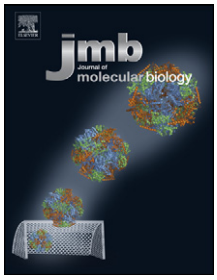

Structural Characterisation Reveals Mechanism of IL-13-Neutralising Monoclonal Antibody Tralokinumab as Inhibition of Binding to IL-13Rα1 and IL-13Rα2

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Abstract

Interleukin (IL)-13 is a pleiotropic T helper type 2 cytokine frequently associated with asthma and atopic dermatitis. IL-13-mediated signalling is initiated by binding to IL-13Rα1, which then recruits IL-4Rα to form a heterodimeric receptor complex. IL-13 also binds to IL-13Rα2, considered as either a decoy or a key mediator of fibrosis. IL-13-neutralising antibodies act by preventing IL-13 binding to IL-13Rα1, IL-4Rα and/or IL-13Rα2. Tralokinumab (CAT-354) is an IL-13-neutralising human IgG4 monoclonal antibody that has shown clinical benefit in patients with asthma. To decipher how tralokinumab inhibits the effects of IL-13, we determined the structure of tralokinumab Fab in complex with human IL-13 to 2 Å resolution. The structure analysis reveals that tralokinumab prevents IL-13 from binding to both IL-13Rα1 and IL-13Rα2. This is supported by biochemical ligand–receptor interaction assay data. The tralokinumab epitope is mainly composed of residues in helices D and A of IL-13. It is mostly light chain complementarity-determining regions that are driving paratope interactions; the variable light complementarity-determining region 2 plays a key role by providing residue contacts for a network of hydrogen bonds and a salt bridge in the core of binding. The key residues within the paratope contributing to binding were identified as Asp50, Asp51, Ser30 and Lys31. This study demonstrates that tralokinumab prevents the IL-13 pharmacodynamic effect by binding to IL-13 helices A and D, thus preventing IL-13 from interacting with IL-13Rα1 and IL-13Rα2.

Introduction

Interleukin (IL)-13 is a cytokine secreted predominantly by CD+ T helper type 2 (Th2) cells that share a receptor component and many biological properties with IL-4. IL-13 mediates its biological effects by initially binding IL-13Rα1 on one side and then recruiting IL-4Rα on the opposite side to form a signal transducer and activator of transcription 6 signalling complex in which IL-13 is in the middle of the two receptors [1]. IL-13 also binds very tightly to IL-13Rα2 [2]. IL-13Rα2 lacks a significant cytoplasmic tail and is generally considered to be a decoy [3,4] involved in removing IL-13 by internalisation [5]. Supporting this decoy hypothesis, we have not yet identified a heterodimeric partner for IL-13Rα2 although other data have suggested IL-13:IL-13Rα2 interactions with activator protein 1 signalling and fibrosis [6].

Asthma is a complex, chronic and heterogeneous inflammatory disease characterised by airway hyper-responsiveness in association with airway inflammation. IL-13 has been shown to drive key

disease mechanisms in asthma including airway hyper-responsiveness, mucus hypersecretion, eosinophilia and fibrosis development [7]. Hence, there has been great interest in neutralising IL-13 as a therapeutic strategy for treating asthma. A key subtype of human asthma is termed Th2-high, characterised by both the presence of lung IL-13 and responsiveness to IL-13 neutralisation with therapeutic antibodies [8–11].

Atopic dermatitis is a chronic and pruritic inflammatory skin disease characterised by atopy, elevated Th2 responses, a defective skin barrier and a predisposition to infection by viruses and bacteria [12]. IL-4 and IL-13 have been implicated as central mediators in atopic dermatitis. Clear clinical benefit has been found following the administration of the IL-4Rα blocking antibody dupilumab [13] in trials of patients with atopic dermatitis. Trials evaluating the role of IL-13–neutralisation alone in atopic dermatitis are on-going (**NCT02347176**, **NCT02340234**).

Tralokinumab (CAT-354) is a fully human, IL-13-neutralising IgG4 monoclonal antibody with a very high affinity [14] for IL-13. Alanine scanning mutation analysis demonstrated that tralokinumab bound to IL-13 in helix D (**PCT/GB2004/003059**). Tralokinumab functionally neutralises IL-13 in a range of cell-based assays (IL-13Rα1:IL-4Rα interactions [15]) and has shown efficacy in moderate–severe asthma [10,11]. It is currently in pivotal phase 3 trials for moderate–severe asthma (STRATOS 1 and 2 [NCT02161757, NCT02194699]) as well as a phase 2 trial for atopic dermatitis (**NCT02347176**).

To support the ongoing clinical development of tralokinumab in both asthma and atopic dermatitis, we crystallised tralokinumab in complex with IL-13 and performed receptor–ligand interaction assays.

Results

Tralokinumab inhibits binding of IL-13 to both IL-13Rα1 and IL-13Rα2

Tralokinumab is a potent inhibitor of IL-13 pharmacodynamics [15]. To define the mechanism by which this occurs, we employed biochemical receptor–ligand interaction assays. Tralokinumab dose-dependently prevented 600 pM IL-13 from interacting with 10 nM IL-13Rα1 with a geometric mean 50% inhibitor

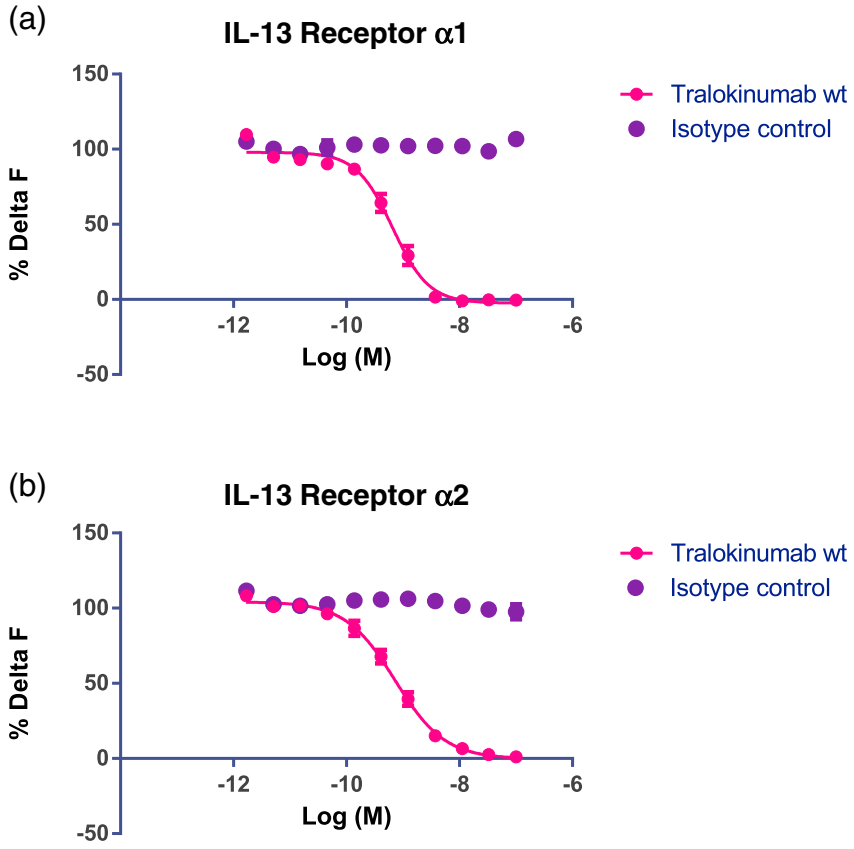

Fig. 1. Tralokinumab prevents IL-13 from interacting with both (a) IL-13Rα1 and (b) IL-13Rα2. Data are shown as mean % DeltaF (standard error of the mean) from two independent experiments each performed in duplicate.