European Journal of Pharmacology 675 (2012) 57–62

Contents lists available at SciVerse ScienceDirect

European Journal of Pharmacology

journal homepage: www.elsevier.com/locate/ejphar

Immunopharmacology and Inflammation

C-Kit controls IL-1β-induced effector functions in HMC-cells

Sebastian Drube *, Frederike Schmitz, Christiane Göpfert, Franziska Weber, Thomas Kamradt

Institut für Immunologie, Universitätsklinikum Jena-Friedrich-Schiller-Universität Jena, 07743 Jena, Germany

ARTICLE INFO

Article history:
Received 25 May 2011
Received in revised form 16 November 2011
Accepted 27 November 2011
Available online 8 December 2011

Keywords:
IL-1β
c-Kit
Cross-activation
Mast cell

ABSTRACT

The receptor tyrosine kinase c-Kit is important for mast cell differentiation, proliferation, and cytokine release. Recently, we reported that c-Kit acts as an intermediate signalling molecule regulating IL-33-induced signalling and effector functions in mast cells. Here, we investigated the influence of c-Kit on the IL-1β-induced signalling and effector functions in HMC mast cell lines. HMC-cells were stimulated with IL-1β and the resulting signalling and cytokine responses were analysed. Furthermore, we used pharmacological inhibitors to investigate the relevance of several signalling molecules for the IL-1β-induced signalling and cytokine responses. Treatment of HMC-cells with the c-Kit inhibitor STI571 blocked the IL-1β-induced activation of Erk1/2 and JNK1/2 but not p38 and NFκB. Furthermore, inhibition of these signalling pathways blocked the IL-6 production in HMC-cells. These findings indicate that IL-1β-induced signalling in mast cells branches into c-Kit- dependent and -independent pathways, both relevant for IL-6 release. Therefore, c-Kit is an important regulator of IL-1 receptor 1-induced signalling and effector functions in HMC-cells.
© 2011 Elsevier B.V. All rights reserved.

1. Introduction

Interleukin (IL)-1 and IL-33 are members of the IL-1 family and their receptors belong to the Toll/interleukin-1 receptor (TIR) superfamily. Members of this superfamily are crucial for protective and pathogenic host responses to injury and infection (Arend et al., 2008; Liew et al., 2010; Nold et al., 2010). IL-33 and IL-1β use similar receptor systems and activate similar signalling pathways. Signalling via the IL-1 receptor type 1 (IL-1 receptor 1) and IL-33 receptor is dependent on the IL-1 receptor accessory protein (IL-1RAcP). Stimulation of the IL-1 receptor 1 or the IL-33 receptor induces a ligand dependent association with the IL-1 receptor accessory protein (Ali et al., 2007; Chackerian et al., 2007; Wesche et al., 1997). Similar to other members of the TIR superfamily, the cytosolic TIR domain of the IL-1 receptor 1 and the IL-33 receptor dimerise with the TIR domain of the adaptor protein MyD88 (Brint et al., 2004; Schmitz et al., 2005). The IL-1 receptor 1-associated kinase (IRAK)-1, IRAK4 and the TNF-receptor-associated factor 6 (TRAF6) are subsequently recruited and activate mitogen-activated protein kinases (MAPK) and the transcription factor NFκB (Ali et al., 2007; Ho et al., 2007; Iikura et al., 2007; Mitcham et al., 1996; Schmitz et al., 2005). Recently, we reported that the IL-33 receptor cross-activates the receptor tyrosine kinase c-Kit in the human mast cell line HMC-1.1, expressing a constitutively active c-Kit mutant. Furthermore, cross-activated c-Kit is required to mediate IL-33-induced cytokine release (Drube et al., 2010). Here we investigated whether mutated and constitutively active c-Kit also mediates IL-1β-induced signalling and effector functions.

Treatment of HMC-1.1-cells with the tyrosine kinase inhibitor STI571 inhibited IL-1β-induced activation of Erk1/2 and JNK1/2 whereas the activation of p38 and NFκB was not affected. This indicates that the IL-1β-induced signalling branches into c-Kit-dependent and -independent signalling in HMC-1.1-cells. Interestingly, both the c-Kit-dependent and -independent signalling pathways are necessary for IL-1β-induced cytokine release in HMC-1.1-cells.

2. Material and methods

2.1. Cell lines

The human mast cell lines HMC-1.1 and HMC-1.2 (provided by Dr. J. H. Butterfield, Mayo Clinic, Rochester, MN) and the murine mast cell line P815 were cultured in RPMI supplemented with 10% foetal calf serum (Sigma), 1% antibiotics (Biochrom) and 50 μM mercaptoethanol as previously described (Drube et al., 2010).

2.2. Flow cytometry

For annexin V staining, cells were left untreated or were treated with the tyrosine kinase inhibitor 4-[(4-methyl-1-piperazinyl)methyl]-N-[4-methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl] amino] phenyl]-methanesulfonate-benzamide (STI571) (5 μM) [provided by Dr. F.-D. Böhmer, Centre of Medical Biomedicine, Jena (Bohmer et al., 2003)] as indicated. Subsequently, cells were harvested and washed with PBA (0.25% BSA; 0.02% Natriumazide in PBS). Apoptotic cells were detected with FITC (5 (6)-fluorescein isothiocyanate)-conjugated annexin V according to manufacturer's instructions (BD Bioscience). For determination of IL-33 receptor- and c-Kit-surface expression, cells were harvested and washed

* Corresponding author at: Institut für Immunologie, Universitätsklinikum Jena, Leutragraben 3, 07743 Jena, Germany. Tel.: +49 3641 938783; fax: +49 3641 938782.
E-mail address: Sebastian.Drube@mti.uni-jena.de (S. Drube).

0014-2999/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejphar.2011.11.035

with PBA (0.25% BSA; 0.02% Natriumazide in PBS). Non-specific binding of antibodies was blocked with anti-CD16/CD32 (clone 2.4G2) and rat-IgG (Jackson). Cells were stained with biotinylated anti-human IL-33 receptor antibody (Baf523; R&D-systems), and PE (Phycoerythrin)-conjugated Streptavidin (eBioscience) or with anti-human-(Allophycocyanin (APC)-conjugated) c-Kit antibody (eBioscience). Cells were acquired using a LSRII flow cytometer (BD Bioscience) and evaluated with FlowJo 8.1.1 (Treestar Inc.).

2.3. Mice

Mice were kept under specific pathogen-free conditions. All experiments were conducted in accordance with federal, state, and institutional guidelines. We used sex- and age-matched wt C57BL/6 or $Kit^{W\text{-}sh/W\text{-}sh}$ (provided by Prof. M. Maurer, Charite, Berlin) for generation of bone marrow-derived mast cells (BMMCs).

2.4. BMMC generation

BMMCs were generated from the femoral bone marrow by culturing in complete IMDM (PAA) supplemented with 20 ng/ml rmIL-3 (Peprotech), 10% foetal calf serum, and 1% antibiotics. BMMCs were used after 4 weeks of culture and consisted of 95% mast cells as identified by the surface expression of Fcε-receptor I, c-Kit, and IL-33 receptor.

2.5. Cell stimulation and lysis

HMC1.1-, HMC1.2- and P815-cells (10^6/ml) were serum-starved over night. BMMCs were cultured in complete IMDM containing 10% foetal calf serum and 1% antibiotics without IL-3 1 h prior to stimulation. Cells were pre-incubated for 30 min with the soluble IL-33 receptor, the soluble IL-1 receptor 1 (both R&D), soluble c-Kit (Symansis), or with the tyrosine kinase inhibitor STI571. Subsequently, cells were stimulated with IL-1β (Peprotec). Cells were lysed with lysis buffer [20 mM HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid), pH7.5; 10 mM EGTA (ethylene glycol tetraacetic acid); 40 mM β-Glycerophosphate; 2.5 mM $MgCl_2$; 2 mM orthovanadate; 1 mM DTT (Dithiothreitol); 1 mM PMSF (phenylmethylsulfonyl fluoride); 20 µg/ml Aprotinin; 20 µg/ml Leupeptin supplemented with 1% Triton]. For ELISA experiments cells were pre-incubated for 30 min with the soluble IL-33 receptor, the soluble IL-1 receptor 1, soluble c-Kit, or inhibitors [the tyrosine kinase inhibitor, STI571, the MEK-inhibitor UO126 (1,4-Diamino-2,3-dicyano-1,4-bis (2-aminophenylthio) butadiene), the JNK-inhibitor SP600125 (Anthra [1,9-cd] pyrazol-6 (2H)-one, 1,9-pyrazoloanthrone), the p38-inhibitor (2-(4-Chlorophenyl)-4-(4-fluorophenyl)-5- pyridin-4-yl-1,2-dihydropyrazol-3-one) or the NFκB-inhibitor (6-Amino-4-(4-phenoxyphenylethylamino) quinazoline) (all Calbiochem)]. Supernatants were collected and analysed by ELISA for IL-4, IL-6, and IL-8 (Immunotools).

2.6. Immunoblotting

Lysates were separated on 10% SDS-Laemmli gels and transferred by electroblotting onto nitrocellulose membranes. Membranes were blocked with dry milk and incubated with primary antibodies detecting phosphorylated signalling molecules [anti-pT183/pY185-JNK-1/2, anti-pT183/pY182-p38, anti-pY719-c-Kit, anti-pS536-p65-NFκB, anti-pT202/pY204-p44/42 (all Cell Signalling Technology)], tubulin [as the loading control (Sigma)] or caspase-3 (Santa Cruz). Membranes were washed in 0.1% Tween/TBS and incubated with the respective HRP-conjugated secondary antibodies: anti-rabbit-Ig or anti-mouse-Ig (Pierce). Detection was performed using enhanced chemiluminescence (ECL) reagent (Pierce).

2.7. Proliferation assays

HMC-1.1-cells were cultured in presence or absence of STI571 for 72 h. [^{3}H]-thymidine (1 µCi) in 25 µl culturing medium was added to each well for the last 18 h. Incorporated radioactivity was measured using a scintillation-gamma counter (Perkin Elmer).

2.8. Statistical analysis

Cytokine concentration is indicated as the mean of quadruple measurements ± standard deviation. The statistical analysis was performed with SPSS 11.5. For significance analysis the Student's *t*-Test was used ($P<0.05$ marked with *).

3. Results

3.1. IL-1β-induced signalling and cytokine release in HMC-1.1-cells

HMC-1.1-cells express the IL-1 receptor 1 (Kandere-Grzybowska et al., 2006). Confirming and extending earlier results (Kandere-Grzybowska et al., 2006), we found that stimulation of HMC-1.1-cells with IL-1β induced a time-dependent activation of Erk1/2, JNK1/2, p38, NFκB (Fig. 1A), and the release of IL-6 (Fig. 1B) and IL-8 (data not shown) but not the release of IL-4 (data not shown). Next, we investigated the IL-1β-induced cytokine response in P815-cells. Stimulation of these cells with IL-1β induced a time-dependent IL-6 production (data not shown). These data show that IL-1β induced the activation of several signalling pathways and the production of cytokines in different mast cell lines.

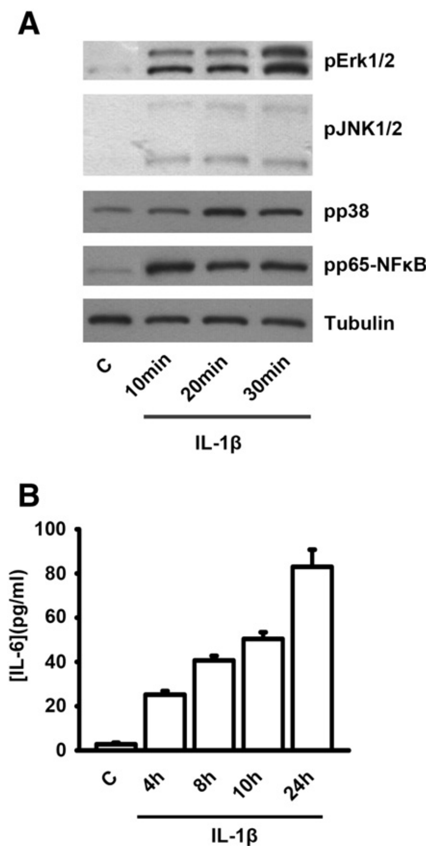

Fig. 1. IL-1β-induced signalling and cytokine release in HMC-1.1-cells. (A) HMC-1.1-cells were stimulated with IL-1β (50 ng/ml). Cell lysates were separated by SDS-PAGE, blotted, and probed with anti-pErk1/2, -pJNK1/2, -pp38, -pp65-NFκB, and -tubulin antibodies. (B) HMC-1.1-cells were stimulated with IL-1β (both 50 ng/ml) as indicated. Supernatants were analysed for IL-6.

Download English Version:

https://daneshyari.com/en/article/2532431

Download Persian Version:

https://daneshyari.com/article/2532431

Daneshyari.com