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Identification of potent antagonist antibodies against mouse IL-13Rα1 using novel bioassays

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ABSTRACT

Interleukin-13 (IL-13) is a cytokine implicated in airway diseases such as asthma and idiopathic pulmonary fibrosis. IL-13 signals through a heterodimeric receptor complex consisting of IL-13Rα1 and IL-4Rα, known as the type II IL-4R. IL-4 also signals through this receptor and as such many of the biological effects of IL-13 and IL-4 are similar. Here we describe the development of two sensitive bioassays to determine the potency of antagonists of the mouse type II IL-4R. Both IL-13 and IL-4 dose-dependently induce CCL17 production from J774 mouse monocytic cells and CCL11 production from NIH3T3 mouse fibroblasts in the presence of TNFα. The assays were optimized to minimize TNFα concentration, cell number and incubation time whilst retaining a suitable signal-to-background ratio. Anti-cytokine antibodies or recombinant soluble receptors completely neutralized IL-13 or IL-4 activity in these bioassays. The J774 assay was used to screen a panel of anti-mIL-13Rα1 antibodies for neutralizing activity against this receptor. We report the identification of the first monoclonal antibodies that bind mouse IL-13Rα1 and neutralize both IL-13-induced and IL-4-induced cellular function. These antibodies should prove useful for determining the effects of neutralizing IL-13Rα1 in mouse models of disease. In addition, these bioassays may be used for measuring the bioactivity of mouse IL-13 and IL-4 and for the discovery of additional antagonists of the mouse IL-13Rα1/IL-4Rα complex.

1. Introduction

Interleukin-13 (IL-13) and IL-4 are closely related cytokines that share a common heterodimeric receptor, termed the type II IL-4R, consisting of IL-13Rα1 and IL-4Rα. As IL-13 and IL-4 can signal through the same receptor many of the functions of these cytokines are shared. However, IL-4 can also signal through a unique receptor termed the type I receptor (IL-4Rα and the common γ-chain (γc)) which is not activated by IL-13.

Many non-hematopoietic cells express the type II receptor, including fibroblasts, smooth muscle cells and mucus secreting goblet cells (Grünig et al., 1998; Wills-Karp et al., 1998; Zhu et al., 1999). Although these cells respond to both IL-4 and IL-13, it appears that IL-13 is the main effector

Abbreviations: CCL, CC chemokine ligand; IL, interleukin; IL-4Rα, interleukin-4 receptor α; IL-13Rα1, interleukin-13 receptor α1; IL-13Rα2, interleukin-13 receptor α2; mAb, monoclonal antibody; HRP, horseradish peroxidase; FBS, fetal bovine serum; PBS, phosphate-buffered saline; γc, common-gamma chain; TNFα, tumor necrosis factor α; TGFβ, transforming growth factor β.

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cytokine acting *in vivo* as neutralizing IL-13 but not IL-4 blocks airway hypersensitivity, mucus production and fibrosis in mouse models of human Th2 inflammatory disease (Chiaramonte et al., 1999; Corry et al., 1996; Grünig et al., 1998; Wills-Karp et al., 1998; Zhu et al., 1999). Macrophages and blood monocytes express both type I and type II receptors and stimulation of these cells with either IL-4 or IL-13 induces an "alternative" activation state (Doherty et al., 1993; Doyle et al., 1994; Stein et al., 1992). Alternative activation of macrophages leads to the production of various chemokines involved in the recruitment of cells associated with a Th2 response and to expression of the low affinity IgE receptor (CD23) on the cell surface. In addition, it results in the production of proteins that contribute to defense against helminths and fungal infections and that mediate tissue remodeling and anti-inflammatory responses (Van Dyken and Locksley, 2013). In mice, T cells express both the IL-4Rα chain and the γc chain but lack expression of IL-13Rα1. Therefore, IL-4 but not IL-13 signaling through the type I receptor can promote differentiation of naïve $CD4^+$ T cells into T helper type 2 (Th2) cells.

Genome wide association studies in humans have identified polymorphisms in the genes encoding both IL-13 and the IL-13R that are associated with asthma (Graves et al., 2000; Howard et al., 2002; Ober et al., 2000). Many studies in mice have also implicated IL-13 as a major cytokine regulating airway hyper-reactivity. Recently, mice with genetic deletions of IL-13Rα1 have been described that define distinct roles for the type II IL-4R in mediating features of human asthma in mouse models of airways disease such as airway hyperreactivity, mucus hypersecretion, chemokine (CCL2, CCL11, CCL17 and CCL24) and TGFβ production, as well as IgE production (Munitz et al., 2008; Ramalingam et al., 2008). The scientific evidence that implicate the IL-13 pathway in airways disease has prompted the development of new monoclonal antibody therapeutics that target the IL-13 pathway, including anti-IL-13, anti-IL-4, anti-IL-4Rα and anti-IL-13Rα1 antibodies (Caruso et al., 2013). Phase 2 clinical trials of anti-IL-13 antibodies have demonstrated efficacy in asthmatic patients, with better responses found in patients with either elevated serum IgE levels and eosinophil counts or elevated serum periostin, a molecule whose expression can be directly regulated by IL-13 (Corren et al., 2011). Correspondingly, in phase 2 trials with an anti-IL-4Rα monoclonal antibody there was an ~80% reduction in asthma exacerbations compared to placebo when controller medications were withdrawn (Wenzel et al., 2013). We have developed an anti-hIL-13Rα1 monoclonal antibody that neutralizes the activity of both human IL-13 and IL-4 signaling through the type II receptor (PCT Patent Publication WO 2008/060813; Redpath et al., 2013). However, this antibody does not cross react with the mouse IL-13Rα1, which prevents the investigation of neutralizing this receptor in mouse models of disease.

In order to screen for antibodies that target mouse IL-13Rα1 and neutralize both mouse IL-13 and IL-4 signaling through the type II IL-4R, we developed bioassays in mouse cell lines that endogenously express IL-13Rα1 and IL-4Rα and that are relevant to airway disease. The mouse J774 macrophage and NIH3T3 fibroblast cell lines were found to produce CCL17 (TARC) and CCL11 (eotaxin) respectively in response to both mouse IL-13 and IL-4 when co-treated with TNFα. These assays were used to determine the potency of known antagonists of mouse IL-4 and IL-13 activity and to identify novel anti-mIL-13Rα1 neutralizing monoclonal antibodies.

2. Materials and methods

2.1. Reagents

J774 and NIH3T3 cell lines were purchased from ATCC (cat. no.: TIB-67 and CRL-1658) and were maintained in RPMI (Sigma, cat. no.: R0883) with 10% fetal calf serum (Thermo Scientific, cat. no.: SV30176.03), 1 X Penicillin/Streptomycin (GIBCO®, Life Technologies, cat. no.: 15140-122), 1 X glutamax (GIBCO®, Life Technologies, cat. no.: 35050-061) at 37 °C, 5% CO_2. FreeStyle™ 293-F (FS293F) cells and the mammalian expression vector pcDNA3.1 were obtained from Invitrogen. FS293F cells were cultured in FreeStyle™ 293 Expression Medium (Life Technologies) supplemented with penicillin/streptomycin/fungizone reagent (GIBCO®, Life Technologies) and cells were maintained at 37 °C in incubators with an atmosphere of 8% CO_2. Mouse CCL11 duoset (cat. no.: DY420) and mouse CCL17 duoset (cat. no.: DY529) ELISA kits were from R&D Systems. Mouse IL-4, IL-13, TNFα and anti-IL-4 (clone 30340) and anti-IL-13 (clone 38213) antibodies were from R&D Systems.

2.2. J774 and NIH3T3 bioassays

Cells were de-attached from tissue culture flasks with accutase® (Sigma), counted and plated at 1×10^5 cells per well (unless stated otherwise) in 96-well flat bottom tissue culture plates (TPP). Cells were incubated at 37 °C, 5% CO_2 for 4 h to allow cells to adhere prior to stimulation with TNFα and IL-4 or IL-13 as indicated for 24 h unless otherwise stated. For J774 assays where the potency of monoclonal antibodies or soluble receptor-Fc fusion constructs were determined cells were pre-incubated with Fc-Block™ (Miltenyi Biotec) for 10 min prior to addition of test article. Supernatants were collected and assayed for either CCL11 or CCL17 by ELISA.

2.3. CCL11 and CCL17 enzyme-linked immunosorbent assays

CCL11 and CCL17 ELISAs were performed using duoset ELISA kits (R&D Systems, cat. no.: DY320 and DY364) according to manufacturer's instructions. Briefly, maxisorb 96 well plates (Nunc) were coated overnight with capture antibody in PBS (Thermo Scientific) then blocked in 1% BSA (Miltenyi Biotec) before standards or test cell culture supernatants were added. Captured chemokine was detected by addition of biotinylated detection antibody followed by streptavidin-HRP and TMB substrate solution (KPL, cat. no.: 50-76-00). Reactions were stopped with 2 N H_2SO_4 and the absorbance at 450 nm measured on a plate reader (Envision, Perkin Elmer).

2.4. Expression and purification of recombinant receptor-Fc proteins

The cDNAs encoding the extracellular domains of mouse interleukin-13 receptor alpha 1 (Mu-sIL13Rα1 aa1-331; GenBank Accession no. NP_598751), mouse interleukin-13

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