

Chemokines in Allergy

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Abstract: Allergic diseases such as atopic dermatitis, asthma, and allergic rhinitis represent a significant healthcare problem. Understanding these diseases as dysregulated inflammatory responses has led to many new targets for therapeutic intervention. Recent data concerning soluble IL-4 receptor, monoclonal antibodies against IL-5 and an antibody toward IgE have led to an appreciation of the crucial role played by Th2 subset of CD4⁺ T cells and their corresponding cytokines. While these potential drugs are presently in clinical trials and may be valuable therapeutics, orally bioavailable small molecule inhibitors of Th2 cell responses would be desirable for treatment of these chronic diseases. One strategy is to prevent effector cell migration (Th2 cells, mast cells, and eosinophils) via chemokine receptor antagonism with a suitable small molecule.

Chemokine receptors are a subset of the seven transmembrane-spanning family, which mediate their effects through interaction with heterotrimeric G-proteins. The ligands are a structurally related set of proteins that are selectively expressed in certain disease settings. Three chemokine receptors CCR3, CCR4, and CCR8 are preferentially expressed by Th2 cells, mast cells and eosinophils and therefore represent therapeutic targets for allergy.

This mini-review will focus on new research involving CCR3, CCR4 and CCR8. The cellular distribution of each receptor, the corresponding chemokine ligands, and various validation studies are discussed. Recent drug discovery advances concerning pharmacological tools and small molecule receptor antagonists will also be presented.

INTRODUCTION

An allergic inflammatory response is a critical feature of several diseases including asthma, atopic dermatitis and allergic rhinitis. Asthma affects ~15 million people in the United States, and is the major cause of preventable childhood hospitalizations [1]. Total asthma related health care costs were estimated to be \$10 billion in 1996 [2]. Atopic dermatitis and allergic rhinitis affect approximately 10-20 million people in the United States and have a significant impact on quality of life [3]. Allergic disease represents a significant healthcare problem and it is increasing in prevalence in the industrialized nations.

The treatment of allergy has evolved over the last 10-15 years reflecting an improved understanding of the underlying disease process. In a clinical setting asthma is characterized by wheezing, airway hyper-responsiveness and reversible airway obstruction [4]. These observations would suggest that asthma is primarily a disorder of the airway smooth muscle cells. Bronchodilators, such as the β_2 -agonists, are widely used in the treatment of asthma and while such treatments are effective in providing symptomatic relief they

do not address the underlying disease biology [5]. A combination of bronchiolar lavage and bronchial biopsies from patients with asthma have shown that the changes in airway physiology are caused by a persistent inflammatory response, which is believed to be central to the pathology of the disease [6].

The recognition of asthma (and other allergic disorders) as being due to a dysregulated inflammatory response has led to changes in therapeutic management. In particular corticosteroids, which are potent anti-inflammatory agents, are widely used in the treatment of moderate to severe asthma, allergic rhinitis and atopic dermatitis [7]. The prolonged use of corticosteroids, particularly at high dose or when used systemically, has been associated with a number of potential adverse effects including suppression of the hypothalamic-pituitary-adrenal axis, bone resorption, dermal thickening, purpura, oral candidiasis and growth retardation [7]. While the shift to locally delivered (inhaled) corticosteroids has dramatically improved their safety profile, these agents are not universally effective and there is a widespread public belief that their prolonged use, especially in children should be avoided [7]. There remains a need for novel therapeutic strategies for the treatment of allergy as demonstrated by the recent introduction of cysteinyl leukotriene inhibitors which represent the first new class of drug for the treatment of asthma in twenty years [8].

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Th2 CELLS AS THERAPEUTIC TARGETS IN ALLERGY

Studies performed over the last ten years on the underlying mechanisms controlling the immune response in allergy have identified new classes of therapeutic targets [9]. The identification of the T cell, and particularly activated CD4⁺ memory T cells in driving the allergic response has been central to establishing the way that we now think about these diseases. CD4⁺ T cells are separated into subsets (Th1 and Th2) based upon the cytokines that they secrete following activation [10]. Th1 cells make IFN γ and TNF, while Th2 cells make IL-4, IL-5, IL-9 and IL-13 and these cell types are involved in distinct pathologies [11]. Rheumatoid arthritis and Crohn's disease are associated with the accumulation of activated Th1 cells, and inhibition of the Th1 cytokine, TNF, has proven to be a highly effective therapeutic strategy for these diseases [12]. In contrast, allergic inflammation is mediated by Th2 cells [11]. Cytokines such as IL-4 and IL-5, which are characteristic of activated Th2 cells, are expressed at sites of allergic inflammation. Inhibition of Th2 cytokines using neutralizing antibodies or gene ablation studies is protective in a number of pre-clinical animal models of allergy [13]. Furthermore, if the differentiation of the Th2 cell is impaired by genetic manipulation of intracellular signaling molecules such as STAT-4 or GATA-3 animals are unable to establish an allergic inflammatory response [14]. Perhaps the most compelling observation has come from analysis of the immunological reactivity of patients undergoing grass pollen immunotherapy. Prolonged clinical remission in these patients was associated with a shift in their T cell cytokine profile from a pro-allergic Th2 to a Th1 type [15].

Based on these observations a number of therapeutics which target aspects of the Th2 response have recently entered the clinic. Nuvance is a soluble form of the human IL-4 receptor (IL-4R) which is under development by Immunex for the treatment of asthma. Nuvance, by binding and inactivating IL-4, should selectively inhibit Th2-dependent inflammation. IL-4 is also required for the generation of IgE, a mediator that is vital for the activation of mast cells. Encouraging results from two multi-dose phase II studies in adults with moderate persistent asthma have been reported. Nuvance was shown to stabilize lung function for up to 12 weeks after discontinuation of the inhaled corticosteroids [9].

The second approach targeting Th2-derived cytokines is to antagonize the cytokine IL-5. IL-5 plays a critical role in the development, survival and function of eosinophils. Eosinophils are thought to play a major role in sustaining the inflammatory response in asthma and, because of the specificity of IL-5 action towards that cell type, IL-5 antagonists will be highly selective therapeutics. Two antibodies to IL-5 are under clinical evaluation, Sch-55700 (Schering Plough) and SB 240563 (Glaxo Smithkline). In a recent placebo control double blind study in patients with asthma SB 240563 failed to show clinical efficacy [16]. The clinical endpoints in this trial were allergen induced bronchoconstriction and airway hyper-reactivity. In this study, blood eosinophils were significantly reduced, and there was a slight reduction in the numbers of eosinophils

present in the sputum. The presence of eosinophils within the airway mucosa was not evaluated. These findings, while disappointing, do not rule out the possibility that long-term suppression of eosinophils will be of clinical benefit in asthma. Clearly additional studies are required to evaluate the role of eosinophils in the pathology of asthma.

While not strictly a Th2 cell product, the generation of IgE is highly dependent upon signals derived from Th2 cells [10]. IgE activates mast cells in response to allergen, which then leads to the release of preformed vasoactive mediators, and to the generation of prostaglandins and leukotrienes. Mast cell activation is the first step in triggering the allergic response in asthma and allergic rhinitis. Support for this view has come from recent clinical trials [17]. RhuMab-E25 is a humanized monoclonal antibody to IgE being developed by Genentech, in conjunction with Novartis and Tanox. RhuMab-E25 binds to IgE and prevents its interaction with the high affinity IgE receptor present on mast cells. RhuMab-E25 has completed two-phase III clinical trials, one in adults (525 patients) and the other in children (334 patients). These patients had moderate to severe asthma and were symptomatic despite taking inhaled corticosteroids. In both studies, patients showed a significant improvement in asthma symptoms including a reduced steroid requirement [18].

Nuvance, Sch-55700, SB 240563 and rhuMab-E25 were well tolerated in humans. The most common reported side effects were pruritis, headaches, and pharyngitis. These findings suggest that interfering with cytokines which mediate the Th2 response (IL-4, IL-5) or immune mediators which are generated in response to these cytokines (IgE), is a safe and potentially efficacious approach for the treatment of allergy [9].

CHEMOKINES AND THEIR RECEPTORS IN ALLERGY

An alternative strategy in these disease settings would be to interfere with the migration of the appropriate effector cell populations (Th2 cells, mast cells and eosinophils). Leukocyte trafficking is regulated by a large family (~50 members) of structurally related proteins that is collectively referred to as chemokines [19]. The receptors for chemokines are members of the seven transmembrane-spanning class which mediate their effects through interaction with heterotrimeric G-proteins [20]. The selective expression of chemokines and of their receptors makes them appealing drug targets. There is evidence that leukocyte migration may be regulated by targeting signaling molecules, which are downstream of the chemokine receptor such as members of the PI-3 kinase family [21]. However, such signaling pathways are shared by many chemokine receptors and the specificity of the response appears to reside at the level of the receptor ligand interaction. Furthermore, members of the G protein-coupled receptor family have proven to be a rich source of targets for the development of orally available small molecule based therapeutics. Three chemokine receptors CCR3, CCR4 and CCR8 are preferentially expressed by Th2 cells, mast cells or eosinophils and therefore represent therapeutic targets in allergy. The

preclinical biology supporting a role for these receptors in the regulation of the allergic response is described below.

CCR3

In the context of allergic inflammation CCR3 is the most extensively studied chemokine receptor. CCR3 has been the focus of considerable efforts to identify small molecule antagonists to this receptor (see below). CCR3 is expressed by eosinophils [22], mast cells [23] and Th2 cells [24]. CCR3 has been shown to mediate the initial trafficking of T cells to the lung in a murine model of asthma [25]. However the expression of CCR3 by human Th2 cells has been controversial and it is the presence of CCR3 on eosinophils that has provoked the greatest interest in this receptor. A number of studies have highlighted the importance of the eosinophil in mediating the allergic response in asthma [26]. CCR3 mediates the arrest of eosinophils on inflamed endothelium under shear flow conditions and can directly activate eosinophils [27]. Levels of eotaxin (a well characterized CCR3 ligand) are elevated in the serum of patients with acute asthma and this correlates with disease severity [28, 29]. The expression of eotaxin is induced in the bronchial epithelia of asthma patients in response to segmental allergen challenge [30] and this is correlated with the accumulation of eosinophils [30]. MCP-4, a second ligand for CCR3, is also expressed in human asthma [31] and in vitro the expression of both eotaxin and MCP-4 is inhibited by glucocorticoids agents that are known to effectively suppress the inflammatory response in asthma [32]. Furthermore CCR3 deficient mice show a reduced migration of eosinophils to the lung in response to allergen challenge in an asthma model [33]. The expression of CCR3 and its ligands is not restricted to asthma. There is enhanced local expression of eotaxin and CCR3 in lesions from patients with atopic dermatitis [34] that suggests that CCR3 may regulate the trafficking of eosinophils in a number of allergic diseases.

CCR3 interacts with a number of ligands including RANTES, MCP-3, MCP-4 and eotaxins. Although many of these ligands interact with receptors in addition to CCR3, studies using blocking antibodies to CCR3 have confirmed that this is the dominant (and perhaps sole) receptor mediating a chemotactic response to these agonists in eosinophils [35]. While recent results using IL-5 antagonists have called into question the role of the eosinophil in asthma [18], these studies used improvements in airway responsiveness to acute (external) allergen challenge as their endpoint. The importance of the eosinophil in the pathogenesis of asthma and other allergic diseases awaits additional clinical trials. CCR3 remains an attractive candidate for the development of novel therapeutics for the treatment of asthma.

CCR4

CCR4 interacts with two ligands, macrophage-derived chemokine (MDC), and T cell activation regulated chemokine (TARC) [36]. The pronounced expression of CCR4 on human Th2 cells suggested that this receptor and its ligands play an important role in the migration of Th2

cells [37]. The expression of both MDC and TARC is induced by a Th2 environment [38], and activated human Th2 cells make MDC and TARC [39]. The expression of MDC by Th2 cells correlates with their ability to make IL-4 and IL-13 suggesting that this may represent a mechanism whereby the production of MDC is amplified during an allergic response [39]. These results suggest that MDC and TARC will be expressed during the allergic response in vivo and this has been confirmed in human disease [39]. Recent studies have shown that MDC and TARC expression is induced on airway epithelial cells following acute allergen challenge [40]. This induction of chemokine expression is associated with the influx of IL-4 producing T cells expressing CCR4 [40].

CCR4 is expressed by a subset of T cells (CLA⁺) that home to inflamed skin but not to systemic or to mucosal sites [41]. MDC and TARC trigger integrin-dependent adhesion of this T cell subset and cause their rapid arrest under physiological flow [41]. TARC, but not MDC, is made by inflamed endothelium [41]. In allergic skin disease such as atopic dermatitis, MDC expression localizes to inflammatory infiltrates within the dermis [39]. These findings suggest that MDC and TARC may act in a coordinated fashion to direct the migration of CCR4⁺ T cells to inflammatory sites. TARC appears to provide the initial signal stimulating firm adhesion of the T cell to the inflamed endothelium. MDC then localizes infiltrating cells within the tissue, and facilitate the interaction of T cells and dendritic cells. This model, which has not been formally proven, is analogous to that described for the chemokines SLC and ELC which regulate the traffic of naive T cells into peripheral lymph nodes [42].

The evaluation of CCR4 and its ligands in the in vivo development of allergic inflammation has yielded mixed results. CCR4 deficient mice develop normal Th2 responses and are not protected from the induction of asthma [43]. In contrast MDC and TARC expression is elevated in both experimental [44] and spontaneous [45] models of Th2 cell mediated inflammation. By using neutralizing antibodies to MDC or TARC, it has been shown that this expression is relevant to the pathology of the disease. For example, antibodies to MDC or TARC block the airway hyper-reactivity, local production of IL-4 and the trafficking of eosinophils in murine models of asthma [25, 44, 46]. The differences in the results obtained in these different model systems remains to be determined. It is possible that the phenotype of the CCR4 knockout mice reflects redundant/compensatory mechanisms that take place in an animal in which the immune system has developed in the absence of CCR4. It should be remembered that these are models of human disease and, while they can be used to evaluate chemokine/chemokine receptor interactions, conclusive, in vivo demonstration of a role for CCR4 in human allergy awaits verification in human clinical trials.

CCR8

When compared to CCR3 or CCR4, less is known concerning the role of CCR8 in the trafficking of cells in allergic inflammation. Th2 cells express CCR8 and this

expression is both sustained and enhanced following T cell activation [47]. I-309 activated T cells make the physiological ligand for CCR8 and this production is inhibited by the Th1-associated cytokine IL-12 [48]. The chemokine vMIP-I (which is encoded by the herpes virus HHV8/KSHV) is an agonist for CCR8 [49], and lesions from individuals with Kaposi's sarcoma are enriched for Th2 cells [50]. These findings suggest that virally encoded agonists for chemoattractant receptor expressed on Th2 cells may represent a means of immune evasion away from an anti-viral Th1 response. CCR8 appears to play an important role in directing the migration of Th2 cells *in vivo*. This is supported by recent studies of CCR8 deficient mice that show significantly reduced pathology in several experimental models of asthma [51]. This protection from disease is due to a failure of the allergen-reactive T cells to migrate to the site of antigen challenge [51]. There is no evidence for any intrinsic defect in the capacity to generate Th2 responses in these mice [51]. Antagonism of CCR8 is therefore a potential target for therapeutic intervention in allergy.

RECEPTOR ANTAGONISTS

Historically, the design and discovery of G protein-coupled receptor antagonists have been a highly successful strategy for therapeutic intervention. Furthermore, in the chemokine field, it appears as though there is a certain amount of ligand redundancy and cross talk while the cellular distribution of the corresponding receptors seems much more specific. As a result, much effort has been focussed on a receptor antagonist approach as opposed to chemokine biosynthesis or release inhibitor strategies [52]. Early drug discovery work in the area of human chemokine receptor antagonists was aimed at validating particular receptor targets and producing pharmacological tools. Antibodies, antisense, and protein-based inhibitors have been reported. Many of these early protein modulators were modified chemokines or chemokines from other species. Recently, non-selective and selective small molecule inhibitors have begun to emerge. Ultimately, efficacious, orally bioavailable antagonists of each key receptor (CCR3, CCR4, and CCR8) are desired for use in these chronic allergic diseases.

Of the three allergy-related chemokine receptors, CCR3 has received the most attention to date because of its strong connection to eosinophil migration and the subsequent tissue damage observed in allergic disease. While CCR3 is similar to CCR5 in that it is a co-receptor for viral entry into the cell, this review will only focus on its role in allergic disease and as a target for such indications.

A selective, antagonistic, monoclonal antibody to CCR3, 7B11, inhibited human eosinophil chemotaxis and calcium flux induced by a variety of chemokines and convincingly validated the role of CCR3 in eosinophil response and the therapeutic potential of a selective receptor antagonist [53]. Subsequent protein-based receptor antagonists have been described such as vMIP-II, Met-RANTES, and Ck 7. Kaposi's sarcoma-associated herpesvirus encodes a chemokine called vMIP-II. This protein possesses antagonistic activity toward CCR2,

CCR5, and CXCR4 and can block the calcium mobilization induced by a variety of human chemokines (including eotaxin) in certain cell systems [54]. While this supports a claim that vMIP-II is a CCR3 antagonist, in other systems it appears to act as a CCR3 agonist [55]. Recently, vMIP-II's role was somewhat clarified as an immunomodulator that drives a Th1-type inflammatory response toward a Th2-type [56]. N-terminal modification of the human chemokine, RANTES, has produced another non-selective CCR3 receptor antagonist [57]. Working through antagonism of CCR1 and CCR3, N-terminal methionylated RANTES (Met-RANTES) has demonstrated efficacy in animal models of arthritis [58] and airway inflammation [59]. Modification of a certain T cell chemoattractant, MIP-4 (a.k.a. PARC, DCK-1, or AMAC-1), provided an extremely potent and selective CCR3 antagonist called Met-chemokine 7 (Ck 7) [60]. This protein is a more potent antagonist than Met-Rantes towards CCR3 and demonstrates no partial agonist activity. Ck 7 completely inhibited eosinophil chemotaxis at 1 nM. As before, the modification of the extreme N-terminal domain of the natural chemokine produced this potent antagonist (alanine to methionine). Recently, a set of CXCR3 agonists was shown to possess pure CCR3 antagonistic properties [61]. I-TAC, Mig, and IP10 competed for eotaxin binding and inhibited chemokine-induced migration and calcium flux via CCR3. This data supports the opposing roles of CCR3 and CXCR3 in polarizing a Th2/Th1 T-cell response. A two-site model for chemokine receptor activation has been proposed based on a compilation of modified chemokine / receptor binding studies [62]. In this model, some chemokines may bind to the same, shared, extracellular site on particular receptors but in other cases, there are different domains on the same receptor for different chemokines. Thus, there may exist a docking domain, which can recognize the N-terminal segments of certain chemokines, and a second site that allows for subsequent receptor activation.

Small molecule antagonists of CCR3 have been derived from natural products and small molecule screening libraries. Distamycin analog NSC 651016 (**1**, see Fig. 1) was shown to inhibit chemokine binding to CCR1, CCR3, CCR5, and CXCR4 [63]. This molecule also blocked chemokine-induced intracellular calcium flux in human monocytes. 4-Aminopiperidine derivative UCB35625 (**2**) has also demonstrated potent, selective antagonism of CCR1 and CCR3 [64]. This ammonium salt inhibited chemokine binding and chemotaxis of CCR3 transfected cells in response to eotaxin ($IC_{50} = 94$ nM). Subsequent studies in which significantly larger concentrations of **2** were required to displace ligand than those required to inhibit receptor function suggest that this compound interacts with a region common to both CCR1 and CCR3 thus preventing a conformational change necessary for signal transduction. Given that CCR1 has the highest homology to CCR3 (62.5%) this model seems plausible. In a follow-up study, focused libraries were designed and synthesized based upon a binding hypothesis for compound **2** to CCR3 [65]. From this library of 770 carboxamide derivatives, compound **3** was identified as a reasonably potent ($IC_{50} = 750$ nM) CCR3 antagonist with ~10X selectivity over CCR1. Further optimization led to compound **4** that was a very potent and selective CCR3 antagonist (CCR3 $IC_{50} = 2.3$ nM; >500X

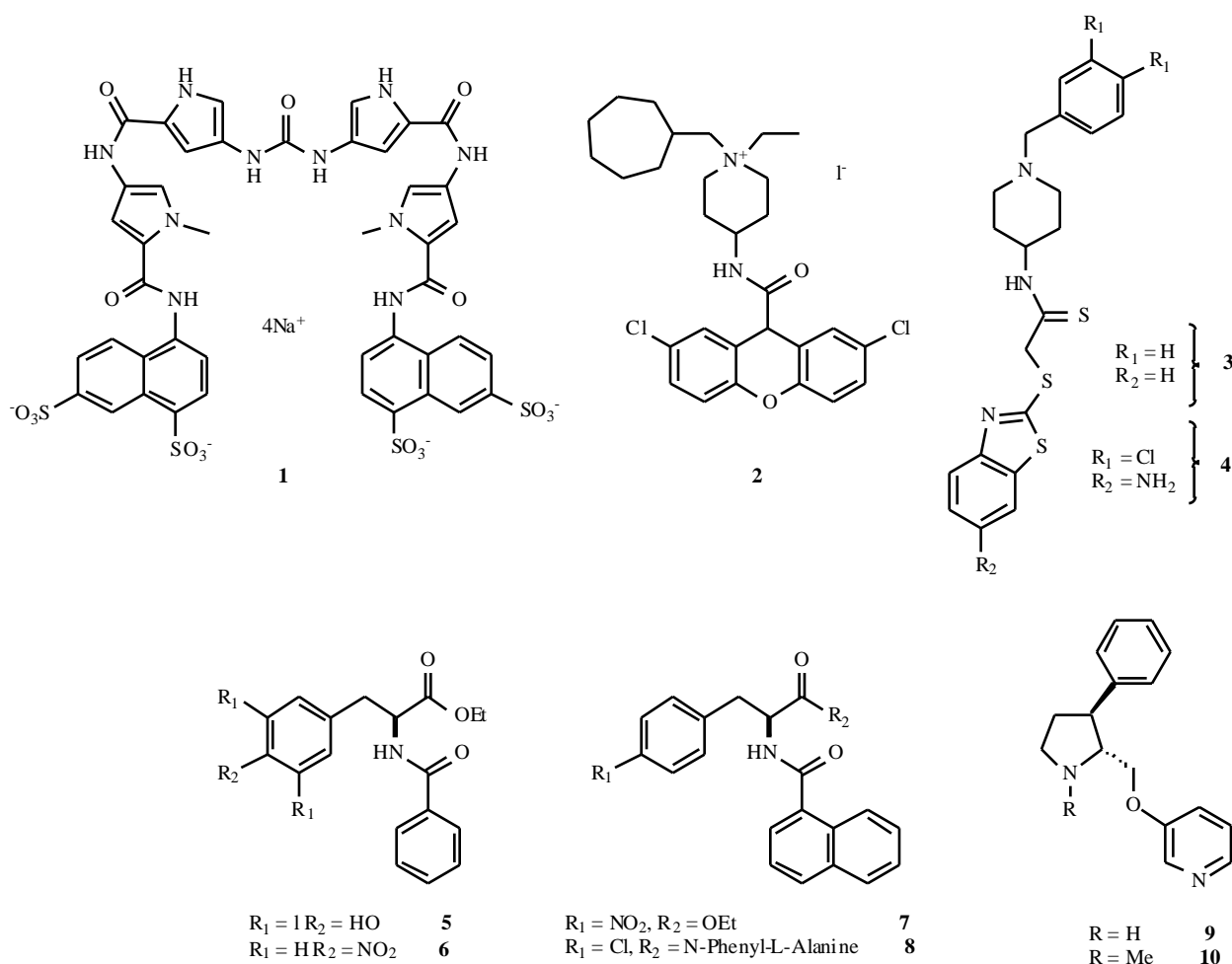


Fig. (1). CCR3 antagonists.

selectivity over CCR1). Compound **4** also inhibited eotaxin- and RANTES-induced calcium mobilization in eosinophils at IC_{50} values less than 30 nM.

In another approach, a whole cell, calcium mobilization FLIPR-based [66] high throughput screen identified a series of phenyl alanine derivatives (SK&F 45523, **5**; SB-297006, **6**; SB-328437, **7**) as potent, selective CCR3 antagonists [67]. While the original HTS hit (**5**) inhibited ^{125}I -eotaxin-binding with human eosinophils with an IC_{50} of 800 nM, optimized compounds **6** and **7** returned IC_{50} values of 60 and 4.5 nM. Interestingly, analogs of opposite stereochemistry were inactive. Compounds **6** and **7** inhibited the cellular calcium response elicited by three different CCR3 agonists (eotaxin, eotaxin-2 and MCP-4). They were also extremely selective for CCR3 versus CCR1 and other chemokine receptors. The lack of a basic nitrogen in these selective compounds distinguishes them from the less selective CCR3/1 antagonist **2** and suggests different binding modes. Some of these CCR3 antagonists (**2**, **5**, **6**, and **7**) failed to block the interaction of guinea pig and murine eotaxin to the corresponding guinea pig or murine CCR3 at concentrations 10,000 times greater than the human IC_{50} values. Thus, despite the 65% and 67% homology of guinea pig and murine CCR3 to the human receptor, such species selectivity of CCR3 antagonists may

limit their utility in animal models of allergic disease. In a follow-up study, compound **7** was further optimized [68]. Attempts to replace the metabolically unstable ester moiety were rewarded with the discovery of amide **8** which displayed an IC_{50} of 5 nM versus CCR3 binding and inhibited eosinophil chemotaxis with an IC_{50} of 15 nM. A disclosure from Abbott described two CCR3 antagonists, A-122057 (**9**) and A-122058 (**10**), which inhibited binding of eotaxin in CCR3 transfectants (IC_{50} 's ~ 1 nM) and blocked the induced calcium signal (IC_{50} 's ~ 0.3 nM) [69]. Finally, recent patents from DuPont [70], Roche [71], Kirin [72] and Tejin [73] along with additional patents from Glaxo-SmithKline [74] indicate a continuing, industry-wide interest in CCR3 antagonists.

Although there has been very little published concerning CCR4 antagonists, this area could attract as much attention as CCR3 because both receptors have limited cell distribution. While CCR3 has been targeted to prevent eosinophil migration, CCR4 will be targeted to block Th2 cell migration in allergic inflammation. A neutralizing antibody to CCR4, 1G1, was shown to potently block ^{125}I -TARC binding to CCR4 transfectants and to prevent chemotaxis of these cells in response to MDC and TARC [75]. Certainly, small molecule CCR4 antagonists will follow.

Antagonists of CCR8 have been reported. Chemokines of viral origin, vMIP-II and vMCC-1 act as potent antagonists and can block the effects of I-309 [49]. MC148, a chemokine derived from human poxvirus molluscum contagiosum, was found to displace ¹²⁵I-I-309 from CCR8 (transiently expressed in COS-7 cells) with an IC₅₀ of 0.47 nM [76]. Interestingly, MC148 could not displace *all* of the radiolabelled chemokine from CCR8 (only 80%) but it could block the entire calcium response elicited by I-309. While MC148 has a high affinity for human CCR8 and blocks subsequent responses, it is unable to bind and block responses through *murine* CCR8 and thus invalidates its use in mouse inflammatory models [77].

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