Cytokine 61 (2013) 469–477

Contents lists available at SciVerse ScienceDirect

Cytokine

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

Modulation of PAR expression and tryptic enzyme induced IL-4 production in mast cells by IL-29

Huiyun Zhang [a], Haiwei Yang [b], Wenjing Ma [b], Zhongfang Zhang [c], Shaoheng He [b,*]

[a] Department of Pathophysiology, Hainan Medical College, Haikou, Hainan 571101, China
[b] Clinical Research Centre, The First Affiliated Hospital of Nanjing Medical University, Nanjing, Jiangsu 210029, China
[c] Department of Physiology, Shantou University Medical College, Shantou 515041, China

ARTICLE INFO

Article history:
Received 28 April 2012
Received in revised form 17 September 2012
Accepted 31 October 2012
Available online 5 December 2012

Keywords:
IL-29
IL-4
Mast cell
Protease activated receptor
Tryptase

ABSTRACT

Interleukin (IL)-29 is a relatively newly discovered cytokine, which has been shown to be actively involved in the pathogenesis of allergic inflammation. However, little is known of the effects of IL-29 on protease activated receptor (PAR) expression and potential mechanisms of cytokine production in mast cells. In the present study, we examined potential influence of IL-29 on PAR expression and cytokine production in P815 and bone marrow derived mast cells (BMMCs) by using flow cytometry analysis, quantitative real time PCR, and ELISA techniques. The results showed that IL-29 downregulated the expression of PAR-1 by up to 56.2%, but had little influence on the expression of PAR-2, PAR-3 and PAR-4. IL-29 also induced downregulation of expression of PAR-1 mRNA. However, when mast cells were pre-incubated with IL-29, thrombin-, trypsin- and tryptase-induced expression of PAR-2, PAR-3 and PAR-4 was upregulated, respectively. IL-29 provoked approximately up to 1.9-fold increase in IL-4 release when mast cells was challenged with IL-29. Administration of IL-29 blocking antibody, AG490 or LY294002 abolished IL-29-induced IL-4 release from P815 cells. It was found that IL-29 diminished trypsin- and tryptase-induced IL-4 release from P815 cells following 16 h incubation. In conclusion, IL-29 can regulate expression of PARs and tryptase- and trypsin-induced IL-4 production in mast cells, through which participates in the mast cell related inflammation.

1. Introduction

IL-29. belongs to IL-28 family, but the protein structure is more closely related to the type I interferon (IFN) than to IL-10 [1,2]. Therefore they have been described independently as IFN-λ1 [3]. Moreover, IL-28 family belongs to the superfamily of human Class II cytokines, which consists of numerous cytokines including IL-10, IFN-γ and IFN-λ [4].

IL-29 has been found to be produced by human peripheral blood mononuclear cells (PBMCs) [5] and monocyte-derived dendritic cells (DCs) [6] in response to viral infection, poly I:C [7] or lipopolysaccharide (LPS) [8]. Recently, it has been observed that large proportion of mast cells [9], eosinophils [10], but not CD4+T cells in human colon, tonsil and lung tissues express IL-29. Apart from anti-viral [11,12] and anti-tumor functions [13], IL-29 has been found to be able to induce maturation of dendritic cells [6], generation of tolerogenic DCs [14], release of IL-6, IL-8, IL-10 [15] and tumor necrosis factor (TNF) [16] from monocytes and macrophages, and recruitment of mast cells in mouse peritoneum by a CD18- and ICAM-1-dependent mechanism [9]. Since high serum levels of IL-29 are detected in the patients with allergic asthma [16] and mast cells have long been recognized to play a pivotal role in allergy, we anticipated that IL-29 may serves as a proinflammatory cytokine in allergy, and investigated potential influence of IL-29 on cytokine release from mast cells in the present study. We have previously reported that extrinsic IL-29 can induce IL-4 release from P815 cells [9], but the potential mechanisms by which IL-29 is able to provoke IL-4 release remains unknown. We therefore investigated the mechanisms of IL-29 induced IL-4 release from P815 cells in the present study.

Protease activated receptors (PARs) named PAR-1, 2, 3 and 4, have been identified as receptors for serine proteases [17]. Activation of PARs has been suggested to be involved in the pathogenesis of inflammation. For example, upregulation of PAR-2 expression has been found in the airways of asthma [18] and allergic rhinitis [19]. As activators of PARs, tryptase and trypsin play a crucial role in allergic inflammation. Tryptase was reported to enhance monocyte chemoattractant protein-1 (MCP-1) and IL-8 production in human endothelial cells through activation of PAR-2 [20]. Trypsin was found to induce histamine release from human mast cells [21] and to stimulate IL-6 release from T cells [22] via PAR related mechanisms. Recently, tryptase has been found to provoke IL-13 release from mast cells, and IL-6 can diminish the influence of

* Corresponding author. Tel./fax: +86 (0)25 83672085.
E-mail address: shoahenghe@hotmail.com (S. He).

1043-4666/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.cyto.2012.10.032

tryptase on mast cells [23]. In order to further understand the effect of IL-29 on mast cells, we investigated the influence of IL-29 on tryptic enzyme induce expression of PARs and production of IL-4 in mast cells. We found that IL-29 can eliminate PAR-1 expression, but enhance protease induced PAR expression on mast cells. IL-29 can also reduce protease induced IL-4 release from mast cells.

2. Materials and methods

2.1. Reagents, cells and animals

Trypsin, leupeptin, paraformaldehyde and bovine serum albumin (BSA, fraction V) and 4-(4-Fluorophenyl)-2-(4-methylsulfinylphenyl)-5-(4-pyridyl)-1H-imidazole (SB203580) were from Sigma Inc. (St. Louis, MO, USA). Recombinant human lung β-tryptase was obtained from Promega (Madison, WI, USA). Recombinant human IL-29, mouse anti-human IL-29 monoclonal antibody and mouse IL-4 ELISA kits were from R&D Systems (Minneapolis, MN, USA). Mouse IL-10 and IL-12 ELISA kits were obtained from Pierce Biotech Inc. (Rockford, IL, USA). N3-cyclopropyl-7-[[4-(1-methylethyl)phenyl]methyl]-7H-pyrrolo[3,2-f]quinazoline-1,3-diamine (SCH 79797) was purchased from Tocris Cookson (Ellisville, Mo, USA). FSLLRY-NH2 was synthesized by CL Bio-Scientific Inc (Xi An, China). Tissue culture reagents including Dulbecco's modified Eagle's medium (DMEM), RPMI 1640 and fetal bovine serum (FBS) were purchased from HyClone (Logan, UT, USA). Mouse IL-3 and SCF were purchased from PeproTech Inc. (Rocky Hill, NJ, USA). TRIzol Reagent was obtained from Invitrogen (Carlsbad, CA, USA). 2-(2-Diamino)-3-methoxyphenyl-4H-1-benzopyran-4-one (PD98059), 1,4-diamino-2,3-dicyano-1,4-bis(2-aminophynyltio) butadiene (U0126), 1,4-diamino-2,3-dicyano-1,4-bis(methylthio) butadiene (U0124), tyrphostin (AG490) and 2-(4-morpholinyl)-8-phenyl-4H-1-benzopyran-4-one (LY294002) were obtained from Cell Signaling Technology (Beverly, MA, USA). ExScript™ RT reagent kit and SYBR® *Premix Ex Taq*™ (perfect real time) was obtained from TaKaRa Biotechnology Co. Ltd. (DaLian, China). Oligonucleotide primers for real time PCR were synthesized by Invitrogen Biotechnology Co. (Shanghai, China). FITC-conjugated goat anti-mouse PAR-2 monoclonal antibody; rabbit anti-mouse PAR-1, rabbit anti-mouse PAR-3 and rabbit anti-mouse PAR-4 polyclonal antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). FITC-conjugated goat anti-rabbit polyclonal antibody was obtained from BD Pharmingen (San Jose, CA, USA). The mouse mastocytoma cell line P815 was obtained from the American Type Culture Collection (Manassas, VA, USA). Most of other reagents such as salt and buffer components were analytical grade and obtained from Sigma. C57BL/6 mice (6 wk old) were obtained from Guangdong Experimental Animal Center, China, Grade II. The animal experiment procedures were approved by the Animal Care Committee at Hainan Medical College.

2.2. Mast cell culture, preparation and challenge

P815 cells were cultured with ATCC complete growth medium at 37 °C in a 5% (v/v) CO_2, respectively as described previously [24]. The procedure for preparation of BMMC was performed as described previously [25], which produced more than 97% purity of BMMC [26,27]. Briefly, the crude bone marrow cells were cultured in RPMI 1640 medium supplemented with 10% FBS, 10 ng/ml of recombinant IL-3 and 10 ng/ml of recombinant SCF in a 5% CO_2-containing atmosphere at 37 °C for 4 wk. Cultured cells at a density of 1×10^6 cells/ml were incubated with the serum-free basal medium for 6 h before challenge.

For challenge experiments, cells were exposed to various concentrations of IL-29 with or without its blocking antibody or antagonists of PARs; tryptase (1 μg/ml = 7.4 nM) or trypsin (1 μg/ml = 42 nM) with or without FSLLRY-NH_2 (500 μM) or an antagonist of PAR-1 SCH 79797 (1.0 μM), respectively. At 2, 6 or 16 h following incubation, the culture supernatants (5 ml) were collected, and the cell pellet containing approximately 5×10^6 cells were resuspended for further analysis. Preliminary experiments showed that accumulated IL-4 was consistently detectable after 6 h challenge period. For priming experiments, cells were preincubated with 0.1 ng/ml of IL-29 for 60 min and washed 3 times before adding thrombin, tryptase or trypsin for 6 h. For cell signalling experiments, cultured cells at a density of 1.5×10^6 cells/ml were treated with the inhibitors of cell signalling pathways including PD98059 (50 μM), U0126 (5 μM), U0124 (5 μM), SB203580 (20 μM), LY294002 (20 μM) and AG490 (40 μM) for 30 min before being washed and challenged with IL-29 (1.0 and 10 ng/ml) for 6 h. Preliminary experiments showed that the inhibitors of cell signalling pathways had maximum effect on IL-4 release at 6 h, not 16 h following challenge. For the experiment with anti-IL-29 antibody, cells were preincubated with 1.0 μg/ml of the antibody for 60 min before adding 10 ng/ml of IL-29 for 2, 6 or 16 h.

2.3. Quantitative real-time PCR

Total RNA was extracted from P815 cells as described previously [24]. Briefly, after synthesizing cDNA from total RNA by using ExScript™ RT reagent kit, real-time PCR was performed by using SYBR® *Premix Ex Taq*™ kit on the ABI Prism 7700 Sequence Detection System (Perkin Elmer Applied Systems, Foster City, CA, USA). Each reaction contains 12.5 μl of 2×SYBR green Master Mix, 300 nM oligonucleotide primers (Table 1), 10 μl of the cDNA or plasmid DNA. The thermal cycling conditions included an initial denaturation step at 95 °C for 2 min, 40 cycles at 95 °C for 30 s, 60 °CC for 30 s, and 72 °C for 30 s. Consequently, at the end of the PCR cycles, the real-time PCR products were immediately analyzed using a ramping rate of 0.03 °C/s from 60 to 95 °C in order to calculate the dissociation curve to verify the correctness of the amplicons. None-template controls for each primer pair were also included to examine potential external DNA contamination. The gene specific threshold cycle (Ct) for each sample was corrected by subtracting the Ct for the housekeeping gene β-actin (ΔCt). Untreated controls were chosen as the reference samples, and the ΔCt for all experimental samples were subtracted by the ΔCt for the control samples (ΔΔCt). The magnitude change of test gene mRNA was expressed as $2^{-\Delta\Delta Ct}$. Each measurement of a sample was conducted in duplicate.

2.4. Flow cytometry analysis

P815 cells were pelleted by centrifugation at 450 g for 10 min, and then fixed in 2% paraformaldehyde for 30 min. After washing, the cells were re-suspended in PBS. For PAR-2 staining, cells were

Table 1
Primer sequences for mouse PARs used in real time PCR.

Primer		Sequence	Size of product (bp)
PAR-1	Sense	5′-GTTGATCGTTTCCACGGTCT-3′	225
	Antisense	5′-ACGCAGAGGAGGTAAGCAAA-3′	
PAR-2	Sense	5′-CACCTGGCAAGAAGGCTAAG-3′	298
	Antisense	5′-CCCAGGGTTACTGACGCTAA-3′	
PAR-3	Sense	5′-TCAATGGCAACAACTGGGTA-3′	205
	Antisense	5′-AAAACCATGACCCACACCAT-3′	
PAR-4	Sense	5′-GCAGACCTTCCGATTAGCTG-3	292
	Antisense	5′-AGGGCTCGGGTTTGAATAGT-3′	
β-actin	Sense	5′-GCTACAGCTTCACCACCACAG-3′	288
	Antisense	5′-GGTCTTTACGGATGTCAACGTC-3′	