding of the mIL-6R (transmembrane domain) and mIL-6R (intracellular domain) chimeras in murine NIH3T3 cells. Again, shedding of mIL-6R (transmembrane domain) and mIL-6R (intracellular domain) in human HEK293 cells by ADAM17 was not affected (Fig. 2 and supplemental Fig. S3, C and F). We further analyzed chimeras in which domain 1 (D1), domain 2 (D2), domain 3 (D3), combinations thereof or the complete stalk region of hIL-6R were introduced into mIL-6R. Again all chimeric proteins were expressed in murine NIH3T3 and human HEK293 cells. Unexpectedly, all extracellular IL-6R chimeras were substrates for PMA-induced ADAM17-mediated shedding not only in human but also in murine cells (Fig. 2 and supplemental Fig. S3, C and F). We concluded from these experiments that IL-6R substrate recognition by ADAM17 does not directly depend on the cleavage site and transmembrane or intracellular domain but on the overall structure of extracellular IL-6R domain architecture. This is in line with recently published data, showing that ADAM17 substrate recognition is not well defined by a cleavage site consensus sequence but by the interaction with the overall structure of the substrate (28, 29).

Apoptosis-induced Shedding of Murine IL-6R Is Mediated by Murine ADAM10 and Not by Murine ADAM17—Recently, we identified apoptosis as a physiological trigger of ADAM17-mediated human IL-6R shedding, leading to locally increased soluble IL-6R levels and induction of IL-6 trans-signaling needed for resolution of acute inflammation (7). This prompted us to investigate which protease triggered apoptosis-induced shedding of mIL-6R. Doxorubicin-induced apoptosis in murine pre-B cells stably transfected with human or murine IL-6R was followed by annexin V FACS analysis (Fig. 3, A and B). ADAM17-mediated shedding of the hIL-6R was induced by apoptosis (Fig. 3C) (7). Importantly, apoptosis-induced shedding of the mIL-6R in murine pre-B cells was reduced by the ADAM10-selective inhibitor GI and the ADAM10/ADAM17 selective inhibitor GW (Fig. 3D). Apoptosis-induced shedding of endogenous mIL-6R in murine primary T cells was inhibited by ADAM10-selective inhibitor GI, the ADAM10/ADAM17-selective inhibitor GW, and the prodomain of ADAM10, indicating that ADAM10 and not ADAM17 was the responsible protease (Fig. 3E). This is the first example of a species-specific switch in ADAM protease/substrate biology.
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**FIGURE 4.** Ionomycin-induced shedding of murine and human IL-6R mediated by murine and human ADAM10. A, NIH3T3 cells were transfected with expression plasmids encoding mIL-6R or hIL-6R. Cells were stimulated with ionomycin (Iono, 1 μM) for 60 min. GI (3 μM) and GW (3 μM) were added 30 min prior to stimulation. Soluble mIL-6R and shIL-6R were measured by ELISA. The murine ADAM10 prodomain (A10 pro, 10 μM) was added 30 min prior to stimulation. The values are the mean ± S.D. of three independent experiments. B, HEK293 cells were transfected with expression plasmids encoding for mIL-6R or hIL-6R. Cells were stimulated with ionomycin (1 μM) for 60 min. Inhibitors GI (3 μM) and GW (3 μM) were added 30 min prior to stimulation. Soluble IL-6R was measured by ELISA. The values are the mean ± S.D. of three independent experiments. C, NIH3T3 cells were transfected with expression plasmid encoding for mIL-6R and a mixture of three siRNAs directed against mADAM10 or with expression plasmid encoding for mIL-6R and unrelated siRNAs. Cells were stimulated with ionomycin (1 μM) for 60 min, and smIL-6R was measured by ELISA. MEFs deficient for ADAM17 were transfected with plasmids encoding hIL-6R (D) or mIL-6R (E) and stimulated with ionomycin (1 μM) for 60 min or PMA for 120 min. Inhibitors were added 30 min before stimulation as indicated. sIL-6Rs were quantified by ELISA. MEFs deficient for ADAM10 were transfected with plasmids encoding hIL-6R (F) or mIL-6R (G) and stimulated with ionomycin (1 μM) for 60 min or PMA for 120 min. Inhibitors were added 30 min before stimulation as indicated. sIL-6Rs was quantified by ELISA. MEFs deficient for both ADAM10 and ADAM17 were transfected with plasmids encoding hIL-6R (H) or mIL-6R (I) and stimulated as described in B. The values are from one representative experiment (n = 3). Wild-type MEFs were transfected with plasmids encoding hIL-6R (J) or mIL-6R (K) and stimulated as described in B. The values are from one representative experiment (n = 3).

the responsible protease. Ionomycin but not PMA induced shedding of human and murine IL-6R in ADAM17−/− MEFs (Fig. 4, D and E), which was blocked completely by the ADAM10-selective inhibitor GI. As a control, in ADAM10−/− but not in ADAM17−/− MEFs, hIL-6R was shed after PMA treatment (Fig. 4, D and F). Shedding of human IL-6R was found after ionomycin stimulation in ADAM10−/− MEFs, which was inhibited by the ADAM10- and ADAM17-specific inhibitor GW but not by the ADAM10 prodomain, indicating that in the absence of ADAM10, ADAM17 mediates compensatory hIL-6R shedding (Fig. 4F). Compensatory shedding by ADAM17 in ADAM10−/− MEFs of ADAM10 substrates was described recently (31). In the same ADAM10−/− MEFs, shedding of the murine IL-6R was also detectable after ionomycin treatment. Compensatory shedding of the murine IL-6R was not inhibited by the ADAM10- and ADAM17-specific inhibitor GW, indicating that this shedding was not mediated by ADAM17 but by a yet unidentified protease (Fig. 4G). Finally, in ADAM10−/−/ADAM17−/− MEFs, no compensatory shedding of human IL-6R was observed after PMA and ionomycin treatment (Fig. 4H), indicating that ADAM10 and ADAM17 are solely responsible for inducible release of hIL-6R. However, ionomycin treatment of ADAM17−/−/ADAM10−/− MEFs still led to increase in soluble murine IL6-R, which again was not inhibited by the ADAM10 and ADAM10/ADAM17-specific inhibitors GI and GW, respectively (Fig. 4I). As a control, wild-type MEFs were treated. Here, ionomycin-induced shedding of human and murine IL-6R was inhibited by the ADAM10 and ADAM10/ADAM17 specific inhibitors and by the prodomain of ADAM10 (Fig. 4, J and K). We conclude from these experiments that ionomycin-induced shedding of murine and human IL-6R was mediated by ADAM10. Compensatory shedding of hIL-6R in absence of ADAM10 was mediated by ADAM17, whereas compensatory shedding of mIL-6R was not mediated by ADAM17 but by an as-yet-unidentified protease.
Physiological Stimulation of Purinergic P2X7 Receptor Signaling Induces ADAM10-mediated Shedding of Murine and Human IL-6R—Previous reports showed that ADAM10-mediated shedding of CD23 is stimulated by activation of the purinergic P2X7R in B cells (34, 35). Furthermore, the stimulation of P2X7R signaling activated ADAM10 to shed common ADAM17 substrates, including TNFα and TGFβ in ADAM17-deficient MEFs (31). However, the shedding of IL-6R was not investigated. Serum level of soluble mIL-6R were reduced significantly by 15.5% in P2X7R−/− mice in comparison with wild-type mice (16.05 ± 0.77 ng/ml versus 13.60 ± 0.13 ng/ml, p < 0.001, Fig. 5A). This data suggested that P2X7R stimulation contributes to serum level concentrations of sIL-6R. NIH3T3 and HEK293 cells were cotransfected with expression plasmids of either murine or human P2X7R and murine or human IL-6R followed by stimulation with 500 μM BzATP. BzATP stimulation led to 3.9-fold increased shedding of mIL-6R in murine NIH3T3 cells (Fig. 5B). Inducible shedding was inhibited by addition of the ADAM10 and ADAM10/ADAM17-selective inhibitors GI and GW, indicating that P2X7R-stimulated shedding was mediated by ADAM10. Comparable results were obtained with hIL-6R in NIH3T3 and with mIL-6R and hIL-6R in human HEK293 cells (Fig. 5, B and C). Accordingly, we show that BzATP induced shedding of endogenous mIL-6R in primary murine spleen cells, which was not detectable in primary murine spleen cells from P2X7R−/− mice as assessed by flow cytometry of cell surface IL-6R and ELISA of sIL-6R (Fig. 5, D and E). The amount sIL-6R was determined by ELISA, I, mouse primary splenocytes were stimulated with 150 μM BzATP in the presence or absence of GI and GW (n = 4). The percentage of IL-6R-positive cells of CD3+ annexin V lymphocytes was determined by flow cytometry. All results represent mean (± S.D.) from individually analyzed mice (n).
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the prodomain of ADAM10 suppressed P2X7R-stimulated IL-6R shedding (Fig. 5, H and I). We conclude from these experiments that physiological stimulation of P2X7R induced ADAM10-dependent shedding of IL-6R.

DISCUSSION

This report describes the first species specific switch of ADAM protease substrate recognition on the basis of IL-6R biology and thus establishes ADAM10 as novel inducible sheddase for IL-6R.

Agonistic and antagonistic soluble cytokine receptors are important inflammatory regulators. Agonistic soluble IL-6R amplifies IL-6-mediated signaling by activation of IL-6-trans-signaling, and levels of sIL-6R were shown to be increased in numerous inflammatory states. Activation of gp130 via IL-6-trans-signaling is critical for inflammatory processes, including lymphocyte trafficking into the inflamed area by controlling chemokine expression (4, 7), promotion of T cell antiapoptosis and proliferation during inflammatory bowel disease, as well as colon cancer development and regulation of adhesion molecule expression on endothelial cells (12, 13).

It was generally accepted that ADAM10 is responsible for the constitutive IL-6R shedding, whereas ADAM17 upon appropriate activation causes rapid IL-6R inducible proteolysis. ADAM17 is expressed by most mammalian cells and gene targeting of ADAM17 resulted in embryonic lethality, indicating the great importance of ectodomain shedding in developmental processes (14, 22). ADAM17 conditional knock-out mice with a specific deletion of the ADAM17 gene in neutrophils and monocytes were protected from LPS-induced endotoxic shock and produced reduced amounts of soluble TNF-α (16). Recently, we generated viable, hypomorphic ADAM17ex/ex mice, which express minimal amounts of ADAM17 protein. These ADAM17ex/ex mice had substantially increased susceptibility to inflammation in the dextran sulfate sodium colitis model of inflammatory bowel disease, caused by impaired shedding of EGF-R ligands and defective regeneration of the intestinal epithelial cell barrier (17). Importantly, the serum level of ADAM17 substrates were significantly reduced in ADAM17-deficient mice (17, 27).

However, the serum sIL-6R levels in ADAM17ex/ex mice were not reduced, indicating that ADAM17 did not contribute to circulating sIL-6R generation. Moreover, ADAM17-mediated shedding of mIL-6R was largely absent in primary splenocytes, and no PMA-induced IL-6R shedding was observed in murine NIH3T3 cells and pro-B cells.

ADAM17 cleaves the hIL-6R between amino acid positions Gln357–Asp358 close to the transmembrane region (8). Close inspection of the ADAM17 cleavage site of IL-6R revealed that only a single amino acid within the core cleavage sequence was different between mice and humans, whereas the remaining stalk region was only poorly conserved. However, introduction of the “perfect” human IL-6R cleavage site into murine IL-6R did not restore ADAM17-mediated shedding. Additionally, the introduction of the hIL-6R transmembrane and intracellular domain into the mIL-6R (hIL-6R transmembrane domain) and IL-6R (intracellular domain) did not restore PMA-stimulated ADAM17-mediated shedding. This was not surprising, as it has been shown previously, that no consensus ADAM cleavage site was found within ADAM substrates and that the intracellular domain of IL-6R was not needed for phorbol ester-stimulated shedding (8). Surprisingly, exchange of any extracellular domain, D1, D2, D3, or the complete stalk region from hIL-6R to mIL-6R restored ADAM17-mediated shedding of the respective mIL-6R chimera, indicating that the overall extracellular structure of IL-6R was needed for substrate recognition and/or cleavage. Interestingly, autoantibodies directed against the cysteine-rich region of ADAM17-related ADAM33TS inhibited shedding (36), and anti-CD40 and anti-HB-EGF antibodies directed against epitopes distal from the cleavage site inhibited ADAM17-mediated ectodomain shedding (37, 38). These data showed that also for IL-6R sites distal to the metalloproteinase domain and the target cleavage site are important for substrate recognition. Until this study, such inhibitory shedding antibodies were not described for IL-6R.

Recently, we demonstrated that intrinsic and extrinsic induction of apoptosis resulted in ADAM17-mediated shedding of the human IL-6R from the cell surface, which was important for recruitment of macrophages, engulfment of apoptotic neutrophils, and resolution of acute inflammation (7). Here, apoptosis-induced shedding of murine IL-6R was found to be mediated by mADAM10, even though human IL-6R was still shed by murine ADAM17 from apoptotic murine cells. Apoptosis-induced shedding was different from PMA-induced shedding because it was PKC-, MAPK-, and reactive oxygen species-independent. Another PKC-independent inducer of shedding is the Ca²⁺ ionophore ionomycin. We have shown for the first time, that ionomycin-induced shedding of the murine and human IL-6R also was dependent on ADAM10. However, under lifelong ADAM10 deficiency in MEFs, ADAM17 compensates for ADAM10-mediated shedding for human IL-6R but not for murine IL-6R, indicating that another as-yet-unidentified protease is able to shed murine IL-6R in the absence of ADAM10.

Finally, physiological stimulation of the purinergic P2X7R with BzATP-induced shedding of murine and human IL-6R, which again was dependent solely on ADAM10. Serum levels of soluble IL-6R were reduced significantly in P2X7R-deficient mice, suggesting that physiological stimulation of purinergic P2X7R contributes to sIL-6R serum level concentration.

Previously, we suggested that ADAM17 is the master regulator for the generation of the soluble IL-6R and initiation of IL-6 trans-signaling under pathophysiological situations. However, in view of the presented data, we have to restrict this working hypothesis only to human cells, and we now defined ADAM10 as master regulator of murine IL-6R shedding in murine cells. Moreover, in humans, ADAM17 is no longer the sole master regulator of IL-6R shedding, as physiological stimulation via P2X7R and ionomycin treatment specifically induced ADAM10- but not ADAM17-mediated IL-6R shedding. In light of this data, we think that results from ADAM17 knockout, ADAM17 conditional, and hypomorphic ADAM17ex/ex mice have to be interpreted with caution because the fundamental IL-6 trans-signaling process is still largely intact in these mice. Therefore, the course of disease models in ADAM17-deficient mice will not reflect the situation in humans. We
hypothesize that ADAM10 conditional knockout mice might fail to conduct IL-6 trans-signaling.

Future work should include functional characterization of IL-6R shedding in conditional ADAM10 knockout mice in murine models of acute and chronic inflammation, which are available now (39). It seems to be of utmost importance that in light of the manifold substrates of ADAM10 and ADAM17, substrate specificity of every substrate has to be examined in mice and men to permit interpretation of results from ADAM-deficient mice.

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