

Tipping the balance: A biased nanobody antagonist of CCR3 with potential for the treatment of eosinophilic inflammation



James E. Pease, PhD, and Timothy J. Williams, PhD London, United Kingdom

Key words: Allergic inflammation, asthma, biased antagonist, CCR3, eosinophil, inflammation

To a large extent, eosinophil recruitment in both homeostasis and allergy is dependent on the eotaxin family of chemokines. CCL11/eotaxin-1 was the first chemokine discovered to have high selectivity for eosinophils through use of a guinea pig model of allergic airway disease.¹ After cloning of mouse and human eotaxin orthologues, CCR3 was identified as the eotaxin-1 receptor, which is expressed at high levels on the surfaces of eosinophils.² Two other eotaxins, CCL24/eotaxin-2 and CCL26/eotaxin-3, were discovered, both showing high selectivity for CCR3. CCR3 is a rather promiscuous receptor, binding almost half of the CC chemokine family, which exhibit varying selectivity and potency as chemoattractants. Consequently, CCR3 has become a magnet for novel therapeutic strategies aimed at targeting the eosinophil trafficking in disease, such as in eosinophilic asthma and esophagitis. One hurdle in drug development has been the limited homology between human and mouse CCR3, which has compromised the testing of some potent human CCR3 antagonists in mouse models of allergic disease.³

In this issue of the *Journal*, Grozdanovic et al⁴ describe the generation and characterization of the peptide-based CCR3 antagonist R321. The peptide has sequence identity with the regions of the second transmembrane (TM) helix and first extracellular loop of both human and mouse CCR3. Targeting G protein-coupled receptors (GPCRs) in such a fashion is not a new idea; indeed, peptide antagonists of GPCRs based on TM helices were reported in the literature as far back as 1999.⁵ However, a novel formulation was used by the authors in which polyethylene glycol was conjugated to the C-terminus, thereby limiting peptide aggregation and resulting in R321 assembling into nanospheres in aqueous conditions.

Assays of CCR3 activation in both human eosinophils and human CCR3 transfectants show that R321 has little if any agonist activity but selectively antagonizes G protein activation when cells are treated with CCR3 ligands. In mouse models of acute

allergic airways disease using a combination of allergens, R321 at a dose of 12 mg/kg administered intravenously shows excellent efficacy when used both prophylactically and therapeutically. This dose compares favorably with other CCR3 antagonists, which have often suffered from problems of bioavailability.³ As the authors state, it will be important to determine the efficacy of R321 in chronic models of allergic disease. Likewise, comprehensive pharmacokinetics are required to assess the half-life of R321 and any inherent toxicity.

Notably, R321 has no inhibitory activity in assays of ligand-mediated CCR3 endocytosis, unlike well-characterized small-molecule CCR3 antagonists, such as UCB35625.⁶ Such bias is unusual for a CCR3 antagonist, although perhaps not entirely unexpected, because there have been numerous reports that *pertussis* toxin treatment (which uncouples GPCRs from G α i proteins) has little or no effect on chemokine receptor endocytosis, suggesting the 2 pathways are clearly divergent. The authors suggest that the inability of R321 to inhibit CCR3 endocytosis means that eosinophils do not become tolerized to the compound because CCR3 is not trapped on the cell surface. Instead, CCR3 is internalized and is likely degraded to be replaced by *de novo*-synthesized CCR3.⁷ Interestingly, R321 has little activity alone in inducing CCR3 endocytosis. This is an important observation because some therapies rely on driving GPCR endocytosis, rendering cells unresponsive to ligand, such as treatment of prostate cancer by the gonadotropin-releasing hormone receptor agonist buserelin.

So how does R321 antagonize CCR3 at the molecular level? Activation of CCR3 is thought to conform to a multistep model in which CCL11 is tethered predominantly by interactions with the N-terminus of CCR3. This serves to orientate the chemokine such that the chemokine N-terminus can interact with the TM helices of the receptor, breaking several intrahelical interactions and ultimately stabilizing a receptor conformation that activates G protein and recruits arrestin. Notably, CCR3 shares considerable homology with CCR1 (64%), which also undergoes G protein-independent endocytosis and has been shown by Gilliland et al⁸ to exist as a homodimer precoupled to both the G α i protein required for signaling and β -arrestin required for endocytosis. Therefore we can surmise that CCR3 also exists as a homodimer (Fig 1, A).

By mimicking 13 amino acids of the upper portion of TM helix II, we can postulate that R321 breaks interhelical interactions within CCR3 that are required for the activation of G proteins but not for β -arrestin-dependent endocytosis (Fig 1, B). Grozdanovic et al⁴ showed that CCL11 is able to bind to CCR3 occupied by R321, which is in keeping with the predominant role of the CCR3 N-terminus in binding CCL11. Similarly, Sabroe et al⁶ showed that inhibitory concentrations of UCB32625, which binds within the CCR3 TM helices, were unable to displace ¹²⁵I-CCL11 from CCR3 transfectants.

Gilliland et al⁸ postulated that drugs that blocked G protein activation but were permissive for endocytosis might form a

From National Heart and Lung Institute, Imperial College London.

Disclosure of potential conflict of interest: J. E. Pease reports personal fees from Heptares Therapeutics outside the submitted work. T. J. Williams declares that he has no relevant conflicts of interest.

Received for publication August 24, 2018; revised October 8, 2018; accepted for publication October 23, 2018.

Available online November 17, 2018.

Corresponding author: James E. Pease, PhD, Inflammation, Repair & Development Section, National Heart & Lung Institute, Faculty of Medicine, Imperial College London, South Kensington Campus, Rm 353, Sir Alexander Fleming Building, London SW7 2AZ, United Kingdom. E-mail: j.pease@imperial.ac.uk.

J Allergy Clin Immunol 2019;143:552-3.
0091-6749/\$36.00

© 2018 American Academy of Allergy, Asthma & Immunology
<https://doi.org/10.1016/j.jaci.2018.10.052>

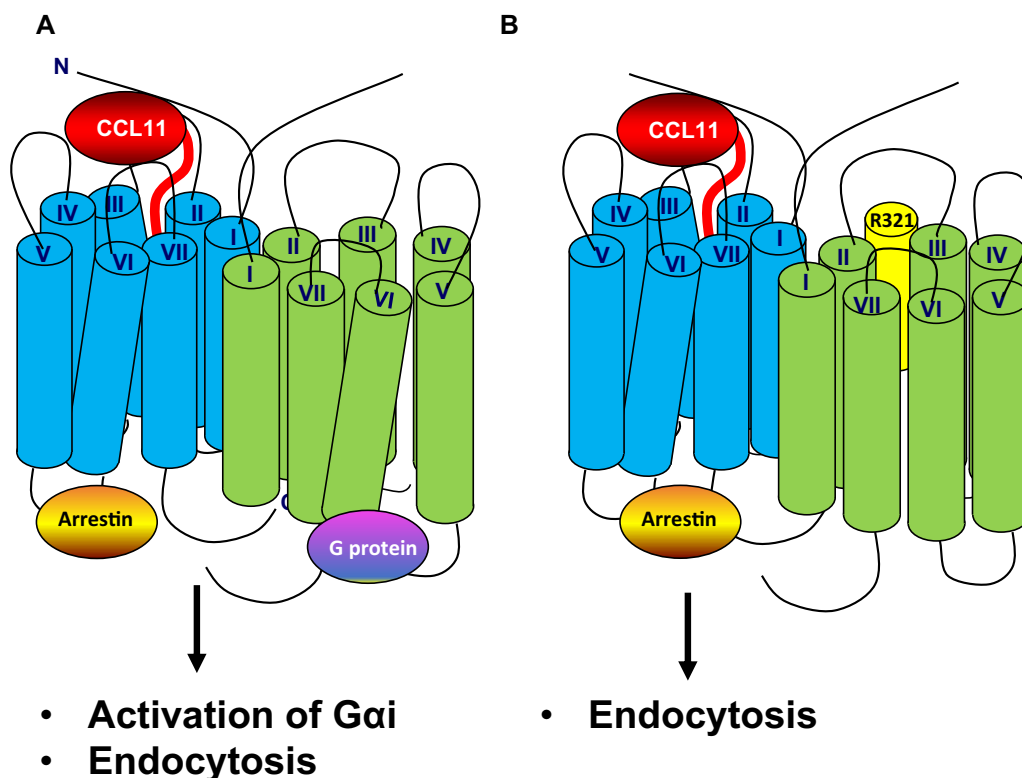


FIG 1. Schematic depicting how R321 could allow ligand-driven endocytosis but prohibit G protein activation. **A**, CCR3 exists as a homodimer that can bind a single molecule of CCL11. Binding induces conformational changes in both CCR3 subunits, which activate both G proteins and β -arrestin, leading to intracellular signaling and endocytosis. **B**, Effects of R321 (yellow). CCL11 can still bind and drive β -arrestin-mediated endocytosis of the complex but is unable to induce conformational changes in the neighboring CCR3 molecule required for G protein signaling.

new pharmacologic axis for drug development. In the case of R321, we would appear to have a prototypic drug that does just that. Given the fact that 15 of the 23 residues in TM helix II of CCR3 are conserved in CCR1, then it is plausible that R321 is also an effective inhibitor of CCR1, which should be examined forthwith. Small-molecule antagonists with specificity for both CCR1 and CCR3 are not uncommon and might also be beneficial, given that hyperresponsiveness of eosinophils to the CCR1 ligand CCL3 has been reported in donors with a history of atopy-associated diseases.⁹ Moreover, because the results of a previous clinical trial targeting CCR3 alone were without efficacy in reducing circulating and sputum eosinophil levels, dual targeting of 2 or more ligand-receptor axes by R321 might provide the long-awaited breakthrough in this area.¹⁰ We eagerly await the progress of this molecule.

REFERENCES

1. Jose PJ, Griffiths-Johnson DA, Collins PD, Walsh DT, Moqbel R, Totty NF, et al. Eotaxin: a potent eosinophil chemoattractant cytokine detected in a guinea pig model of allergic airways inflammation. *J Exp Med* 1994;179:881-7.
2. Ponath PD, Qin S, Post TW, Wang J, Wu L, Gerard NP, et al. Molecular cloning and characterization of a human eotaxin receptor expressed selectively on eosinophils. *J Exp Med* 1996;183:2437-48.
3. Pease JE, Horuk R. Recent progress in the development of antagonists to the chemokine receptors CCR3 and CCR4. *Expert Opin Drug Discov* 2014;9:467-83.
4. Grozdanovic M, Laffey KG, Abdelkarim H, Hitchinson B, Harijith A, Moon H-G, et al. Novel peptide nanoparticle-biased antagonist of CCR3 blocks eosinophil recruitment and airway hyperresponsiveness. *J Allergy Clin Immunol* 2019;143:669-80.
5. Tarasova NI, Rice WG, Michejda CJ. Inhibition of G-protein-coupled receptor function by disruption of transmembrane domain interactions. *J Biol Chem* 1999;274:34911-5.
6. Sabroe I, Peck MJ, Van Keulen BJ, Jorritsma A, Simmons G, Clapham PR, et al. A small molecule antagonist of chemokine receptors CCR1 and CCR3. Potent inhibition of eosinophil function and CCR3-mediated HIV-1 entry. *J Biol Chem* 2000;275:25985-92.
7. Zimmermann N, Conkright JJ, Rothenberg ME. CC chemokine receptor-3 undergoes prolonged ligand-induced internalization. *J Biol Chem* 1999;274:12611-8.
8. Gilliland CT, Salanga CL, Kawamura T, Trejo J, Handel TM. The chemokine receptor CCR1 is constitutively active, which leads to G protein-independent, β -arrestin-mediated internalization. *J Biol Chem* 2013;288:32194-210.
9. Phillips RM, Stubbs VEL, Henson MR, Williams TJ, Pease JE, Sabroe I. Variations in eosinophil chemokine responses: an investigation of CCR1 and CCR3 function, expression in atopy, and identification of a functional CCR1 promoter. *J Immunol* 2003;170:6190-201.
10. Neighbour H, Boulet LP, Lemiere C, Sehmi R, Leigh R, Sousa AR, et al. Safety and efficacy of an oral CCR3 antagonist in patients with asthma and eosinophilic bronchitis: a randomized, placebo-controlled clinical trial. *Clin Exp Allergy* 2014;44:508-16.