Identification of Potent, Selective Non-peptide CC Chemokine Receptor-3 Antagonist That Inhibits Eotaxin-, Eotaxin-2-, and Monocyte Chemotactic Protein-4-induced Eosinophil Migration*

Received for publication, July 25, 2000, and in revised form, August 31, 2000
Published, JBC Papers in Press, August 31, 2000, DOI 10.1074/jbc.M006613200

John R. White‡‡, Judithann M. Lee‡, Kimberly Dede‡, Christina S. Imburgia‡, Anthony J. Jurewicz‡, George Chan‡, James A. Forwald†, Dashyant Dhanak**, Lisa T. Christmann**, Michael G. Darcy**, Katherine L. Widdowson**, James J. Foley‡‡, Dulcie B. Schmidt‡‡, Henry M. Sarau‡‡

From the Departments of Immunology, Biomolecular Discovery, Pulmonary Biology, Gene Expression Sciences, and Medicinal Chemistry, SmithKline Beecham Pharmaceuticals, King of Prussia, Pennsylvania 19406

Eosinophils have been implicated in the pathogenesis of asthma and other allergic diseases. Several CC chemokines including eotaxin (CCL-11), eotaxin-2 (CCL-24), RANTES (CCL-5), and monocyte chemotactic protein-3 (MCP-3, CCL-7) and 4 (MCP-4, CCL-13) are potent eosinophil chemotactic and activating peptides acting through CC chemokine receptor-3 (CCR3). Thus, antagonism of CCR3 could have a therapeutic role in asthma and other eosinophil-mediated diseases. A high throughput, cellular functional screen was configured using RBL-2H3 cells stably expressing CCR3 (RBL-2H3-CCR3) to identify non-peptide receptor antagonists. A small molecule CCR3 antagonist was identified, SK&F 45523, and chemical optimization led to the generation of a number of highly potent, selective CCR3 antagonists including SB-297006 and SB-328437. These compounds were further characterized in vitro and demonstrated high affinity, competitive inhibition of $^{125}$I-eotaxin and $^{125}$I-MCP-4 binding to human eosinophils. The compounds were potent inhibitors of eotaxin- and MCP-4-induced Ca$^{2+}$ mobilization in RBL-2H3-CCR3 cells and eosinophils. Additionally, SB-328437 inhibited eosinophil chemotaxis induced by three ligands that activate CCR3 with similar potencies. Selectivity was affirmed using a panel of 10 seven-transmembrane receptors. This is the first description of a non-peptide CCR3 antagonist, which should be useful in further elucidating the pathophysiological role of CCR3 in allergic inflammatory diseases.

The recruitment of inflammatory cells into sites of inflammation is a normal physiological response designed to fight infection, remove damaged cells, and stimulate healing. However, the excessive recruitment of such cells often exacerbates tissue damage, slows healing, and in some cases leads to host death. Therefore, inhibition of inflammatory cell recruitment may be an appropriate therapeutic strategy in a number of inflammatory diseases, such as asthma, reperfusion injury, arthritis, and inflammatory bowel disease.

Chemokines are a superfamily of approximately 30 distinct small secreted proteins, and additional members continue to be identified (1, 2). They are classified into two major groups, CXC and CC, based on the position of the first two of their four invariant cysteines (3). The actions of chemokines are mediated via interactions with 7-TM G protein-coupled receptors on the surface of immune and inflammatory cells. To date, 18 unique chemokine receptors, including 11 CC chemokine receptors, have been cloned (4, 5).

The properties of the chemokines suggest that they are essential for leukocyte trafficking and inflammatory processes and thus are important components in a number of disease states (6, 7). Eosinophils are proinflammatory granulocytes that play a major role in allergic diseases, such as bronchial asthma (8), allergic rhinitis (9), atopic dermatitis (10), and eosinophilic gastroenteritis (11). Upon activation, eosinophils release lipid mediators, cytotoxic proteins, oxygen metabolites, and cytokines, all of which have the potential to produce pathophysiologically. Recent studies have clearly demonstrated the presence of eosinophilic or eosinophil-specific products in inflamed lung biopsy tissues in human asthma (10).

Although the molecular mechanism responsible for the selective infiltration of eosinophils into inflamed tissue has not been elucidated, recently the CC chemokine eotaxin was identified in guinea pig lung following antigen challenge in sensitized guinea pigs (12, 13). Furthermore, neutralizing antibodies to eotaxin in a mouse model of allergy demonstrated inhibition of eosinophil recruitment when administered before the antigen challenge (14). Five CCR3 ligands have been shown to induce eosinophil transendothelial migration using human umbilical vein endothelial cells. This migration is inhibited by pretreatment with anti-CCR3. In addition, a human lung epithelial cell line (BEAS-2B), stimulated with proinflammatory cytokines, has been shown to produce eotaxin and MCP-4 (15, 16). In humans, biopsies obtained from asthmatic lung have shown increased levels of CCR3 and its ligands, eotaxin, eotaxin-2, RANTES, and MCP-4, both at the mRNA and protein levels (17). The abbreviations used are: 7-TM, seven-transmembrane; MCP-3 and 4, monocyte chemotactic protein-3 and -4, respectively; CCR1, CCR3, CCR5, and CCR7, CC chemokine receptor-1, -3, -5, and -7, respectively; RANTES, regulated on activation of normal T cell expressed and secreted; RBL, rat basophilic leukemia; MIP-1α, macrophage inflammatory protein-1α; C5a and C5a, complement fragment 3a and 5a, respectively; FLIPR, fluorescence imaging plate reader; MS, mass spectrometry; ES, electrospray; CHO, Chinese hamster ovary; CHAPS, 3-[3-(cholamidopropyl)dimethylammonio]-1-propanesulfonic acid; PIPES, 1,4-piperazinediethanesulfonic acid; IL, interleukin; LDT, leukotriene D4.
tein levels (17). Other data are more controversial, including targeted deletion of the eotaxin gene, which results in either a reduction (18) or no effect on eosinophil recruitment (19).

CCR3 was cloned from a human monocyte (20) or an eosinophil library by two groups (21, 22) and subsequently shown to bind eotaxin, RANTES, and MCP-3 (21–23). More recently, eotaxin-2 and MCP-4 (24–26) were also reported as functional ligands for CCR3. Monoclonal antibodies raised to CCR3 demonstrate that the receptor is primarily localized to eosinophils and a subset of Th2 T-cells (21, 22, 27). This restricted expression pattern may be responsible for the selective recruitment of eosinophils and Th2 T-cells in allergic inflammatory diseases. In addition to antibodies, a dual CCR1/CCR3 low molecular weight compound (28) and a number of proteins (Met-RANTES, vMIP-II, and MIP-4) have also been reported as antagonists of CCR3 (29–31). Some of these proteins appear to be specific (30, 31), while others bind multiple chemokine receptors (29).

The above delineated studies provided a rationale to identify selective antagonists of CCR3, which could be therapeutically beneficial in the treatment of eosinophil-mediated diseases. Here, the identification and initial characterization of the first potent and selective non-peptide small molecule antagonists of CCR3 is described. These compounds were utilized to demonstrate that inhibition of CCR3 is sufficient to prevent eotaxin-, eotaxin-2-, and MCP-4-induced eosinophil chemotaxis in vitro. Potent and selective CCR3 antagonists, like SB-328437, should be useful to help define the pathophysiological role of CCR3 and eosinophils in allergic diseases.

**EXPERIMENTAL PROCEDURES**

**Cloning of CCR3**

CCR3, which was initially identified from an expressed sequence tag library, and was obtained from Human Genome Sciences (Rockville, MD). The expressed sequence tag was converted to a full-length cDNA and subcloned into a mammalian expression vector, pCDN, and stably expressed in rat basophilic leukemia (RBL-2H3) cells (ATCC, Manassas, VA). Stable clonal lines, produced by serial dilution in selection medium containing G418 (400 µg/ml), were screened for eotaxin-induced Ca²⁺ mobilization to identify the best responding cell line.

**FLIPR Calcium Mobilization Assay**

CCR3 Ca²⁺ mobilization studies were carried out using Fluo 3-loaded RBL-2H3-CCR3 cells and a microplate fluorometer using a fluorescent imaging plate reader (FLIPR, Molecular Devices, Sunnyvale, CA). RBL-2H3-CCR3 cells were grown to confluence in RPMI 1640 medium containing 10% fetal calf serum (Hyclone, Logan, Utah) in T-150 flasks with 5% CO₂ at 37 °C. Cells were removed from the T-150 flask by removing the medium and then treating the cells with 5 ml of Versene (Life Technologies, Inc., Rockville, MD) for 5 min at room temperature. Cells were washed once in RPMI 1640 medium, 10% fetal calf serum and then plated into sterile 96-well black ViewPlates™ (Packard, Meriden, CT) at 40,000 cells/well and incubated for 18–24 h. On the day of assay, the medium was aspirated and replaced with 100 µl of Earle's minimal essential medium with Earle’s salts containing l-glutamine, 0.1% bovine serum albumin, 4 µM Fluo-3-acetoxymethyl ester (Fluo-3/AM; Molecular Probes, Inc., Eugene, OR), and 1.5 mM dextran to help define the pathophysiological role of CCR3 and eosinophils in allergic diseases.

**Calcium Mobilization Selectivity Assays**

Calcium mobilization assays using freshly isolated monocytes loaded with Fura-2 and stimulated with RANTES (10 nM), MCP-1 (2 nM), and MIP-1α (CCL-3, 10 nM) were utilized to assess functional selectivity of antagonists (33).

**Human Eosinophil Isolation and 125I-Eotaxin and 125I-MCP-4 Binding**

Human eosinophils were isolated from peripheral blood of allergic volunteers (~5% eosinophils) as described previously (34, 35). Binding assays were performed in 96-well microtiter plates containing 1 x 10⁶ human eosinophils/well in a final volume of 0.1 ml. Assay buffer (RPMI 1640; 25 mM HEPES (pH 7.4), 0.1% gelatin, 0.1% sodium azide, and 0.08% CHAPS) and compounds were added at the indicated concentrations in a volume of 25 µl; the final Me₆SO concentration was <1%.

Binding was initiated by the addition (25 µl) of either 125I-eotaxin (Amersham Pharmacia Biotech; 2.200 Ci/mmol) or 125I-MCP-4 (PerkinElmer Life Sciences; 2,000 Ci/mmol) and 0.2 nM final concentration. After a 1-h incubation at room temperature, the plate was harvested using a 96-well filter plate harvester (Packard) onto a Unifilter-96 G/F (Packard) filtermate blocked with 1% polyethyleneimine (Sigma) and washed eight times (0.2 ml/wash) with 20 µM HEPES (pH 7.4) in 0.5 µl NaCl. The filter plate was dried, and the bottom was sealed. Microcristant-20 (Packard) (50 µl) was placed into each well, and the plate was sealed with Topseal. The plate was counted using a Packard Top Count NXT.

Other binding assays used to assess selectivity were performed according to previously published reports (C5a (35) and LTB₄ (36)). The C3a binding was carried out as described previously (37) except that RBL-2H3-3a receptor cells were used (4 x 10⁶ cells/well) with a final 125I-C3a concentration of 100 pM in a volume of 100 µl. MIP-1α binding was carried out using HEK-293 cells expressing CCR1 (Receptor Biology, Beltsville, MD), which were reconstituted and used as described by the manufacturer (38). CXCR1 and CXCR2 binding experiments were carried out as described previously (39).

**Inhibition of Ca²⁺ Mobilization in Eosinophils**

Human eosinophils, isolated as described above, or RBL-2H3-CCR3 cells were loaded with Fura-2/AM as previously described (40). For antagonist studies, compounds were added at the indicated concentrations (final Me₆SO <0.35%) to 1 x 10⁶ eosinophils/ml in Krebs Ringer Henseleit buffer, followed 15 s later by agonist at the designated concentration. The maximal calcium mobilization attained after agonist stimulation was quantitated as described previously (40).

**Inhibition of Eosinophil Chemotaxis**

Eosinophil motility was determined using a modified Boyden chamber procedure as described (40). Briefly, for the measurement of chemotaxis, lower chambers were filled with 30 µl of eotaxin (3.3 nM), MCP-4 (10 nM), or C5a (10 nM) separated from the upper chamber by a 5-µm polycarbonate filter (Poretics, Livermore, CA). Into the upper chamber was placed 50 µl of an eosinophil suspension (5 x 10⁶ cells/ml) in PAGCM buffer (147 mM NaCl, 5 mM KCl, 20 mM PIPES (pH 7.4) with 0.03% human serum albumin, 0.1% CHAPS) and compounds were added at the indicated concentrations (final Me₆SO <0.35%). Eosinophil migration proceeded for 30 min at 37 °C in a 5% CO₂ incubator, after which the chamber was disassembled. Following fixation of the filter (75% methanol) and staining (Diff-Quick), the migrated cells were counted in four successive high power fields.

**Synthesis of S&K-F-1-45523, SB-297006, and SB-328437**

S&K-F-1-45523: (S)-Ethyl-2-benzoylamoino-3-(3,5-dii-odo-4-hydroxyphenyl)propionate

Bi was prepared in a 0.24 mg, 0.64 mmol reaction mixture in 3.5 ml. N-Benzoyl-L-tyrosine ethyl ester (0.10 g, 0.32 mmol) was then added, immediately causing the color of the solution to turn from pale pink to pale yellow. The solution was stirred at room temperature under nitrogen for 1 h. Aqueous saturated Na₂S₂O₃ was poured into the flask, and the product was extracted with CH₂Cl₂. The organic portion was dried over MgSO₄, filtered, and concentrated to a yellow oil. Trituration with diethyl ether induced precipitation of a white solid (0.11 g, 61%) which was filtered, washed with diethyl ether, and dried. MS (ES+) m/z 566 [M + H]⁺, 588.

SB-297006: (S)-Ethyl-2-benzoylamoino-3-(4-nitrophenyl)propionate

(S)-Ethyl-2-amino-3-(4-nitrophenyl)propionate Hydrochloride—4-Nitro-L-phenylalanine monohydrate (1.02 g, 4.47 mmol) was suspended in ethanol (20 ml), and HCl gas was bubbled into the suspension until a
clear solution formed. The mixture was refluxed for 6 h, cooled to room temperature, and concentrated to yield the title compound as a white solid (1.23 g, 100%). MS (ES +) mle 239 [M + H].

(S)-Ethyl-2-benzoylamino-3-(4-nitrophenyl)propionate—(S)-Ethyl-2-benzoylamino-3-(4-nitrophenyl)propionate hydrochloride (1.23 g, 4.47 mmol) was suspended in dichloromethane (25 ml) and treated with benzoyl chloride (0.628 g, 4.47 mmol) and triethylamine (1.34 g, 13.4 mmol). The mixture was stirred overnight at room temperature under nitrogen. The crude reaction was washed with 5% HCl, water, brine; dried (Na2SO4); concentrated; and purified by flash chromatography (silica gel, 2:1 hexanes/ethyl acetate) to afford SB-297006 as a white powder (0.97 g, 92%). MS (ES +) mle 343 [M + H]; melting point 99–101 °C.

SB-328437: (S)-Methyl-2-naphthoylamino-3-(4-nitrophenyl)propionate

(S)-2-Naphthoylamino-3-(4-nitrophenyl)propionic Acid—A suspension of 4-nitro-L-phenylalanine (5.0 g, 22 mmol) in water (45 ml) was treated with a solution of sodium hydroxide (0.9 g, 20 mmol) in water (5 ml). 1-Napthoyl chloride (3.8 g, 20 mmol) in toluene (10 ml) was added followed by a solution of sodium hydroxide (0.9 g, 20 mmol) in water (5 ml). The mixture was stirred at room temperature for 30 min and acidified with 1 N sulfuric acid. The resulting solid was isolated; washed with water, diethyl ether; and dried to a white powder (0.865 g, 57%). MS (ES +) mle 386 (Na2SO4); concentrated; and purified by flash chromatography (silica gel, 2:1 hexanes/ethyl acetate) to afford SB-297006 as a white powder (0.97 g, 92%). MS (ES +) mle 343 [M + H]; melting point 99–101 °C.

A high throughput screen was configured using RBL-2H3-CCR3 cells and a FLIPR-based 96-well calcium mobilization assay utilizing eotaxin as the activating ligand (42, 43). One compound identified in this screen was a diiodotyrosine ester, SK&F-L-45523 (Fig. 1), which selectively inhibited eotaxin-induced calcium mobilization in RBL-2H3-CCR3 cells with an IC50 of 800 ± 60 nM, 60, and 4.5 nM, respectively. Results are expressed as a percentage of control specific binding and are the mean ± S.E. of multiple experiments (n = 3–4). B, competition binding of [125I]MCP-4 by SK&F-L-45523, SB-297006, and SB-328437 in human eosinophils. SK&F-L-45523 (●), SB-297006 (○), and SB-328437 (▲) inhibit binding with IC50 values of 800, 60, and 4.5 nM, respectively. Results are expressed as a percentage of control specific binding ± S.E. (n = 3–4).

Chemical modifications to SK&F-L-45523 led to a number of more potent derivatives including SB-297006 (Fig. 1B) and the most potent CCR3 antagonist, SB-328437 (Fig. 1C), which reversibly inhibited 125I-eotaxin binding to human eosinophils with IC50 values of 60 ± 5.5 and 4.5 ± 1.6 nM, respectively (Fig. 2A). In addition, all three antagonists inhibited binding of 125I-MCP-4 to human eosinophils with IC50 values of 360 ± 64 nM, 44 ± 14.4, and 7 ± 0.5 nM for SK&F-L-45523, SB-297006, and SB-328437, respectively (Fig. 2B).

To determine the specificity of these compounds for CCR3, we assessed whether these compounds could inhibit the binding of a number of agonists to their respective cognate 7-TM receptors. At concentrations up to 33 μM, SB-297006 failed to significantly inhibit the binding of [125I]-IL-8 to CHO-CXCR1 cell or CHO-CXCR2 cell membranes, 125I-SLC (CCL-21) to HEK-293-CXCR7 membranes, or [3H]LTB4 to guinea pig lung membranes (Fig. 3). A number of other receptors were similarly insensitive at a single concentration (10 or 33 μM) of SB-297006 or SB-328437 (Table I) in Ca2+ mobilization or binding assays. SB-297006 and SB-328437 were therefore, at a minimum, 250-fold selective for CCR3 over the other 7-TM receptors tested.

To determine if SB-297006 and SB-328437 were functional CCR3 antagonists, we monitored their effects on intracellular calcium mobilization induced by eotaxin, eotaxin-2, or MCP-4. In RBL-2H3-CCR3 cells, SB-297006 and SB-328437 concentration dependently inhibited calcium mobilization induced by all three CCR3 agonists (Fig. 4, A and B). Thus, these two antagonists inhibited calcium mobilization induced by
CCR3 Antagonist Blocks Eotaxin-induced Eosinophil Migration

A new technology has been developing over the last couple years to characterize 7-TM receptor calcium mobilization responses in a high throughput 96-well format. Several recent reports from our laboratories and others have shown the utility of this technology for identifying activating ligands of "orphan" 7-TM receptors (32, 49, 50). In addition, the FLIPR calcium assays have been used to pharmacologically characterize known 7-TM receptors using standard agonists and antagonists (51). In this study, we utilized this technique to configure a high throughput screen to identify novel 7-TM receptor antagonists from an in-house compound bank. To our knowledge, this is the first report utilizing the FLIPR technology and a cellular functional assay to identify selective 7-TM receptor antagonists.

The antagonists identified were capable of inhibiting the binding of eotaxin and MCP-4 to CCR3, suggesting that these antagonists inhibit the receptor-ligand interaction by binding to the chemokine receptor rather than binding to the chemokine itself. Thus, these compounds serve as a starting point to potentially inhibit binding and receptor activation by all CCR3 agonists.

These antagonists were able to inhibit a cellular calcium functional response induced by three different CCR3 agonists with similar potencies, to their inhibition of eotaxin binding. Again, this result demonstrates that a single antagonists can bind the receptor in such a way that it can inhibit a functional response induced by multiple CCR3 ligands. This would suggest that the CCR3 ligands, which vary in amino acid identity between 34 and 64%, interact with the receptor in a similar manner. Furthermore, the results support a pharmacological approach that may have better disease-modifying activity than an antibody to a single CCR3 ligand, which would inhibit only a single agonist and may not deal with the redundant nature of the chemokine family. Moreover, unlike the recently reported CCR1 antagonist (28), these antagonists were highly selective and did not interact with several other 7-TM receptors including other chemokine receptors, indicating that their interaction is specific and presumably represents a unique site within the receptor for antagonist binding. Interestingly, the compounds described in the present work are structurally unrelated to the recently disclosed, nonselective, aminopiperidine CCR1 antagonists (28). The lack of a basic center in the CCR3-selective compounds reported here coupled with the apparent requirement for a quaternary nitrogen in the nonselective compounds suggests that the two series of antagonists may interact with distinct amino acid residues(s) in the receptor. In particular, to confirm selectivity, we studied the binding of 125I-MIP-1α to CCR1 because CCR1 has the highest homology to CCR3 (62.5% identity) and is therefore the most likely receptor to be inhibited by these compounds. In this respect, none of these compounds inhibited MIP-1α binding to CCR1 membranes at concentrations up to 10 μM. In addition, these compounds like UCB 35625 failed to antagonize the binding of murine or guinea pig eotaxin to murine or guinea pig CCR3 at concentrations up to 10,000 times the IC50 for inhibiting human CCR3. This indicates that although mouse and guinea pig CCR3 are 67 and 65% identical to human CCR3, respectively, there is still sufficient divergence in the receptor sequence to prevent effective binding of these compounds.

The present study represents the first report of a potent and selective non-peptide functional antagonist of human CCR3 and is the sixth reported small molecule antagonist of a chemokine receptor (28, 39, 46–48). Although small molecule antagonists of other 7-TM G protein-coupled receptors including...
Eotaxin, IL-5, IL-6, IL-8, MCP-1, and C3a binding assays were performed as described under “Experimental Procedures.” Whole cell calcium mobilization assays used the ligand and cell types indicated. RANTES, MCP-1, and MIP-1α were used in freshly isolated monocytes loaded with Fura-2 (33), while C5a used HEK-293 cells transfected with the C5a receptor.

| Ligand (receptor) | Assay design | Assay configuration | Cell type | IC50 SB-297006 | Ratio | IC50 SB-328437 | Ratio |
|------------------|--------------|---------------------|-----------|---------------|-------|---------------|-------|
| Eotaxin (CCR3)   | Binding      | Eosinophils (whole) |           | 0.039         | 1     | 0.004         | 1     |
| IL-8 (CXCR1)     | Binding      | CHO (membr.)        |           | >27           | >700  | >27           | >700  |
| IL-8 (CXCR2)     | Binding      | CHO (membr.)        |           | >27           | >700  | >27           | >700  |
| RANTES           | Ca2+        | Monocyte (whole)    |           | >10           | >250  | >10           | >250  |
| MCP-1            | Ca2+        | Monocyte (whole)    |           | >10           | >250  | >10           | >250  |
| MIP-1α (CCR1)    | Binding      | HEK-293 (membr.)    |           | >10           | >250  | >10           | >250  |
| C5a (C5aR)       | Ca2+        | RBL-2H3 (whole)     |           | >33           | >850  | >10           | >2500 |
| SLC (CCR7)       | Binding      | HEK-293 (membr.)    |           | >33           | >850  | >33           | >8250 |
| LTD4             | Binding      | gp-trachea          |           | >10           | >256  | >10           | >2500 |
| C3a (C3aR)       | Binding      | RBL-2H3 (whole)     |           | >33           | >850  |               |       |

**FIG. 4. Effect of SB-297006 and SB-328437 on agonist-induced calcium mobilization.** A, inhibition of Ca2+ mobilization in RBL-2H3-CCR3 cells by SB-297006 stimulated with 3.3 nM eotaxin (●), eotaxin-2 (■), or MCP-4 (△). B, inhibition of Ca2+ mobilization in RBL-2H3-CCR3 cells by the higher affinity antagonist SB-328437, stimulated by 3.3 nM of eotaxin (●), eotaxin-2 (■), or MCP-4 (△). Cells were pretreated for 15 s with the indicated concentrations of SB-297006 (10–3000 nM) or SB-328437 (3.3–1000 nM) before the addition of agonist. Data shown in A and B are the mean of three individual experiments ± S.E.

**FIG. 5. Effect of SB-328437 on agonist-induced calcium mobilization in human eosinophils.** Freshly isolated human eosinophils were loaded with Fura-2 and stimulated with a single concentration (3.3 nM) eotaxin (●), eotaxin-2 (■), MCP-4 (△), or C5a (□). Cells were pretreated for 15 s with the indicated concentrations of SB-328437 (10–3000 nM) before the addition of agonist. Intracellular calcium mobilized was monitored until peak intracellular calcium was reached, at which point the measurement was terminated, and minimum and maximum fluorescence were determined as described under “Experimental Procedures.” Data are the average of three individual experiments ± S.E.

**FIG. 6. Effect of SB-328437 on eotaxin-, eotaxin-2-, MCP-4-, or C5a-induced human eosinophil chemotaxis.** Chemotaxis was measured in 48-well Boyden chambers as described under “Experimental Procedures.” The effect of SB-328437 on chemotaxis induced by 3.3 nM eotaxin (●), 3.3 nM eotaxin-2 (■), 10 nM MCP-4 (△), or 10 nM C5a (□) was evaluated with eosinophils. Control agonist responses were 270 ± 16.5, 235 ± 9.5, 254 ± 14.4, and 522 ± 18.8 eosinophils/high power field for eotaxin, eotaxin-2, MCP-4, and C5a, respectively. The negative unstimulated response was 36.5 ± 1.7 eosinophils/high power field. Results are expressed as a percentage of control cells responding to their respective ligands. Each point represents a mean of three determinations from four individual donors ± S.E.

Short peptide receptors (e.g., tachykinin (52), angiotensin (53), and endothelin (54)) have been reported, chemokines and other large protein-agonist interactions with their receptors have been more difficult to antagonize. To our knowledge, the only antagonist reported for a large peptide receptor other than the chemokine receptors was a micromolar antagonist of the C5a receptor (55). Since chemokine receptors are part of the 7-TM receptor family, which have traditionally been productive targets for drug discovery, it is anticipated that small molecule CCR3 receptor antagonists may have potential as novel therapeutics. The availability of potent and selective non-peptide antagonists, such as SB-297006 and SB-328437, will help de-
fine the apparent overlap in activities of the chemokines and their receptors and elucidate their relative importance. In particular, SB-328437, the most potent CCR3 antagonist, will be an important tool to compound to assess the role of CCR3 in eosinophil recruitment, a process that is thought to be crucial in the pathology of several inflammatory diseases including asthma, allergic rhinitis, and eczema.

REFERENCES

1. Baggilini, M., Mosen, B., and Clark-Lewis, I. (1994) Chest 105, Suppl. 3, 105A–106A.
2. Oppenheim, J. J., Zachariae, C. O., Mukaida, N., and Matsushima, K. (1991) Annu. Rev. Immunol. 9, 617–48.
3. Schall, T. (1994) in The Cytokine Handbook (Thompson, A., ed) 2nd Ed., pp. 419–460, Academic Press, Inc., San Diego.
4. Mackay, C. R. (1996) J. Exp. Med. 184, 799–802.
5. Mackay, C. R. (1997) Curr. Biol. 7, R348–R356.
6. Bargou, R. (1996) J. Clin. Invest. 97, 887.
7. Kita, H., and Gleich, G. J. (1996) J. Exp. Med. 183, 2421–2426.
8. Kroegel, C., Liu, M. C., Hubbard, W. C., Lichtenstein, L. M., and Bochner, B. S. (1997) J. Allergy Clin. Immunol. 99, 632–639.
9. Durham, S. R. (1998) Clin. Exp. Allergy 28, Suppl. 6, 20–24.
10. Leung, D. Y. (1999) J. Allergy Clin. Immunol. 104, (suppl.) 599–5108.
11. Bischoff, S. C., Mayer, J., Nguyen, Q. T., Stolte, M., and Manns, M. P. (1999) Nature 400, 657–663.
12. Stellato, C., Collins, D. A., Walsh, D. T., Moqbel, R., Tottt, N. F., Truong, O., Hsuan, J. J., and Williams, T. J. (1994) J. Exp. Med. 179, 881–887.
13. Ying, S., Meng, Q., Zeibecoglou, K., Robinson, D. S., Macfarlane, A., Humbert, M., and Kay, A. B. (1999) J. Immunol. 163, 6321–6329.
14. Roberton, M. E., MacLean, J. A., Pearlman, R., Luster, A. D., and Leder, P. (1997) J. Exp. Med. 185, 785–790.
15. Yang, Y., Loy, J., Ryan, R. P., Carrasco, D., and Bravo, R. (1998) Blood 92, 2054–2060.
16. Comedricia, C., Auha, S. K., and Murphy, P. M. (1995) J. Biol. Chem. 270, 16491–16494.
17. Forgherty, B. L., Siciliano, S. J., DeMartino, J. A., Malkovitz, L., Sirotina, O., and Springer, M. S. (1996) J. Exp. Med. 183, 2349–2354.
18. Ponath, P. D., Qin, S., Post, T. W., Wang, J., Wu, L., Gerard, N. P., Newman, W., and Springer, M. S. (1996) J. Biol. Chem. 271, 7725–7730.
19. Heath, H., Qin, S., Rao, P. W., Lu, L., Laffos, G., Kassam, N., Ponath, P. D., and Wells, T. N. (1997) J. Exp. Med. 185, 179–184.
20. Forssmann, W. G., Ueguczioni, M., Loetscher, P., Dahinden, C. A., Langen, H., Thelen, M., and Baggiolini, M. (1997) J. Exp. Med. 185, 2171–2176.
21. White, J. R., Imburgia, C., Del, E., Appuhamy, E., O'Donnell, K., O'Shannessy, D. J., Brawner, M., Jaffe, S. T., and Smart, D. (1999) J. Med. Chem. 42, 1283–1289.
22. Clozel, M., Breu, V., Burri, K., Cassal, J. M., Fischli, W., Gray, G. A., Hirth, G., Loffler, B. M., Muller, B. M., and Neidhart, W. (1999) Nature 397, 759–761.
23. Lanza, T. J., Durette, P. L., Rolins, T., Siciliano, S., Cianciarulo, D. N., Kobayashi, S. V., Caldwell, C. G., Springer, M. S., and Hamgann, W. K. (1992) J. Med. Chem. 35, 252–258.