Cytokine 71 (2015) 238–245

Contents lists available at ScienceDirect

Cytokine

journal homepage: www.journals.elsevier.com/cytokine

ADAM17 regulates IL-1 signaling by selectively releasing IL-1 receptor type 2 from the cell surface

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ARTICLE INFO

Article history:
Received 18 May 2014
Received in revised form 22 September 2014
Accepted 21 October 2014

Keywords:
ADAM17/TACE
IL-1 receptors
Ectodomain shedding

ABSTRACT

Interleukin (IL)-1 is one of the most evolutionarily conserved cytokines and plays an essential role in the regulation of innate immunity. IL-1 binds to two different receptors, IL-1R1 and IL-1R2, which share approximately 28% amino acid homology. IL-1R1 contains a cytoplasmic domain and is capable of transducing cellular signals; by contrast, IL-1R2 lacks a functional cytoplasmic domain and serves as a decoy receptor for IL-1. Interestingly, IL-1R2 is proteolytically cleaved and also functions as a soluble receptor that blocks IL-1 activity. In the present study, we examined the shedding properties of IL-1R2 and demonstrate that ADAM17 is *de facto* the major sheddase for IL-1R2 and that introducing a mutation into the juxta-membrane domain of IL-1R2 significantly desensitizes IL-1R2 to proteolytic cleavage. IL-1R1 was almost insensitive to ADAM17-dependent cleavage; however, the replacement of the juxta-membrane domain of IL-R1 with that of IL-1R2 significantly increased the sensitivity of IL-1R1 to shedding. Furthermore, we demonstrate that ADAM17 indirectly enhances IL-1 signaling in a cell-autonomous manner by selectively cleaving IL-1R2. Taken together, the data collected in the present study indicate that ADAM17 affects sensitivity to IL-1 by changing the balance between IL-1R1 and the decoy receptor IL-1R2.

1. Introduction

Interleukin (IL)-1 is one of the most crucial regulators of innate immunity and a mediator of inflammatory diseases in humans [1–4]. IL-1 belongs to the IL-1 family of genes, which consists of 11 members with both pro- and anti-inflammatory activities [2–5]. Among the members of the IL-1 family, two agonists, IL-1α and IL-1β, and one receptor antagonist, IL-1Ra, bind to IL-1 receptor type 1 (IL-1R1). The cytoplasmic domain of IL-1R1 contains the Toll/IL-1 receptor (TIR) domain, which is highly conserved among the Toll-like receptors and allows the binding of signal transduction components downstream of IL-1R1 and Toll-like receptors [6]. Upon binding of IL-1α or IL-1β, IL-1R1 forms a heterodimer with the co-receptor IL-1 receptor accessory protein (IL-1RAcP), which enables the recruitment of signal transduction molecules, including myeloid differentiation primary response protein 88 (MyD88), IL-1R-associated kinase 4 (IRAK4), and TNFR-associated factor 6 (TRAF6), to the TIR domain of the IL1-R1 and IL-1RAcP heterodimer [7,8]. IL-1α and IL-1β can also bind to another member of the IL-1 receptor family: IL-1 receptor type 2 (IL-1R2). The extracellular domain of IL-1R2 is homologous to that of IL-R1; however, IL-1R2 has a short cytoplasmic tail and lacks the TIR domain. Therefore, IL-1R2 is incapable of inducing intracellular signaling and functions as a decoy receptor for IL-1α and IL-1β [9–12].

Interestingly, IL-1R2 can also be cleaved from the cell surface and released as a soluble receptor, which traps free IL-1α and IL-1β and inhibits the activities of these molecules [13]. Past studies have demonstrated that the proteolytic activity that mediates the cleavage of IL-1R2 is metalloprotease-dependent and that ADAM17, which is also known as TNFα-converting enzyme (TACE), exhibits shedding activity towards IL-1R2 [14–17]. ADAM17 is a membrane-bound metalloprotease that was originally identified as a protease responsible for the ectodomain shedding of membrane-bound pro-TNFα [18,19]. Since its identification, a

Abbreviations: AP, alkaline phosphatase; JM, juxta-membrane; mEFs, mouse embryonic fibroblasts; PMA, phorbol 12-myristate 13-acetate.

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http://dx.doi.org/10.1016/j.cyto.2014.10.032
1043-4666/

number of different molecules have been identified as target substrates for ADAM17 [20–23]. These targets include not only membrane-bound ligands but also receptors and adhesion molecules. However, despite the potential importance of the ectodomain shedding of IL-1R2 in the regulation of IL-1 activity, it remains unclear whether proteases other than ADAM17 are also involved in this activity or whether the shedding of IL-1R2 by ADAM17 affects IL-1 signaling at a cellular level. The main goals of the present study were to further characterize the shedding properties of IL1-R2 and to identify the potential roles of ADAM17 in IL-1 signaling. Novel insight into the involvement of ADAM17 in the regulation of IL-1 activity may have implications for our understanding of the pathology of inflammatory diseases in which ADAM17 participates by regulating multiple cellular signaling pathways, including the TNFα/TNFR and IL-6/IL-6 receptor pathways [21,22,24,25].

2. Materials and methods

2.1. Reagents and cells

The generation of immortalized wild type (WT), *Adam10*$^{-/-}$, and *Adam17*$^{-/-}$ mouse embryonic fibroblasts (mEFs) has been previously described [24,26]. Recombinant human IL-1β was obtained from PeproTech (Rocky Hill, NJ, USA). The following antibodies were used in the present study: phycoerythrin-conjugated anti-IL1-R2 antibody (Ab) (clone 4E2, BD Bioscience, San Jose, CA, USA), biotin-conjugated anti-L-selectin Ab (MEL-14, Biolegends, San Diego, CA, USA), anti-phospho-JNK Ab (#4668, Cell Signaling Technology, Danvers, MA, USA), anti-Myc Ab (9E10, Santa Cruz Biotechnology, Dallas, TX, USA), anti-HA-tag Ab (3F10, Roche Applied Science, Penzberg, Upper Bavaria, Germany), anti-human alkaline phosphatase (AP) Ab (Sigma–Aldrich, St. Louis, MO, USA), anti-IL1-R2 (AF563, R&D systems, Minneapolis, MN, USA), and anti-β-Actin Ab (C4, Santa Cruz Biotechnology). Recombinant TIMPs 1–3 were purchased from R&D Systems. The protease inhibitor cocktail (Sigma–Aldrich) used in the present study contains AEBSF, aprotinin, bestatin hydrochloride, E-64, leupeptin hemisulfate salt, and pepstatin A. siRNAs were purchased from Dharmacon (Lafayette, CO, USA). All other reagents were obtained from Sigma–Aldrich unless otherwise indicated.

2.2. Cloning of IL-1 receptors and generation of AP-tagged vectors and mutants

Myc-tagged (IL-1R2^{Myc}) and dual HA- and Myc-tagged IL-1R2 (IL-1R2$^{HA\text{-}Myc}$) expression vectors were generated by cloning the full-length coding sequence of *Il1r2* into the pCDNA4-Myc/His expression vector (Life Technologies, Carlsbad, CA, USA) and inserting nucleotides encoding the HA-epitope next to the signaling sequence using a PCR-based method. To generate AP fusion expression vectors, the cDNA fragments of IL-1 receptors (i.e., *Il1r1* (nucleotides 964–1521), *Il1r2* (nucleotides 697–1230), *Il1rap* (nucleotides 679–1253), and human *IL1R2* (nucleotides 697–1254)) were cloned using RT-PCR and subcloned into pAPtag5 (GenHunter, Nashville, TN, USA). A schematic of the vectors used in the present study is presented in Fig. 1A. The mutant AP-tagged IL-1R1 and IL-1R2 expression vectors (see Figs. 3B and 4B) were generated using the KOD-plus Mutagenesis Kit (Toyobo, Osaka, Japan) according to the manufacturer's instructions. All constructs were sequenced to rule out the presence of any undesired mutations. Cell surface expression, which indicates the maturation of the transgenically expressed protein, was confirmed by immunostaining using an anti-AP antibody (Fig. 1C and data not shown).

2.3. Flow cytometry

Bone marrow cells were collected from polyinosinic–polycytidylic acid-treated 8-wk-old *Adam17* mutant and control mice, as previously described [24]. The cells were filtered through a cell strainer (BD Biosciences) to remove debris. The RBCs were removed using RBC lysis buffer (Roche). The cells were incubated with anti-CD16/32 Ab (clone 2.4G2, BD Biosciences) to block non-specific binding and subsequently stained with phycoerythrin-conjugated anti-IL1-R2 Ab and biotinylated anti-L-selectin Ab, followed by streptavidin allophycocyanin. The frequency of surface IL-1R2-positive cells in the bone marrow was determined via direct immunofluorescence using the FACSCalibur system (BD Biosciences).

2.4. Cell culture, transfection, and shedding assay

COS-7 cells and mEFs were grown in DMEM supplemented with 10% FBS and antibiotics. The cells were transfected with expression vectors using Fugene HD (Roche). Fresh Opti-MEM (Life Technologies) medium with or without 25 ng/ml of phorbol 12-myristate 13-acetate (PMA) and/or 20 μM GM6001 (Merck Millipore, Darmstadt, Germany) was added 18–24 h after transfection, and the cells were incubated for 1 h. The evaluation of AP activity in the supernatants and cell lysates was performed using in-gel visualization and colorimetry, as previously described [27,28]. In the colorimetric assays, the shedding efficiency of a given construct is presented as a ratio of AP activity in the supernatant to that in the cell lysate to adjust the difference in the transfection efficiency. All experiments were repeated at least three times with similar results.

2.5. Generation of stable transfectants

Stable transfectants were generated using the pMX-IG retroviral vector and Plat-E packaging cells, which were generously provided by Dr. T. Kitamura from the Institute of Medical Science at the University of Tokyo [29]. Briefly, full length IL-1R2 with a Myc-His sequence attached to its 3′ end was subcloned into the pMX-IG vector. Retrovirus was produced using Plat-E packaging cells and transfected into WT mEFs. Single cell cloning was performed using the serial dilution technique. The expression of IL-1R2 in the selected clones was confirmed by western blotting using an anti-Myc antibody.

2.6. Amino acid sequence analysis

All amino acid sequence data were collected from the Ensembl web site (http://www.ensembl.org/index.html). Sequence analysis was performed using Genetyx 10 software (Genetyx Corp., Tokyo, Japan).

2.7. Statistical analysis

All data are presented as the mean ± SEM. Student's t-test (Figs. 1D, 2A, 2E, and 5C) and the Mann–Whitney U test (Fig. 5F) were used to calculate p values. Values of $p < 0.05$ were considered statistically significant.

3. Results

3.1. IL-1R2 but not IL-1R1 or IL-1RAcP is sensitive to PMA-induced proteolytic activity

To evaluate the shedding properties of IL-1R2, we first generated an HA and Myc epitope dually tagged IL-1R2 construct