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Original Contribution

IL-4 and IL-13 employ discrete signaling pathways for target gene expression in alternatively activated monocytes/macrophages

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ABSTRACT

Monocytes/macrophages are innate immune cells that play a crucial role in the resolution of inflammation. In the presence of the Th2 cytokines interleukin-4 (IL-4) and interleukin-13 (IL-13), they display an anti-inflammatory profile and this activation pathway is known as alternative activation. In this study we compare and differentiate pathways mediated by IL-4 and IL-13 activation of human monocytes/macrophages. Here we report differential regulation of IL-4 and IL-13 signaling in monocytes/macrophages starting from IL-4/IL-13 cytokine receptors to Jak/Stat-mediated signaling pathways that ultimately control expression of several inflammatory genes. Our data demonstrate that although the receptor-associated tyrosine kinases Jak2 and Tyk2 are activated after the recruitment of IL-13 to its receptor (containing IL-4Rα and IL-13Rα1), IL-4 stimulates Jak1 activation. We further show that Jak2 is upstream of Stat3 activation and Tyk2 controls Stat1 and Stat6 activation in response to IL-13 stimulation. In contrast, Jak1 regulates Stat3 and Stat6 activation in IL-4-induced monocytes. Our results further reveal that although IL-13 utilizes both IL-4Rα/Jak2/Stat3 and IL-13Rα1/Tyk2/Stat1/Stat6 signaling pathways, IL-4 can use only the IL-4Rα/Jak1/Stat3/Stat6 cascade to regulate the expression of some critical inflammatory genes, including 15-lipoxygenase, monoamine oxidase A (MAO-A), and the scavenger receptor CD36. Moreover, we demonstrate here that IL-13 and IL-4 can uniquely affect the expression of particular genes such as dual-specificity phosphatase 1 and tissue inhibitor of metalloprotease-3 and do so through different Jaks. As evidence of differential regulation of gene function by IL-4 and IL-13, we further report that MAO-A-mediated reactive oxygen species generation is influenced by different Jaks. Collectively, these results have major implications for understanding the mechanism and function of alternatively activated monocytes/macrophages by IL-4 and IL-13 and add novel insights into the pathogenesis and potential treatment of various inflammatory diseases.

Interleukin-4 (IL-4)[1] and IL-13 are Th2 cytokines in the immune system that exhibit a wide range of activities in regulating inflammatory responses and are thought to play significant roles during allergic reactions [1]. However, these cytokines primarily act as anti-inflammatory molecules [2]. IL-13 shares many biological activities with IL-4. Both cytokines reduce production of IL-1, tumor necrosis factor-α (TNF-α), and other proinflammatory mediators, whereas they upregulate the expression of several markers such as IL-1Rα, mannose receptor, dectin-1, CD23, etc., by monocytes/macrophages [3]. Both IL-4 and IL-13 antagonize the actions of interferon-γ (IFN-γ) [3], significantly enhance the ability of activated human monocytes to oxidize low-density lipoprotein (LDL) [4], and reduce inflammation in various animal models of arthritis [5–7]. In addition, IL-4 has been shown to downregulate the production of prostaglandin E2 by synovial macrophage-type cells through the inhibition of cyclooxygenase 2 [8], whereas IL-13 has been reported to induce tissue fibrosis by the stimulation and activation of TGF-β [9].

IL-4 and IL-13 are hallmark cytokines of Th2-associated diseases, including asthma. IL-13 mediates central characteristics of allergic asthma, including immunoglobulin E synthesis, mucus hypersecretion, airway hyperresponsiveness (AHR), and fibrosis [10]. Both IL-4 and IL-13 signal through a complex receptor system including the IL-4Rα chain. In human monocytes, IL-4 mediates its effect through the type I IL-4R, composed of the IL-4Rα and common γ-chain (IL-2Rγ) [11]. In contrast, IL-13 primarily exerts its IL-4Rα-dependent effect via type II IL-4R

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[1] *Abbreviations used:* IL, interleukin; 15-LO, 15-lipoxygenase; ODN, oligodeoxyribonucleotide; MAO-A, monoamine oxidase A; ROS, reactive oxygen species; Jak, Janus kinase; Stat, signal transducer and activator of transcription.

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containing a different component, IL-13Rα1 [11]. Previously IL-13Rα1 was identified as a fundamental receptor mediating IL-13- and IL-4-induced AHR, mucus production, and fibrosis in response to the "classical" experimental asthma model [12]. Further studies have demonstrated a key role for IL-13Rα1 and the type II IL-4R in lung Th2 responses and asthma pathogenesis [12,13]. Recent studies show the differential regulation of IL-13Rα1 in aeroallergen-induced lung responses [14]. Strategies targeting IL-13Rα1 for anti-asthma therapy are currently under way [15] and require further elucidation.

IL-4 and IL-13 have been classified as alternative macrophage (M2a-phenotype) activators [16–18]. Previous studies suggested that in human peripheral blood monocytes IL-4/13 significantly downregulate the expression of classical proinflammatory signal transducers, such as IL-1, IL-6, IL-8, IL-18, monocyte chemotactic protein-1, and TNF-α [19]. Expression of enzymes involved in the biosynthesis of proinflammatory eicosanoids, e.g., cyclooxygenase-2 and 5-lipoxygenase, is also attenuated [19]. In contrast, expression of some gene products involved in inflammatory resolution (15-lipoxygenase (15-LO), fibronectin (FN), monoamine oxidase-A (MAO-A), coagulation factor XIII (FXIII), annexin 1, collagen 1α2, laminin α5, heme oxygenase-1, C-C motif chemokine 22, heat shock protein 8, etc.) were upregulated in monocytes upon exposure to IL-4/IL-13 [19]. Among the most strongly upregulated gene products in alternatively activated monocytes/macrophages with potential anti-inflammatory properties are 15-LO [19–21], MAO-A [19,22,23], scavenger receptor CD36 [23–25], FN [19,26], and FXIII [19].

Our recent studies also identify Hck as the essential Src kinase isoform that regulates the expression of a panel of genes including 15-LO, MAO-A, and CD36 in alternatively activated monocytes/macrophages [23]. Moreover, our recent results present evidence that Stat transcription factors that regulate 15-LO expression are also involved in controlling both CD36 (V. Yakubenko et al., submitted for publication) and MAO-A (data presented in this article) expression in IL-13-activated monocytes/macrophages.

In this study we explore both IL-4 and IL-13 signaling in monocytes/macrophages starting from the level of the IL-4/IL-13 receptor to Jak/Stat-mediated signaling pathways and investigate the differential expression of several inflammatory genes mediated by these two cytokines. Our data demonstrate that whereas IL-13 utilizes both IL-4Rα/Jak2/Stat3 and IL-13Rα1/Tyk2/Stat1/Stat6 signaling cascades to regulate 15-LO, MAO-A, and CD36 gene expression, IL-4 can use only the IL-4Rα/Jak1/Stat3/Stat6 axis to control the expression of these genes. Furthermore, we present evidence that IL-13 and IL-4 uniquely induce the gene expression of dual-specificity phosphatase 1 (DUSP1) and tissue inhibitor of metalloprotease-3 (TIMP3), respectively. Our results further show that generation of MAO-A-mediated reactive oxygen species (ROS) in monocytes/macrophages is also regulated by different Jaks upon exposure to IL-4 and IL-13. These results add novel insights into the regulation of IL-4/IL-13-mediated asthma pathogenesis as well as in the control of inflammation and atherosclerosis.

Materials and methods

Materials

Recombinant human IL-13 and IL-4 were purchased from Biosource International (Camarillo, CA, USA). The rabbit reticulocyte 15-LO antibody, cross-reacting with human 15-LO, was raised in sheep and was obtained as a gift from Dr. Joseph Cornicelli (Molecular Imaging). Anti-phosphotyrosine-Stat (pY701-Stat1, pY705-Stat3, and pY641-Stat6) antibodies were purchased from Cell Signaling Technology (Beverly, MA, USA). Stat6 antibody was purchased from BD Pharmingen (San Diego, CA, USA). Anti-CD36 polyclonal antibody was from Cayman Chemical (Ann Arbor, MI, USA). The other primary antibodies used in this study were mouse anti-human p-Tyr (PY99) and anti-Jak1, Jak2, Jak3, Tyk2, MAO-A, and β-tubulin from Santa Cruz Biotechnology (Santa Cruz, CA, USA). The ROS-sensitive fluorescent probe 6-carboxy-2′,7′-dichlorodihydrofluorescein diacetate, diacetoxymethyl ester (H_2DCFDA), was from Life Technologies (Carlsbad, CA, USA). The Amplex red monoamine oxidase assay kit (Cat. No. A12214) was from Molecular Probes (Invitrogen, Eugene, OR, USA). MAO-GLO assay kit (Cat. No. V1401) and MAO-A enzyme (human recombinant enzyme expressed in yeast, Cat. No. V1452) were purchased from Promega (Madison, WI, USA). Tyramine and pharmacological inhibitors such as pargyline (a pan-MAO inhibitor) and moclobemide (specific inhibitor for MAO-A) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). The inhibitors were stored either at room temperature or at −20 °C as concentrated stock solutions (either in water or in dimethyl sulfoxide) according to the manufacturer's instructions.

Isolation of human monocytes

Human peripheral blood monocytes (PBMs) were isolated either by separation of mononuclear cells followed by adherence to bovine calf serum (BCS)-coated flasks as described earlier [27] or by Ficoll–Hypaque sedimentation followed by countercurrent centrifugal elutriation [28,29]. PBMs purified by these two methods were identical in response to IL-13 and IL-4 and consistently >95% $CD14^+$. These studies complied with all relevant federal guidelines and institutional policies regarding the use of human subjects.

Immunoprecipitation (IP) and immunoblotting

PBMs were routinely treated with IL-13 (2 nM) or IL-4 (670 pM) for various time intervals (these doses were chosen because they demonstrated comparable 15-LO induction in monocytes in numerous previous studies performed by our group). In some specific experiments, primary monocytes were stimulated with both IL-13 and IL-4 at 2 nM and results qualitatively similar to those reported here were observed. Total and postnuclear extracts were prepared by previously published protocols [11,30]. After determining the protein concentration using the Bio-Rad protein assay reagent (Hercules, CA, USA), lysate proteins (50 μg/well) were resolved by 8% SDS–PAGE and subjected to immunoblotting as described previously [31]. 15-LO protein was detected on Western blots after a previously described protocol [27]. Immunoprecipitation experiments were performed according to our previously published method [32] using prewashed protein A–Sepharose beads (Sigma) at 4 °C overnight. Immunoblots were stripped and reprobed to assess equal loading according to our previously published protocol [11].

RNA extraction and real-time RT-PCR

Monocytes (5×10^6 in 2 ml 10% BCS/Dulbecco's modified Eagle's medium) were plated in six-well culture plates, treated with antisense or decoy oligodeoxyribonucleotides (ODNs), and finally treated with IL-13 (2 nM) or IL-4 (670 pM) for 24 h. Total cellular RNA was extracted using the RNeasy mini kit from Qiagen (Valencia, CA, USA) and real-time quantitative RT-PCR was performed according to established protocols [33]. The sequences of the primers used were 15-LO forward, 5′-GCTGGAAGGATCTAGATGACT-3′, and 15-LO reverse, 5′-TGGCTACAGAGAATGACGTTG-3′; CD36 forward, 5′-CAGAGGCTGACAACTTCACAG-3′, and CD36 reverse, 5′-AGGGTACGGAACCAAACTCAA-3′; MAO-A forward, 5′-GCCAAGATTCACTTCAGACCAGAG-3′, and MAO-A reverse,