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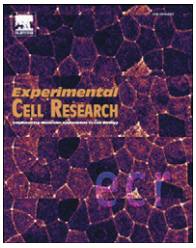

Research Article

Oncostatin M (OSM) primes IL-13- and IL-4-induced eotaxin responses in fibroblasts: Regulation of the type-II IL-4 receptor chains IL-4Rα and IL-13Rα1

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ABSTRACT

Oncostatin M (OSM), a pleiotropic cytokine and a member of the gp130/IL-6 cytokine family, has been implicated in regulation of various chronic inflammatory processes. Previous work has shown that OSM induces eosinophil accumulation in mouse lungs in vivo and stimulates the eosinophil-selective chemokine eotaxin-1 synergistically with IL-4 in vitro. To examine the role of receptor regulation by OSM in synergistic eotaxin-1 responses, we here examine the modulation of the type-II IL-4 receptor (IL-4Rα and IL-13Rα1) by OSM and other gp130/IL-6 cytokine family members using NIH3T3 fibroblasts and primary mouse lung fibroblasts. We first show that OSM with either IL-13 or IL-4 synergistically induces eotaxin-1 expression in a dose-dependent fashion. Analysis of IL-4Rα expression at the protein (Western blot and FACS) and RNA (TAQMAN) levels showed that OSM markedly elevates expression by 3 h. OSM enhanced IL-13Rα1 mRNA and induced a smaller but detectable increase in total IL-13Rα1 protein. Priming fibroblasts with OSM for 6 h markedly enhanced subsequent IL-13 and IL-4-induced eotaxin-1 responses and STAT6 tyrosine-641 phosphorylation. Regulation of IL-4Rα by OSM was sensitive to inhibition of the PI3′K pathway by LY294002. These studies provide novel mechanistic insights in OSM role in regulation of synergistic eotaxin-1 responses and IL-4Rα expression in fibroblasts.

Introduction

The T_H2 cytokines IL-4 and IL-13 are members of the IL-4 cytokine family [1] that posses redundant biological activities including induction of IgE synthesis and expression of the low affinity IgE receptor (CD23) on B cells [2]. IL-4 and IL-13, products of T_H2 lymphocytes, are implicated in mechanisms of allergic disease processes. Indeed, IL-4 and IL-13 protein levels are increased in bronchoalveolar lavage fluid (BALF) of asthmatics following segmental allergen provocation [3] as are levels of the eosinophil-selective chemokine eotaxin-1 [4]. Both IL-4 and IL-13 stimulate the expression of eotaxin-1 by various airway structural cells [5–8], which induces tissue eosinophilia in vivo [9,10]. Selective activity of eotaxin-1 on eosinophil chemoattraction has been demonstrated in eotaxin-1 deficient mice that display significantly attenuated peripheral blood and airway eosinophilia following allergen exposure in animal models of airway disease [11]. In clinical studies, patients with atopic asthma have elevated expression of eotaxin-1 in plasma [12], BALF [13] and bronchial mucosa [13–15], which is correlated with increased number of eosinophils at sites of inflammation [14] and compromised lung function [12,15].

Both IL-4 and IL-13 engage and signal through the type-II IL-4 receptor (IL-4R) (also known as IL-13R) which is comprised of the

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IL-4Rα [16–19] and IL-13Rα1 [20–22] chains. In addition, IL-4, but not IL-13, binds the type-I IL-4R composed of IL-4Rα and IL-2Rγ (common gamma (γc)) chains [23,24]. It is believed that the type-II IL-4R is the main route by which IL-4 and IL-13 signal in non-hematopoietic cells whereas the type-I IL-4R is thought to be mainly expressed on hematopoietic cells [22]. Interleukin-4 and IL-13 are known to induce tyrosine (Y)-641 phosphorylation of signal transducer and activator of transcription (STAT)6 in an IL-4Rα-dependent manner [25]. Studies have shown that STAT6 is necessary, but not sufficient, for activation of IL-4Rα gene transcription in response to IL-4 stimulation [26]. Furthermore, STAT6 has been demonstrated to mediate eotaxin-1 expression in response to IL-4 or IL-13 [5–8] and studies have conclusively shown that both STAT6 [27–30] and IL-4Rα [30] are critical factors in the induction of asthma-like phenotype in animal models of airway disease.

Oncostatin M (OSM) can be expressed by activated macrophages, T cells and granulocytes and thus has potential roles in chronic inflammation involving activation of such cells at tissue sites. OSM is a member of the gp130/IL-6 family of cytokines that bind and signal through receptor complexes that include the 130-kDa glycoprotein subunit designated as gp130 [31]. In the mouse system, mouse (m) OSM binds only the type-II OSM receptor (OSMR) complex comprised of the OSMRβ and gp130 chains, whereas in human cells, human OSM can engage both type-II and type-I OSMRs, the latter known as the LIFR (OSMRβ/LIFRβ) [32]. Stimulation through type-II OSMR leads to the recruitment and activation of three primary signaling pathways: (a) Janus-activated kinase (JAK)/signal transducer and activator of transcription (STAT) (STAT1, STAT3, STAT5 and STAT6) [33,34]; (b) Ras/MAPK (extracellular-signal regulated kinase, ERK) [33]; and (c) insulin receptor substrate (IRS)/phosphoinositol-3′-kinase (PI3K) pathway [35,36].

Recent studies show that OSM may participate in the pathogenesis of chronic allergic eosinophilic disease states including allergic rhinitis [37], atopic dermatitis [38] and possibly asthma [34,39–41]. We have demonstrated previously through an overexpression system that transient ectopic expression of OSM in C57BL/6 mouse lungs induces lung tissue remodeling and airway eosinophilic inflammation in vivo and this was associated with OSM induction of eotaxin-1 in vitro [41]. OSM induces eotaxin-1 in NIH3T3 cells and in primary mouse lung fibroblasts (in an STAT6-independent manner) in vitro [34,41]. Studies also demonstrated that treatment of mouse lung fibroblasts [34] or human airway smooth muscles cells [40] with OSM in combination with IL-4 induced eotaxin-1 expression synergistically. Faffe and colleagues [40] found that OSM was able to induce surface IL-4Rα upregulation in human airway smooth muscle cells [40]; however, regulation of the IL-4R in other cells or by other gp130 cytokines was not investigated. Furthermore, the mechanism by which OSM enhances eotaxin-1 expression in the context of IL-4/IL-13 costimulation was not thoroughly explored.

In this study, we demonstrate that mOSM in combination with either mIL-13 or mIL-4 synergistically induces eotaxin-1 expression in mouse NIH3T3 fibroblasts. Unlike other gp130/IL-6 cytokines, mOSM was an effective inducer of IL-4Rα and IL-13Rα1 expression and primed fibroblasts for enhanced mIL-13 and mIL-4 induced STAT6 activation or eotaxin-1 expression. We go on to examine the regulation of IL-4Rα and IL-13Rα1 chains by pharmacological means and in fibroblasts lacking functional STAT6 signaling.

Materials and methods

Cell lines

NIH3T3 embryonic fibroblasts cells were obtained from the American Type Culture Collection (ATCC) and maintained in DMEM culture media supplemented with 10% calf serum (Invitrogen Life Technologies), 1.0% penicillin–streptomycin and 0.1% fungizone. Primary wild-type (wt) or STAT6$^{-/-}$ C57BL/6 mouse lung fibroblast (MLF) cell lines were derived from explants of finely minced lung tissue from wt C57BL/6 mice (10–12 weeks old; Charles River Laboratories, Wilmington, MA) or STAT6$^{-/-}$ mice (10–12 weeks old; provided by Dr. Z. Xing and Dr. Y. Wan, McMaster University, Ontario, Canada). MLF were cultured in Earle's modified MEM (F-15) medium supplemented with 10% fetal bovine serum (FBS), 1.0% penicillin–streptomycin, 1.0% L-glutamine and 0.1% fungizone.

Cytokines and reagents

Stimulations were performed in vitro on fibroblast monolayers using recombinant mouse (rm) OSM, rmLIF, rmIL-6, rmCT-1, rmIL-11, rmIL-4 or rmIL-13 purchased from R&D systems. Pharmacological inhibitors PD98059 (PD), SB203580 (SB) and LY294002 (LY) were purchased from Calbiochem, whereas cycloheximide was from Sigma-Aldrich. In experiments involving pharmacological inhibitors, fibroblasts were pretreated with 25 μM of PD98059 for 2.0 h; 10 μM of SB203580 for 1.0 h; 20 μM of LY294002 for 0.5 h; or 50 μg/ml of cycloheximide for 0.5 h before cytokine stimulation. Primary antibodies (Abs) specific for phospho-tyrosine (pY)-705 STAT3, pY-641 STAT6 and total STAT3 were purchased from Cell Signaling Technology (New England Biolabs). Primary Abs specific for total STAT6 (M-20), IL-4Rα (M-300), IL-13Rα1 (H-300) and Actin (I-19) were purchased from Santa Cruz Biotechnology. Primary Abs against phospho-STAT and total STAT3 were diluted 1:1000 in 5.0% BSA (Sigma-Aldrich). Primary Abs against total STAT6, IL-4Rα and Actin were diluted at 1:1000 and primary Ab against IL-13Rα1 was diluted at 1:800 in 5.0% milk. For detection of primary Abs against phospho-STAT, total STAT, IL-4Rα (M-300) and IL-13Rα1 (H-300), secondary goat anti-mouse IgG HRP Ab was purchased from Santa Cruz Biotechnology. Goat anti-mouse IgG HRP Ab was diluted 1:2500 in 5.0% milk. For detection of primary Actin (I-19) Ab, rabbit anti-goat IgG HRP Ab was purchased from Sigma-Aldrich. Rabbit anti-goat IgG HRP Ab was diluted 1:4500 in 5.0% milk.

Cell lysates, Western blot and ELISA

Cell lysates were prepared and Western blots generated as published previously [34]. Culture supernatant levels of mouse eotaxin-1 were measured using ELISA. Subconfluent NIH3T3 fibroblast cultures (~40%) were stimulated for 18–24 h in 2% serum-supplemented medium; supernatants were collected and stored at −20°C until analysis. Mouse eotaxin-1 DuoSet ELISA kits were purchased from R&D Systems and used according to the manufacturer's instructions. Sensitivity of mouse eotaxin-1 DuoSet ELISA kit is ≤7.5 pg/ml.

RNA purification and analysis by RT-PCR (Taqman)

Subconfluent NIH3T3 fibroblast cultures (~40%) were stimulated for 6 or 18 h with the indicated cytokines in culture medium