CCR3 Expression and Function in Asthmatic Airway Smooth Muscle Cells

Philippe Joubert, Stéphane Lajoie-Kadoch, Isabelle Labonté, Abdelilah Soussi Gounni, Karim Maghni, Vincent Wellemans, Jamila Chakir, Michel Laviolette, Qutayba Hamid and Bouchaib Lamkhioued

*J Immunol* 2005; 175:2702-2708; doi: 10.4049/jimmunol.175.4.2702

http://www.jimmunol.org/content/175/4/2702

---

**References**

This article cites 40 articles, 15 of which you can access for free at:

http://www.jimmunol.org/content/175/4/2702.full#ref-list-1

**Subscription**

Information about subscribing to *The Journal of Immunology* is online at:

http://jimmunol.org/subscription

**Permissions**

Submit copyright permission requests at:

http://www.aai.org/About/Publications/JI/copyright.html

**Email Alerts**

Receive free email-alerts when new articles cite this article. Sign up at:

http://jimmunol.org/alerts
CCR3 Expression and Function in Asthmatic Airway Smooth Muscle Cells

Philippe Joubert,* Stéphane Lajoie-Kadoch,* Isabelle Labonté,† Abdelilah Soussi Gounni,‡ Karim Maghni,§ Vincent Wellemans,‖ Jamila Chakir,‡ Michel Laviolette,‡ Qutayba Hamid,* and Bouchaib Lamkihoued‖

Asthma is characterized by an increase in airway smooth muscle mass and a decreased distance between the smooth muscle layer and the epithelium. Furthermore, there is evidence to indicate that airway smooth muscle cells (ASMC) express a wide variety of receptors involved in the immune response. The aims of this study were to examine the expression of CCR3 on ASMC, to compare this expression between asthmatic and nonasthmatic subjects, and to determine the implications of CCR3 expression in the migration of ASMC. We first demonstrated that ASMC constitutively express CCR3 at both mRNA and protein levels. Interestingly, TNF-α increases ASMC surface expression of CCR3 from 33 to 74%. Furthermore, using FACS analysis, we found that ASMC CCR3 is expressed to a greater degree in asthmatic vs control subjects (95 vs 75%). Functionality of the receptor was demonstrated by calcium assay; the addition of CCR3 ligand eotaxin to ASMC resulted in an increase in intracellular calcium production. Interestingly, ASMC was seen to demonstrate a positive chemotactic response to eotaxin. Indeed, ASMC significantly migrated toward 100 ng/ml eotaxin (2.2-fold increase, compared with control). In conclusion, the expression of CCR3 by ASMC is increased in asthmatics, and our data show that a CCR3 ligand such as eotaxin induces migration of ASMC in vitro. These results may suggest that eotaxin could be involved in the increased smooth muscle mass observed in asthmatics through the activation of CCR3. The Journal of Immunology, 2005, 175: 2702–2708.

Materials and Methods

Cell culture

Human ASMC were obtained from two sources. Bronchial/tracheal smooth muscle cells (B/TSMC) were purchased from Cambrex and were positively stained for α-smooth muscle actin and negatively for factor VIII, CD45, and CD3, as indicated by the manufacturer. B/TSMC were grown in their optimal medium (SmGM-2; Cambrex) containing 5% FBS at 37°C in a humidified incubator with 5% CO₂, as recommended by the supplier. The
second source of ASM were from human bronchiol epithelial, ASM were isolated and purified from biopsies as described by Labonte et al. (15). Briefly, bronchial biopsies obtained from mild steroid-naive asthmatic subjects (mean age 25 years, methacholine provocative concentration 20 lower or equal to 4.33 mg/ml, positive skin prick test for at least one inhaled allergen) and nonasthmatic, nonallergic subjects (mean age 29.3 years, methacholine provocative concentration 20 higher than 24.4 mg/ml, negative skin prick test for the inhaled allergens) underwent four consecutive cycles of enzymatic digestion with collagenase (Roche) and/or elastase (Sigma–Aldrich) for 20 min at 37°C. Cells were then plated in flasks and cultured in complete DMEM-F12 medium containing 10% FBS, 20 U/ml penicillin, 20 μg/ml streptomycin, 25 ng/ml fungizone, 5 mg/ml insulin, 10 ng/ml epidermal growth factor, 5 μg/ml transferrin, 10−10 M cholera toxin, and 2 × 10−3 M 3,5,3′-triiodo-l-thyronine sodium salt. The medium was replaced every 2–3 days, and cells were passaged with 0.5% trypsin and 1 mM EDTA once confluence was reached. Cell identity was assessed by measuring the expression of smooth muscle actin, calponin, smooth muscle myosin H chain (SM-1 and SM-2), tropomyosin, desmin, and vimentin (16). ASM demonstrated the characteristic hill and valley appearance, with an elongated and spindle shape and possessing a central nucleus.

**Cell stimulation**

Confluent B/TSMC from Cambrex were used in passages 3–6 while ASM from biopsies were used in passages 2–3. Cells were growth arrested by FBS deprivation for 24 h before stimulation with cytokines. After serum deprivation, cells were stimulated in fresh, serum-free medium containing either TNF-α, IL-4, IL-13, or IFN-γ (R&D Systems) in a concentration- and time-dependent manner. Cell viability was assessed by the trypan blue dye exclusion test.

**RNA extraction and RT-PCR**

Total cellular RNA was isolated from B/TSMC, epithelial cells (Calu-3), purified peripheral blood eosinophils and neutrophils, and CCR3-transfected Ghost cells (National Institutes of Health, AIDS reagent program, no. 3682). RNA was extracted using the RNeasy mini kit extraction columns (Qiagen) as directed by the manufacturer. RNA was eluted in 30 μl of nuclease-free water, and cDNA was generated in a 30 μl reaction, using 0.5 μg of total RNA, oligo(dT)12–18 primers (Amersham Pharmacia Biochem), and SuperScript II (Invitrogen Life Technologies), in the presence of RNAGuard (Amersham Pharmacia Biotech).

Genes of interest were amplified using conventional PCR. The PCR mixture consisted of 1.5 mM MgCl2, 1× PCR buffer, 0.25 mM dNTP, 2.5 U Taq Platinum polymerase (Invitrogen Life Technologies), 0.4 μM each sense and antisense primers, and 1 μl of cDNA. Primers for the housekeeping gene ribosomal protein S9 and CCR3 were commercially generated (GeneWorks, Nottingham, U.K.). The following sequences: sense 5′-TGC TGA CGC TTC ATG AGA AG-3′ and antisense 5′-CGC AGA GAG TCG ATG TG-3′ and CCR3 sense 5′-TCC TCT CCT CTT ATC AAT C-3′ and antisense 5′-GCC ATT TTT CTG CAT CTG-3′. The samples were amplified in a thermal cycler (PTC-100 Programmable Thermal Controller; MJ Research) for 35 cycles, consisting of denaturation at 94°C for 1 min, annealing at 57°C, and extension at 72°C. The PCR products were visualized on a 2% agarose gel containing 0.2 μg/ml ethidium bromide, and clear band size was determined by comparison with a 100-bp DNA ladder (Invitrogen Life Technologies). Amplicons were purified using the QIAquick PCR purification kit (Qiagen), ligated in pGEM-T (Promega), and used for transformation into XL-1 blue bacteria. Plasmids representing each insert were purified using a HiSpeed Plasmid Maxi Kit (Qiagen) and commercially sequenced to confirm integrity and identity (Pavillon de synthèse et d’analyse d’acides nucléiques, Université Laval).

**Immunohistochemistry**

To determine whether ASM have the capacity to produce CCR3 in vivo, immunohistochemistry was performed on sections of major airways (large bronchi) from four asthmatics and four normal subjects. The subjects were obtained from the Tissue Bank (MCI/Meakins–Christie Tissue Bank, McGill University). A clinical diagnosis of asthma was based on the evaluation of the patient’s medical file by a respiratory physician. Diagnostic criteria included prior inclusion and treatment for asthma, documented evidence of variable airflow obstruction (>15%), and bronchial hyperresponsiveness. Immediately following biopsies, lung specimens were prepared for immunohistochemistry. Briefly, formalin-fixed tissues were paraffin embedded, and 5-μm-thick sections were prehydrated in xylene, and hydrated through a graded alcohol series. Endogenous peroxidase was quenched by incubating the slides in 0.5% hydrogen peroxide in PBS for 30 min. After rinsing in PBS, sections were blocked with block-
inverted-phase microscope (IX-81) using a mounted digital camera (CoolSnapPro CF monochrome) equipped with a CRI filter. Images were analyzed by using ImagePro Plus software (Carsen Group).

Measurement of intracellular-free Ca\(^{2+}\)

For calcium measurements, glass coverslips with confluent serum-deprived B/TSMC were rinsed twice with prewarmed (37°C) HEPES-buffered (10 mM, pH 7.4) HBSS-1% BSA, and incubated for 1 h at 37°C in a buffer containing the calcium-sensitive dye fura 2-AM (10 \(\mu\)M) as previously described (17). Thereafter, the fura 2-loaded cultures were washed twice with HEPES-HBSS and then incubated in the dark at RT for 30 min before measurement of intracellular calcium. Glass slides were mounted on an inverted microscope (Olympus IX70) equipped with an OlymPix TE3/A/S digital camera controlled through a PC workstation. Cells were alternatively exited at 340 and 380 nm using a A 10 filter (Sutter Instruments). Emitted fluorescence (510 nm) was measured for 350 ms at each excitable wavelength, and collected data were used to calculate calcium concentrations (in nanomolars) at each pixel from an in vitro calibration curve of known free Ca\(^{2+}\) (0–1.35 \(\mu\)M) and pentapotassium fura 2 (50 \(\mu\)M). An Olympus UAPON/340 \(\times 200\) objective was used in all studies, and image size was set to 540 \(\times\) 540 pixels. Calcium responses within individual cells were determined using UltraView version 4.0 software (Olympus), by circumscibing single myocytes and spatially averaging fura 2 fluorescence within each cell. At the beginning of each experiment, each chamber contained 200 \(\mu\)l of HEPES-HBSS. Intracellular free Ca\(^{2+}\) was first recorded for 30 s to establish a baseline; the cells were then stimulated by adding an equal volume of HEPES-HBSS containing either recombinant human eotaxin-1 or MCP-4 (both at 50 ng/ml). Intracellular-free Ca\(^{2+}\) was recorded for at least 200 s to characterize peak and plateau responses, and acetycholine was used as a positive control. Studies were performed in duplicate using three cell lines, each acquired from a different donor.

Chemotaxis assay

Migration of B/TSMCs in response to different concentrations of eotaxin (1, 10, 100, and 1000 ng/ml) was assessed in a 24-well microchemotaxis chamber (NeuroProbe) using a polycarbonate filter (8-\(\mu\)m pore size), as previously described (9). Briefly, B/TSMCs were resuspended in Ham's F-12 medium supplemented with 0.1% BSA, and 5 \(\times\) 10\(^5\) cells were then loaded into the upper chambers and tested for chemotraction to medium supplemented with either 0.1% BSA (negative control) or increasing doses of eotaxin. In some of the experiments, cells were incubated for 1 h with 10 \(\mu\)g/ml anti-CCR3 Ab (MAB155, clone 61828; R&D Systems) before the addition of a CCR3 ligand: FITC-labeled eotaxin (see Fig. 1). This signal was completely suppressed when anti-human eotaxin blocking Ab. A representative experiment of two is shown.

Statistical analysis

Statistical significance was determined using a Student's t test. Values of \(p < 0.05\) were considered statistically significant.

Results

ASMC constitutively express CCR3 at mRNA and protein levels

To demonstrate the expression of CCR3 by ASMC at both mRNA and protein levels, we performed RT-PCR and Western blot on RNA and proteins obtained from cultured ASMC, epithelial cells, and CCR3-transfected cells, as well as from eosinophils and neutrophils purified from blood. Gel electrophoresis (Fig. 1A) revealed bands corresponding to the expected size of the CCR3 cDNA product (313 bp). At the protein level, Western blot revealed comparable expressions of CCR3 by ASMC, Calu-3 epithelial cells, and CCR3-transfected cells, whereas eosinophils demonstrated greater signal intensity (Fig. 1B). Surface expression of CCR3 by ASMC was confirmed using flow cytometry and revealed a high percentage of unstarved serum cells expressing the receptor (see Fig. 1C). In the left panel of Fig. 1C, a PE-labeled Ab against CCR3 was used. The specificity of the signal was confirmed by the addition of a CCR3 ligand: FITC-labeled eotaxin (see Fig. 1C, right panel). This signal was completely suppressed when anti-eotaxin was added prior to eotaxin (see Fig. 1C, right panel). Immunofluorescence was performed on unstimulated B/TSMC and demonstrated an increased signal compared with the isotype control. Also, a high percentage of cells stained positive for CCR3 (Fig. 1D), confirming the previous results obtained by flow cytometry (Fig. 1C).

FIGURE 1. Detection of CCR3 in B/TSMC. RT-PCR (A) and Western blot (B) analysis of constitutive CCR3 expression by B/TSMC. Epithelial cells (EC), eosinophils (Eosino), and CCR3-transfected cells (CCR3\(^+\)) were used as positive controls, whereas purified neutrophils (Neuto) were used as a negative control (representative of \(n = 4\)). C. Determination of CCR3 surface expression by B/TSMC using flow cytometry. Confluent B/TSMC (passages 3–6) obtained from Cambrex were cultured and analyzed by flow cytometry for cell surface expression of CCR3. C. Left panel, B/TSMC were labeled using a rat anti-CCR3 Ab; as a negative control, cells were labeled with isotype matched IgG2A. A representative experiment of five is shown. C. Right panel, biotinylated human recombinant eotaxin was added to cultured B/TSMC in the absence or presence of anti-human eotaxin blocking Ab. A representative experiment of two is shown. D. Immunofluorescent staining of CCR3 in unstimulated permeabilized B/TSMCs. Nuclei are stained blue with 4,6-diamidino-2-phenylindole.
**CCR3 is expressed by ASMC in vivo**

To further investigate the protein expression of CCR3 in human airways, immunocytochemistry was performed. CCR3 immunoreactivity was detected in ASMC in subjects with (Fig. 2A) and without (Fig. 2B) asthma. In asthmatic specimens, CCR3 protein was localized in the smooth muscle bundles, airway epithelium, and infiltrating cells found within the submucosa, as previously demonstrated (data not shown) (13, 18).

**TNF-α up-regulates the expression of CCR3 by ASMC at the protein level**

Expression of chemokine receptors has been shown to be regulated by different cytokines (19). In asthma in particular, the cytokine environment is characterized by increased TNF-α, IL-4, and IL-13 and decreased IFN-γ levels (20). We investigated the effects of the addition of IL-4, IL-13, IFN-γ, and TNF-α on B/TSMC surface expression of CCR3. Addition of IL-4, IL-13, and IFN-γ downregulated surface expression of CCR3 (33 ± 5% to 23 ± 8% of positive cells for IL-4 (p = 0.14); 33 ± 5% to 13 ± 9% for IL-13 (p = 0.06); and 33 ± 5% to 25 ± 7% for IFN-γ (p = 0.15)), whereas TNF-α was shown to cause significant up-regulation (33 ± 5% to 74 ± 8% of positive cells (p = 0.001)) (Fig. 3). The combination of TNF-α with either IL-4 or IL-13 did not modify the surface expression of CCR3, as seen with TNF-α, IL-4, IL-13, and IFN-γ alone (data not shown).

**ASMC from asthmatics express more CCR3 than control ASMC**

Another objective was to compare the surface expression of CCR3 on ASMC between asthmatic and control patients using flow cytometry. Cells were used soon after initial passaging (passages 2–3), following isolation from asthmatic and nonasthmatic patients. From both groups, we examined the percentage of cells expressing the CCR3 receptor, as well as the intensity of this expression, via a mean assessment of their signal fluorescence. Our observations concluded a higher percentage of CCR3 expression (95 ± 6% vs 75 ± 2%; p < 0.05) as well as a greater mean average fluorescence (21.5 ± 4.3 vs 14.3 ± 0.7; p < 0.01) for ASMC isolated from asthmatic subjects compared with cells isolated from nonasthmatics (Fig. 4).

**Addition of CCR3 ligands induces an increase in intracellular Ca\(^{2+}\) concentration**

To assess the functionality of CCR3, we stimulated B/TSMC with eotaxin and MCP-4. We observed a sharp increase in intracellular Ca\(^{2+}\) concentration within smooth muscle cells following the addition of both eotaxin and MCP-4 (Fig. 5). However, upon adding MCP-4 alone, we obtained a much weaker signal, presumably due to receptor desensitization. A similar desensitization phenomenon was observed when MCP-4 was used first to stimulate the cells, followed by addition of eotaxin, suggesting that the induction of intracellular Ca\(^{2+}\) concentration is through the activation of CCR3 (results not shown).

**Eotaxin induces migration of B/TSMC**

Characteristic of asthma is the increase in smooth muscle mass and the reduction in the distance between the smooth muscle layer and the epithelium, suggested to be associated with the migration of smooth muscle cells toward the smooth muscle layer. Consequently, we wanted to examine whether eotaxin could promote the migration of B/TSMC. As shown in Fig. 6, eotaxin increased the migration of B/TSMC in a dose-dependent manner with a maximal response at 100 ng/ml (2.2 ± 0.32-fold, compared with baseline). No significant differences between 100 ng/ml and 1000 ng/ml (2.06 ± 0.48-fold) were observed when these two doses were compared, suggesting that 100 ng/ml is the peak dose for inducing migration of B/TSMC. Platelet-derived growth factor (PDGF) was used as a positive control and increased cell migration by 2.5 ± 0.30-fold, compared with the medium. Migration of B/TSMC was totally abrogated (1.11 ± 0.07) when the cells were preincubated with blocking anti-CCR3. Collectively, these data demonstrate the
capacity of eotaxin to induce the migration of B/TSMC toward an
increasing gradient of eotaxin through the activation of CCR3. To
address whether the obtained findings were the result of chemo-
taxis or chemokinesis, eotaxin was added to both upper and lower
wells, and migration was examined after identical conditions of
stimulation. As shown in Fig. 6, the absence of a concentration
gradient did not stimulate the migration of B/TSMC, confirming
that eotaxin acts as a chemoattractant for B/TSMC. To assess the
viability of the cells, ASMC were incubated with the same con-
centrations of cytokines or anti-CCR3 for 24 h in six-well plates.
No differences in cell viability were observed between the different
conditions used.

Discussion
In the last few years, studies have shown that ASMC possess prop-
erties that would indicate a potential involvement in airway re-
modeling and inflammation. These properties include the expres-
sion of a variety of cytokines and chemokines as well as their
receptors. The CC and CXC chemokines are important chemotac-
tic molecules that control leukocyte trafficking and functions.
These molecules also play an important role in regulation of leu-
kocyte development, expression of adhesion molecules, cell pro-
liferation, and angiogenesis. CCR3 is a CC chemokine receptor
that has been traditionally associated with recruitment of inflam-
matory cells implicated in the pathophysiology of asthma (21, 22).
At sites of allergic inflammation, increased expression of CCR3
and CCR3 ligands, such as eotaxin and RANTES, by inflammatory
cells have been well characterized (14). However, recent work has
demonstrated that the expression of chemokine receptors is not
restricted to leukocytes. Stellato et al. (18) have shown that airway

FIGURE 3. Regulation of CCR3 expression by
B/TSMC using flow cytometry. Confluent B/TSMC
(passes 3–6) cultured in serum-free medium were
stimulated with or without TNF-α (10 ng/ml), IL-4 (20
ng/ml), IL-13 (20 ng/ml), or IFN-γ (10 ng/ml) for 24 h.
Expression of CCR3 was measured by flow cytometry
using a rat anti-CCR3 Ab. As a negative control, cells
were labeled with isotype-matched IgG2A, and the per-
centage of positive cells was calculated by subtracting
the isotype control from the specific signal.

FIGURE 4. CCR3 expression by asthmatic and nonasthmatic ASMC.
ASMC (passage 3) were obtained from M. Laviolette and J. Chakir’s group
as previously described in Materials and Methods. CCR3 surface expres-
sion was assessed by flow cytometry using a rat anti-CCR3 Ab. As a
negative control, cells were labeled with isotype-matched IgG2A. The per-
centage of positive cells and mean fluorescence intensity were calculated
by subtracting the isotype control from the specific signal. *, p < 0.01 (n =
3 for asthmatic group and n = 4 for control group).

FIGURE 5. Measurement of Ca2+ in cultured B/TSMC in response to
eotaxin and MCP-4. For calcium measurements, confluent serum-fed
B/TSMC were loaded with fura 2 as described in Materials and Methods.
Cells were stimulated with either eotaxin or MCP-4 (50 ng/ml), and intra-
cellular Ca2+ was measured for at least 350 s thereafter. Acetylcholine
(ACh; 10−60 M) was used as a positive control. Data shown are repre-
sentative of three experiments.
epithelial cells could also express CCR3. In this study, we examined the expression of CCR3 by ASM and showed that the receptor is expressed both in vivo and in vitro. Functional studies demonstrate that CCR3 is a functional receptor, as it transduces intracellular calcium mobilization and induces ASM migration. We have also demonstrated that CCR3 is up-regulated in bronchial smooth muscle cells of individuals with asthma, compared with normal control subjects.

Asthma is a disease characterized by marked structural changes of the airway wall. Benayoun et al. (23) and C. Pepe et al. (manuscript in preparation) have recently demonstrated that there is a decrease in the distance between the airway smooth muscle and epithelial layers of asthmatic individuals. The possible migration of ASM, either from the interstitium compartment or from a circulating precursor stem cell population, has been suggested as a possible mechanism to explain the increase in smooth muscle mass observed in the airways of asthmatics (8, 9). The mediators involved in the migration of ASM, however, must still be determined. We hypothesized that the increased levels of eotaxin in asthmatic airways could promote the chemotaxis of ASM. In the present study, we demonstrate that eotaxin is able to induce the migration of ASM in a dose-dependent manner. Similar observations have recently been made concerning the migration of vascular smooth muscle cells in atherosclerosis (24). In vivo, both epithelial and airway smooth muscle cells are potent producers of eotaxin (25–27) and thus may be responsible for the generation of an eotaxin gradient, allowing migration of ASM toward the smooth muscle bundles and the epithelium. To support this hypothesis, C. Pepe et al. (manuscript in preparation) have recently shown that there is an increased production of eotaxin by ASM in severe asthmatics compared with control subjects. Furthermore, this increased production of eotaxin was seen to correlate significantly with the amount of smooth muscle found in the airways of asthmatic patients.

Recent studies strongly suggest that chemokine receptor expression in many cell types can be modulated by both inflammatory and anti-inflammatory signals (18, 22, 28, 29). Proinflammatory Th1 and Th2 cytokines have been shown to be potent mediators regulating the expression of CCR3 in lymphocytes, eosinophils, and neutrophils, whereas TNF-α was shown to augment the expression of CCR3 transcripts in epithelial cells. We examined the effects of IL-4, IL-13, IFN-γ, and TNF-α on the expression of CCR3 in ASM. Using flow cytometry analysis, we found that the expression of CCR3 was increased 24 h after stimulation with TNF-α. Interestingly, IFN-γ, IL-4, and IL-13 acted to slightly down-regulate the cell surface expression of CCR3, suggesting that the Th2-biased immunological state observed in asthmatic individuals is not likely to play a role in the migration of ASM within airways. Rather, proinflammatory cytokines, such as TNF-α, are more likely to control the migration of ASM toward the epithelium. Vijh et al. (30) reported that the CCR3 promotor contains several transcription factor-binding sites for AP-1, a transcription factor implicated in the regulation of genes involved in the pathogenesis of asthma (31). Also, AP-1 is strongly activated by TNF-α (32) and may explain the induction of CCR3 expression by ASM. It is also possible that ASM might be able to migrate toward the smooth muscle layer as a result of interactions between eotaxin and CCR3.

We demonstrated in our study that a higher percentage of asthmatic ASM express CCR3 compared with nonasthmatic patients. This increased expression by asthmatic ASM might be an inducible phenomenon related to the augmented expression of TNF-α in the airways of asthmatics (33, 34). The increased expression of CCR3 on the cell surface attributable to an increased expression of TNF-α might render ASM more responsive to the greater eotaxin levels observed in asthmatic airways (35). These observations suggest that the cytokine environment in asthma could contribute to increased CCR3 production by ASM and their increased responsiveness to eotaxin. A different phenotype linked to a genetic duality between asthmatics and nonasthmatics could also explain this increased expression of CCR3 by ASM.

We have also demonstrated that the addition of various CCR3 ligands, such as eotaxin and MCP-4, to cultured ASM induced the release of intracellular calcium, suggesting efficient signaling through CCR3, as shown in epithelial cells (18). Eotaxin alone was able to trigger an increase in intracellular calcium, supporting the requirement of a G protein-coupled receptor (GPCR) for this effect (36). However, because RANTES, MCP-3, and MCP-4 are able to activate other GPCR potentially expressed by ASM, we chose to focus our attention on eotaxin, which solely binds to CCR3. A decrease in the effect of a second ligand stimulus was also observed independent of whether the same chemokine was used in both instances. This phenomenon, known as desensitization, is a well-documented feature of the GPCR response and is an indication of receptor specificity because only ligands that interact with the same GPCR can desensitize its response (36). Further studies are underway to establish the implication of other intracellular pathways possibly involved in the activation of ASM.

The signaling pathways that mediate chemokine-induced trafficking are not well understood. In lymphocytes, activation of Gαi and release of Gβγ subunits have been shown to be crucial for induction of chemotaxis in response to a chemokine (37, 38), whereas, in tracheal smooth muscle, p38MAPK/HSP27 seems to be involved in initiating migration in response to cytokines and growth factor such as TNF-α, IL-1β, and PDGF (39). In eosinophils, multiple signaling pathways activated by CCR3 participate in the inflammatory response and the initiation of migration. Eotaxin stimulates intracellular calcium release, production of reactive oxygen species, and changes in actin polymerization through a pertussis-sensitive pathway. Rho and Rho-associated coiled coil-forming protein kinase, a protein kinase activated by Rho, regulate actin stress fiber formation and are required for eosinophil chemotaxis (40). MAPK pathways are also involved in

![Cellular migration of B/TSMC in response to eotaxin.](image-url)
chemotaxis (41). However, whether one of these intracellular pathways is involved in the induction of ASMC migration toward a gradient of eotaxin will need to be investigated.

In conclusion, we have demonstrated for the first time the expression of functional CCR3 by ASMC. Our results suggest that the activation of CCR3 by eotaxin could participate in the increased smooth muscle mass observed in the airways of asthmatic subjects, inducing the migration of ASMC toward the smooth muscle layer and contributing to the airway hyperresponsiveness characterizing an episode of asthma. Further work will be required to demonstrate the migration of ASMC in an in vivo model of asthma, as well as to determine the intracellular pathways involved in the activation of CCR3 in ASMC.

Acknowledgments

We sincerely thank Bertrand Lefort for immunofluorescence, Zöe Müller and Wendy Somerville for correction of the manuscript, Fabienne Bellessort for Western blot, and Elsa Schotman for technical support.

Disclosures

The authors have no financial conflict of interest.

References

27. Mitchell, R. W., A. J. Halayko, S. Kahraman, J. Solway, and M. E. Wylam. 2000. Increased smooth muscle mass observed in the airways of asthmatic subjects, inducing the migration of ASMC toward the smooth muscle layer and contributing to the airway hyperresponsiveness characterizing an episode of asthma. Further work will be required to demonstrate the migration of ASMC in an in vivo model of asthma, as well as to determine the intracellular pathways involved in the activation of CCR3 in ASMC.

The authors have no financial conflict of interest.

Downloaded from http://www.jimmunol.org/ by guest on May 1, 2017