

EDITORIAL

Eosinophils on trial

Although eosinophils constitute a minor population of blood granulocytes, they are recruited in significant numbers at tissue sites of worm infection and allergic reactions. These cells, distinguished by their cytoplasmic granules staining pink with eosin, have an impressive armamentarium of secretory products that are employed to combat parasitic helminths. These same products have been associated with tissue damage in allergic asthma, making them an attractive target for drug development.

IL-5 has been the lead target in the development of drugs directed at eosinophils. This cytokine is important for eosinophil development and release from the bone marrow and eosinophil survival once the cells are in peripheral tissues. Deletion of the IL-5 gene or use of anti-IL-5 antibodies in animal models of allergic asthma results in low blood and lung eosinophil counts, which is associated with improved lung function.¹ Hence, therapeutic anti-IL-5 antibodies have been developed which have proven efficacy in reducing some aspects of the disease.

An alternative approach is to identify the molecules produced in the asthmatic lung that recruits eosinophils from small blood vessels to airway tissues. IL-5 is not thought to act in this way. IL-5 drives the chemokinesis of eosinophils, that is, an increased cell motility, but not chemotaxis, that is, directional migration along a chemical gradient. Initially, some non-selective chemoattractants were identified using *in vitro* chemotaxis assays, but these could not account for the enriched eosinophil accumulation observed in allergic reactions *in vivo*. Subsequently, a highly potent CC chemokine with high selectivity for eosinophils was discovered using a guinea-pig model of allergic airway disease, and named eotaxin.² The sequence of eotaxin was subsequently used to determine eotaxins in several species, including human.³ The receptor for eotaxin, CCR3, was then identified⁴ and found to be highly expressed on eosinophils as well as on other cells involved in allergy, such as basophils and mast cells. Two more eotaxins were then discovered showing similar activity on eosinophils, but low sequence similarity.^{5,6} Hence, there are now known to be 3 eotaxins: eotaxin-1/CCL11, eotaxin-2/CCL24 and eotaxin-3/CCL26. All 3 eotaxins stimulate CCR3 as agonists, although they can exert inhibitory effects on other cells via additional chemokine receptors. These findings held out the promise of an orally available drug aimed at blocking CCR3 and thus preventing eosinophil effects by inhibiting access to airway tissues. From the mid-1990s, many biotechnology and pharmaceutical companies established programmes to identify such antagonists.

The paper by Gauvreau et al⁷ in this issue describes a small, short-term safety study of a CCR3 antagonist, AXP1275, given to

patients with allergic asthma and control subjects. At the doses used, there was no significant effect on allergen-induced early or late bronchoconstriction in the patient group. This was disappointing, but the authors point out that the serum levels of the compound did not reach the levels envisaged to have efficacy. Furthermore, there was no significant effect on the numbers of eosinophils in sputum, as was observed in another study using a chemically distinct CCR3 antagonist, GW766994.⁸ The lack of effect could be due to inadequate serum levels of drug (and therefore low receptor occupancy) or, as discussed by the authors, the involvement of other chemoattractants *in vivo* acting on receptors other than CCR3. Notable in this respect is the receptor CCR1 that has been shown to be expressed on the eosinophils of some individuals.⁹ Early studies *in vitro* explored the effects of a dual antagonist that blocked both CCR3 and CCR1.¹⁰

Interestingly, the study by Gauvreau and colleagues showed that the CCR3 antagonist suppressed airway hyperresponsiveness to methylcholine, an effect also observed in the previous study with GW766994. At first sight, this is difficult to reconcile with the lack of effect on sputum eosinophil numbers. However, the drug may affect the distribution of eosinophils in lung tissue. As the authors mention, there is evidence that eotaxin-1 can attract eosinophils to nerves and released major basic protein is able to block inhibitory M2 muscarinic receptors.¹¹ Furthermore, the authors have previously put forward data which support the notion that CCR3 receptors and acetylcholine M3 receptors physically interact with one another, which may modulate their respective functions in eosinophils.¹²

These results are discouraging; but to some extent, they mirror the early results with a therapeutic antibody to IL-5.¹³ In this small study with mepolizumab, the antibody did not induce significant changes in lung function after allergen challenge, although eosinophil counts in blood and sputum were down. This study was followed by several detailed investigations over a longer period with larger, selected patient groups and optimized dosing. These studies demonstrated a clear effect in suppressing exacerbation rates in patients with severe, eosinophilic asthma and the ability to spare the glucocorticoid dose given to patients.¹⁴⁻¹⁷ An antibody to the IL-5 receptor, benralizumab, has also proved effective.¹⁸ There is additional evidence from studies using Mepolizumab that tissue remodelling in the airways is dependent on IL-5.¹⁹ It is possible that optimal dosing over longer periods in selected patient groups with CCR3 antagonists could also prove effective, but this raises broader issues which are discussed below.

The exciting era of chemokine research, starting in the mid-1980s, resulted in the discovery of approximately 50 chemokines and 20 receptors.²⁰ The roles of these molecules were investigated

in animal models in vivo, in test systems in vitro and in patients. This in turn resulted in a "gold rush" to discover therapeutic agents aimed at neutralizing-specific chemokines or blocking their receptors. The prospect was a medicine chest of new selective therapeutic agents designed to treat different inflammatory diseases. This prospect was used to justify a huge investment world-wide. The benefits from all this activity have been substantial in terms of knowledge of basic mechanisms underlying infection, immunity and inflammation. This knowledge will be invaluable in the development of future medicines, but, so far, therapeutic returns have been surprisingly limited with only 2 drugs on the market to date, a CCR5 antagonist used in the treatment of HIV infection²¹ and a CXCR4 antagonist used to mobilize stem cells from the bone marrow.²²

In retrospect, there are several possible reasons why many more effective anti-inflammatory drugs have not come from the chemokine field. To effectively suppress the migration of a particular cell type, a sustained high serum level of drug is likely to be necessary.²³ Furthermore, recruitment mechanisms are intrinsically complex, with often several different chemokine receptors expressed on each cell type that are modulated in a dynamic fashion. Chemokines also often act on several receptors linked to stimulatory or inhibitory pathways. This complexity emphasizes the importance of inflammation for survival following injury or infection. Pathogenic organisms have evolved evasion strategies to bypass inflammatory processes, countered by backup host defence mechanisms. In this respect, potent chemokine-blocking mechanisms have been identified in viruses, bacteria and helminths. Thus, effective anti-inflammatory therapy may require 2 or more chemokine receptors to be blocked, although the risk of compromising host defence is always in the background.

CCR3 remains an attractive target for the treatment of allergic asthma, but this may not succeed given the above caveats. Various CCR3 antagonists (reviewed in²⁴) and an Eotaxin-1-neutralizing antibody bertilimumab²⁵ have been developed and are under investigation for diseases other than asthma, for example, inflammatory bowel disease,²⁶ bullous pemphigoid²⁷ and age-related macular degeneration.²⁸

Hence, where exactly does this leave the eosinophil? Despite the association of eotaxins and eosinophils with the allergen-induced late bronchoconstrictor reaction in asthma, to date there is no evidence that these cells mediate the late response. The studies on AXP1275 and GW766994 suggest that eosinophils may have an influence on hyperresponsiveness, but the clinical significance is unclear. However, it is more firmly concluded from the studies with anti-IL-5 antibodies in severe asthmatics that eosinophils are involved in disease exacerbations. The detailed mechanisms underlying these components of allergic asthma remain to be established.

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REFERENCES

1. Foster PS, Hogan SP, Ramsay AJ, Matthaei KI, Young IG. Interleukin 5 deficiency abolishes eosinophilia, airways hyperreactivity, and lung damage in a mouse asthma model. *J Exp Med*. 1996;183:195-201.
2. Jose PJ, Griffiths-Johnson DA, Collins PD, et al. Eotaxin: a potent eosinophil chemoattractant cytokine detected in a guinea pig model of allergic airways inflammation. *J Exp Med*. 1994;179:881-887.
3. Ponath PD, Qin S, Ringler DJ, et al. Cloning of the human eosinophil chemoattractant, eotaxin. Expression, receptor binding, and functional properties suggest a mechanism for the selective recruitment of eosinophils. *J Clin Invest*. 1996;97:604-612.
4. Ponath PD, Qin S, Post TW, et al. Molecular cloning and characterization of a human eotaxin receptor expressed selectively on eosinophils. *J Exp Med*. 1996;183:2437-2448.
5. Forssmann U, Ugucioni M, Loetscher P, et al. Eotaxin-2, a Novel CC Chemokine that Is Selective for the Chemokine Receptor CCR3, and Acts Like Eotaxin on Human Eosinophil and Basophil Leukocytes. *J Exp Med*. 1997;185:2171-2176.
6. Kitaura M, Suzuki N, Imai T, et al. Molecular cloning of a novel human CC chemokine (Eotaxin-3) that is a functional ligand of CC chemokine receptor 3. *J Biol Chem*. 1999;274:27975-27980.
7. Gauvreau GM, FitzGerald JM, Boulet LP, et al. The effects of a CCR3 inhibitor, AXP1275, on allergen-induced airway responses in adults with mild-to-moderate atopic asthma. *Clin Exp Allergy*. 2018;42:706-707.
8. Neighbour H, Boulet LP, Lemiere C, 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;44:508-516.
9. Sabroe I, Hartnell A, Jopling LA, et al. Differential regulation of eosinophil chemokine signaling via CCR3 and non-CCR3 pathways. *J Immunol*. 1999;162:2946-2955.
10. Sabroe I, Peck MJ, Van Keulen BJ, 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;275:25985-25992.
11. Fryer AD, Stein LH, Nie Z, et al. Neuronal eotaxin and the effects of CCR3 antagonist on airway hyperreactivity and M2 receptor dysfunction. *J Clin Invest*. 2006;116:228-236.
12. Bacon K, Moss E, Bingham K, et al. LATE-BREAKING ABSTRACT: a novel role for CCR3 in promoting airways hyperreactivity; Role for CCR3-muscarinic M3 receptor heterodimers. *Eur Respir J*. 2015;46 (suppl 59), OA288. <https://doi.org/10.1183/13993003.congress-2015.OA288>.
13. Leckie MJ, Brinke AT, Khan J, et al. Effects of an interleukin-5 blocking monoclonal antibody on eosinophils, airway hyper-responsiveness, and the late asthmatic response. *Lancet*. 2000;356:2144-2148.
14. Haldar P, Brightling CE, Hargadon B, et al. Mepolizumab and exacerbations of refractory eosinophilic asthma. *N Engl J Med*. 2009;360:973-984.

15. Nair P, Pizzichini MMM, Kjarsgaard M, et al. Mepolizumab for prednisone-dependent asthma with sputum eosinophilia. *N Engl J Med*. 2009;360:985-993.
16. Pavord ID, Korn S, Howarth P, et al. Mepolizumab for severe eosinophilic asthma (DREAM): a multicentre, double-blind, placebo-controlled trial. *Lancet*. 2012;380:651-659.
17. Bel EH, Wenzel SE, Thompson PJ, et al. Oral glucocorticoid-sparing effect of mepolizumab in eosinophilic asthma. *N Engl J Med*. 2014;371:1189-1197.
18. Nair P, Wenzel S, Rabe KF, et al. Oral Glucocorticoid-Sparing Effect of Benralizumab in Severe Asthma. *N Engl J Med*. 2017;376:2448-2458.
19. Flood-Page P, Menzies-Gow A, Phipps S, et al. Anti-IL-5 treatment reduces deposition of ECM proteins in the bronchial subepithelial basement membrane of mild atopic asthmatics. *J Clin Invest*. 2003;112:1029-1036.
20. Zlotnik A, Yoshie O. The chemokine superfamily revisited. *Immunity*. 2012;36:705-716.
21. Wood A, Armour D. The discovery of the CCR5 receptor antagonist, UK-427,857, a new agent for the treatment of HIV infection and AIDS. *Prog Med Chem*. 2005;43:239-271.
22. De Clercq E. Recent advances on the use of the CXCR4 antagonist plerixafor (AMD3100, Mozobil™) and potential of other CXCR4 antagonists as stem cell mobilizers. *Pharmacol Ther*. 2010;128:509-518.
23. Schall TJ, Proudfoot AEI. Overcoming hurdles in developing successful drugs targeting chemokine receptors. *Nat Rev Immunol*. 2011;11:355-363.
24. Pease JE, Horuk R. Recent progress in the development of antagonists to the chemokine receptors CCR3 and CCR4. *Expert Opin Drug Discov*. 2014;9:467-483.
25. Main S, Handy R, Wilton J, et al. A potent human anti-eotaxin1 antibody, CAT-213: isolation by phage display and in vitro and in vivo efficacy. *J Pharmacol Exp Ther*. 2006;319:1395-1404.
26. Adar T, Shteingart S, Ben-Ya'acov A, et al. The Importance of Intestinal Eotaxin-1 in Inflammatory Bowel Disease: new Insights and Possible Therapeutic Implications. *Dig Dis Sci*. 2016;61:1-10.
27. Günther C, Wozel G, Meurer M, Pfeiffer C. Up-regulation of CCL11 and CCL26 is associated with activated eosinophils in bullous pemphigoid. *Clin Exp Immunol*. 2011;166:145-153.
28. Takeda A, Baffi JZ, Kleinman ME, et al. CCR3 is a target for age-related macular degeneration diagnosis and therapy. *Nature*. 2009;460:225-230.