

Original article

Differential pattern of CCR1 internalization in human eosinophils: prolonged internalization by CCL5 in contrast to CCL3

Background: Whereas recent studies underlie the fundamental importance of the CC chemokine receptor 3 (CCR3) for the recruitment of eosinophils in allergic diseases, controversial data exist about the relevance of CCR1 on eosinophils. Therefore, the purpose of this study was to investigate the expression and regulation of CCR1 on eosinophils.

Methods: Flow cytometric analysis of whole blood eosinophils and CD16-negative selected eosinophils from healthy nonatopic donors and from patients with atopic disorders was performed and CCR1 receptor internalization and re-expression were studied.

Results: Flow cytometric analysis of whole blood eosinophils revealed that 17.8% of the donors expressed high levels of CCR1 (CCR1^{high}) and 82.2% low levels of CCR1 (CCR1^{low}). A significant down-regulation of CCR1 was induced by 24 h preincubation of isolated eosinophils from CCR1^{high} donors either with IL-3, CC chemokine ligand 3 (CCL3), CCL5, CCL7, or CCL13. Internalization experiments using eosinophils from CCR1^{high} donors revealed that CCL5 is more effective to induce CCR1 internalization than CCL3. Whereas CCR1 re-expression after stimulation with CCL3 reached prestimulation levels (120 min: 81.3% relative CCR1 surface expression) CCL5 induced a prolonged CCR1 internalization (120 min: 15.7%).

Conclusions: This study demonstrates a distinct pattern of CCR1 internalization and re-expression in human eosinophils between CCL3 and CCL5, as CCL5 induces a prolonged CCR1 internalization and the basic value is not reached after 24 h. Since prolonged receptor internalization plays a central role in chemokine-mediated inhibition of receptor function, CCR1 seems to be an attractive target on human eosinophils for chemokine receptor blockade besides CCR3.

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Chemokines play a central role in autoimmune, inflammatory, viral and allergic diseases due to their potential to attract and activate leukocytes (1). They can be divided into four subclasses, which can be distinguished on the basis of the arrangement of the two amino terminal cysteine residues. The major classes are CXC chemokines (CXC Ligands, CXCL) and CC chemokines (CCL) (2). Chemokines mediate their effects by binding to cell-surface receptors, the chemokine receptors CXCR and CCR that belong to the seven-transmembrane domain G protein-coupled receptors (GPCR) (3). Initially it was generally accepted that the chemokine subclasses differ in their biological activity to stimulate different kinds of leukocytes and that CCLs act only on CCRs and CXCLs on CXCRs (2). However, this paradigm has recently been shown to have certain exceptions and these led to a new understanding of the chemokine network. In this model, regulation of leukocyte recruitment by chemokines is based on a combination of agonistic and antagonistic activity (4).

Allergic diseases such as allergic asthma, allergic rhinitis and atopic dermatitis are characterized by a complex interaction of different cell types and mediators. In this context, Th₂ cells, mast cells, basophils and eosinophils can be found in the inflamed tissue due to the attraction of chemokines (5). Different chemokine receptors such as CCR5 (6), CCR6 (7), CCR8 (8), CXCR4 (9, 10) have been suggested to play a pivotal role in allergic diseases and are therefore of interest as drug targets to reduce cellular infiltration (1). Of all the chemokine receptors known, the chemokine receptor CCR3 plays a major role in allergic diseases (11). This is supported by recent studies using a CCR3 knock out mouse model in which the authors underline the importance of the chemokine receptor CCR3 for eosinophil recruitment into the skin and lung tissue as well as the airway hyperresponsiveness in murine models of allergic airway inflammation (12, 13).

Whereas the role of CCR3 for eosinophil activation is unambiguous, only few data about the role of CCR1 in

eosinophil biology exist. Most of the published studies do not mention the expression of CCR1 on human eosinophils or report controversial data about its expression on eosinophils (14–16). This conflict might be due to the observation that eosinophils from some donors respond to the CCR1 ligand CCL3 (MIP-1 α) and eosinophils from other donors do not (17–20). In this context, two recent studies demonstrated that eosinophils from a subgroup of donors express CCR1 and respond to CCL3 (21, 22). Since CCR1 expression seems to be nonnormally distributed in atopic patients (21) and elevated levels of CCL3 has also been found in the asthmatic lung (23), CCR1 besides CCR3 could also represent a relevant drug target for inhibiting the accumulation and activation of eosinophils.

Regulation of chemokine receptors might play an important role for cellular inactivation as reported in several studies. For instance, down-regulation of CCR3 by IL-3 leads to loss of responsiveness of eosinophils to CCL11 (eotaxin) stimulation (24). Another example is the prolonged internalization of CCR5 by the CCL5 derivative AOP-RANTES that results in inhibition of HIV entry (25). Since the regulation of CCR1 on human eosinophils is poorly understood, the aim of this study was to investigate the modulation of CCR1 surface expression by cytokines and of receptor recycling by chemokines in human eosinophils. From the data presented, we found a donor-to-donor variety of CCR1 expression on human eosinophils, a CCR1 down-regulation by IL-3 among the cytokines tested, and a prolonged internalization of CCR1 induced by CCL5. Therefore, CCR1 besides CCR3 might also represent an attractive target to stop the recruitment and activation of human eosinophils.

Materials and methods

Chemokines, cytokines, and monoclonal antibodies (mAbs)

The following reagents were purchased as indicated: Recombinant human CCL3, CCL5, CCL7, CCL11, and CCL13 (PeproTech, London, UK); IL-3, IL-4, IL-5, GM-CSF, IL-12, IL-13, IFN- γ , and TNF- α , anti-human CCR1Ab (clone 53504.111; mouse IgG2b) and anti-human CCR3Ab (clone 61828.111; rat IgG2a) (R&D Systems, Wiesbaden, Germany); isotype control mAbs IgG2a (mouse), IgG2b (rat), FITC-conjugated goat anti-mouse Ab (for anti-CCR1), FITC-conjugated goat anti-rat Ab (for anti-CCR3), and anti-human CD16-PE mAb (clone 3G8) were obtained from Beckman-Coulter (Krefeld, Germany).

Eosinophil isolation

Human granulocytes were isolated from heparin-anticoagulated venous blood from normal nonatopic healthy or atopic donors using Ficoll (Pharmacia, Erlangen, Germany) density gradient centrifugation and CD16-negative selection as described previously (10). The resulting eosinophil purity was 99% as determined by microscopic examination with Kimura staining and flow cytometric

analysis (FACScan[®], Becton Dickinson, Heidelberg, Germany) using anti-CD16. There were no differences in viability of the cells (> 95%) between the fractions, as judged by trypan blue exclusion.

Donors

A total of 73 volunteers (healthy nonatopic donors and atopic donors) were recruited in this study under approval of the local research ethic committee from the Hannover Medical School. For the diagnosis of atopy, a clinical history (asthma, rhinoconjunctivitis, and atopic eczema) was taken from the donors, and increased serum total IgE as well as specific IgE against birch pollen, rye pollen, Timothy grass pollen, cat dander, house dust mite, and aspergillus fumigatus by radioallergosorbent test (RAST) have been measured in our department. All subjects participating in this study were not taking systemic or topic immunosuppressive drugs. Whole blood samples were taken from 73 donors and CCR1 expression of eosinophils in the whole blood sample was analyzed as described below.

Immunofluorescence staining of eosinophils

Immunofluorescence staining of isolated eosinophils was carried out as described previously (10). Aliquots (100 μ l) containing 1×10^5 highly purified eosinophils were incubated at 4°C for 30 min the specific mAbs (anti-CCR1 mAb or anti-CCR3), stained in a second step by a FITC-conjugated goat anti-mouse Ab for CCR1 or FITC-conjugated goat anti-rat for CCR3, and analyzed by flow cytometry.

For staining of eosinophils in whole blood, samples were taken from different healthy donors. After double staining with anti-CCR1 and anti-CD16-PE mAb according to standard techniques, erythrocytes were lysed and remaining cells were fixed simultaneously with Simultest[™] IMK-Lymphocyte Lysing solution (Becton Dickinson, Heidelberg, Germany). Cells were analyzed by flow cytometry and granulocytes were discriminated on the basis of different forward/side scatter properties and electronic gates were set on CD16-negative cells to exclude neutrophils.

Data are expressed as original flow cytometry graph, scattergram (specific mAb vs isotype control), ratio of fluorescence intensity of specific mAb (median) and isotype control (median) or relative fluorescence intensity (%) calculated as: (median channel fluorescence [stimulus] – median channel fluorescence [isotype control]) / (median channel fluorescence [medium] – median channel fluorescence [isotype control]) $\times$ 100%.

Down-regulation of chemokine receptors

These experiments were carried out as described in detail previously (24). Eosinophils from CCR1^{high} donors were incubated in a total volume of 1000 μ l of RPMI for 24 h at 37°C with the indicated cytokine and chemokine. The concentrations of cytokines and chemokines were selected due to their ability to modulate CD69 and CCR3 expression as described previously (24, 26). RPMI medium cultured eosinophils were used as control. Aliquots were taken different points of time and stained for analysis of receptor surface expression as described above.

Flow cytometric measurement of CCR1 internalization

These experiments were performed as previously described in detail (24, 27). For internalization of CCR1, eosinophils from CCR1^{high}

donors were preincubated for 60 min at 37°C with the indicated stimuli in a total volume of 100 µl RPMI 1640 medium prior to staining. Thereafter, cells were fixed on ice and immediately washed with cold PBS. After the washing step, 10^5 eosinophils were stained by anti-CCR1 mAb as described before. Inhibition of CCR1 internalization is achieved by treatment of eosinophils with the stimuli at 4°C. This strategy is suitable to inhibit internalization of seven-transmembraneous receptors from cell surfaces as described earlier (24, 27).

Flow cytometric measurement of CCR1 re-expression

For re-expression experiments, highly purified eosinophils from CCR1^{high} donors were incubated for 60 min with CCL3 or CCL5 to induce maximal down-regulation of the receptor as described previously (24, 27). Thereafter, cells were fixed on ice and immediately washed with cold PBS. After this washing step, 10^5 eosinophils were stained by anti-CCR1 mAb as described above to get the '0 min' basic value. The residual eosinophil sample was further incubated in chemokine-free medium (RPMI) for the indicated time at 37°C. For data analysis only cells with a viability > 95%, as judged by trypan blue exclusion, were taken.

Statistical analysis

Unless otherwise stated, the data in the text and figures were expressed as mean $\pm$ SE of the mean (SEM) as determined by SigmaStatTM (SPSS Inc., Chicago, IL, USA) analysis. Values of $P < 0.05$ were accepted as significant using paired *t*-test or Student's *t*-test: * $P < 0.05$, ** $P \leq 0.01$, and *** $P \leq 0.001$.

Results

Donor to donor variety of CCR1 on human eosinophils

In the first set of experiments we investigated the expression of CCR1 on the surface of human eosinophils from 73 different healthy donors. Flow cytometric analysis of whole blood eosinophils, by gating the CD16-negative granulocyte population in the characteristic FSC/SSC profile, resulted in a mean of 66.9 ± 5.0 for CCR1 and a mean of 53.4 ± 2.6 for the control antibody IgG2b ($P = 0.001$, paired *t*-test) (Fig. 1A). With respect to the 95% confidence interval (7.2–19.8) we found that 17.8% of the donors ($n = 13$) expressed high levels of CCR1 (CCR1^{high}) and 82.2% ($n = 60$) expressed low levels of CCR1 (CCR1^{low}).

To exclude that the donor-to-donor variety is due to an atopic state of the donors we compared the expression of CCR1 and CCR3 of isolated eosinophils from nonatopic donors with that of atopic donors as defined in 'Materials and methods'. No statistical difference ($P = 0.534$) of CCR1 expression on isolated eosinophils between healthy donors (Mean Fluorescence Intensity ratio 1.87 ± 0.30 , $n = 8$) and atopic donors (Mean Fluorescence Intensity ratio 2.19 ± 0.43 , $n = 6$) was detected (Fig. 1B). Moreover, the dominant chemokine receptor on eosinophils, CCR3, is also not expressed differently on atopic donors. In contrast to CCR1, CCR3 is higher expressed on the

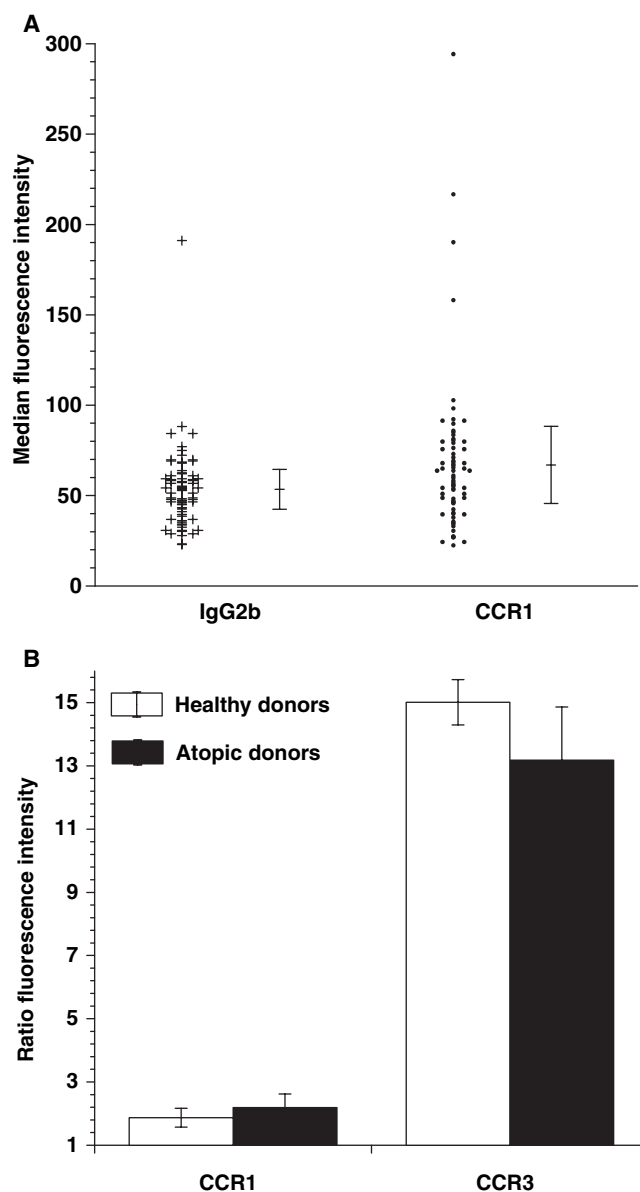


Figure 1. Donor to donor variety of CCR1 expression on human whole blood eosinophils is not related to the status of atopy. (A) Whole blood samples were collected from 73 different donors and stained by anti-CCR1 and isotype control, respectively, and subsequently stained by the second FITC-labeled Ab. Thereafter, the probe was stained by anti-CD16-PE antibody and double-gated on the granulocyte population as well as on the CD16-negative granulocyte population (eosinophils). Data are shown in a scattergram in which every symbol represents one donor. The median fluorescence intensity $\pm$ SD of specific mAb against CCR1 and isotype control is given. The 95% confidence interval for difference of means ranges from 7.2–19.8 ($P < 0.001$ using paired *t*-test). (B) Highly purified CD16-negative selected eosinophils from healthy and atopic donors were stained with anti-CCR1 and anti-CCR3. Data are expressed as the ratio of fluorescence intensity between specific mAb and isotype control.

surface of human eosinophils (Fig. 1B). These data indicate a donor-to-donor variety of CCR1 expression on human eosinophils, which cannot be explained by an atopic status of the donor.

Down-regulation of CCR1 on human eosinophils by chemokines and cytokines

To get more insight into the differential expression pattern of CCR1 on human eosinophils we investigated the influence of several cytokines and chemokines. The CD16-negatively selected eosinophils from CCR1^{high} donors were incubated for 24 h with medium and the indicated cytokine or chemokine at optimal concentrations, which have been tested for eosinophils in a previous study (24). From the cytokines tested, only IL-3 significantly ($P < 0.05$) induced a down-regulation of CCR1 whereas IL-4, IL-5, IL-12, IL-13, TNF- α , GM-CSF, and IFN- γ did not (Fig. 2A, B). From the chemokines tested, CCL3 ($P < 0.01$), CCL5 ($P < 0.001$), CCL7 ($P < 0.01$), and CCL13 ($P < 0.01$) induced a significant down-regulation of CCR1 from the surface of human eosinophils, whereas CCL11 did not (Fig. 2A, B). To find out whether the down-regulation of CCR1 by CCL3 and CCL5 as well as IL-3 depends on internalization of CCR1, we incubated eosinophils from CCR1^{high} donors at 37 and 4°C, a temperature at which internalization of seven-transmembrane receptors does not occur (24). As seen in Fig. 3, down-regulation of CCR1 after treatment with CCL3, CCL5 and IL-3 occurred only at 37°C, suggesting that these stimuli induce an internalization of CCR1 from the surface of human eosinophils.

CCL5 but not CCL3 induces a prolonged internalization of CCR1 in human eosinophils

Since receptor recycling of chemokine receptors is one key mechanism for cell inactivation, we investigated the time dependence of CCR1 internalization and re-expression. For this purpose we stimulated isolated CCR1^{high} eosinophils with optimal concentrations of CCL3 and CCL5. As seen in Fig. 4, CCL5 is significantly more effective to induce CCR1 internalization from the surface of human eosinophils than CCL3 after 2, 5, 10, 30, and 60 min. For instance, CCL5 treatment decreased relative CCR1 surface expression down to $32.2\% \pm 1.8$ (5 min, $P = 0.001$) and $17.0\% \pm 5.4$ (60 min, $P = 0.038$) in contrast to CCL3, which reduces relative CCR1 surface expression down to $55.3\% \pm 1.8$ (5 min) and $34.7\% \pm 4.6$ (60 min).

To investigate CCR1 re-expression, eosinophils from the same donors were stimulated for 60 min with optimal concentrations of CCL5 and CCL3, respectively, and immediately resuspended in chemokine-free medium for different periods of time. Whereas CCR1 surface expression after stimulation with CCL3 reached prestimulation

level, CCL5 showed a prolonged CCR1 internalization (Fig. 5). In contrast to CCL3, CCL5-treated eosinophils expressed much less CCR1 receptors on their surface ($< 20\%$ relative expression) after 30, 60, 90, and 120 min ($P < 0.001$). Even re-suspension of eosinophils in CCL5-free medium for 24 h resulted only in the expression of $45.2\% \pm 9.8$ CCR1 receptors of basic values compared to $97.0\% \pm 12.0$ of basic values in the CCL3-treated group ($P = 0.01$) (Fig. 5). These data demonstrate that CCL5, in contrast to CCL3, induces a prolonged internalization of CCR1 in human eosinophils.

Discussion

Chemokines and their corresponding receptors play an important role in allergic diseases. Whereas the function of the chemokine receptor CCR3 is well accepted as a principal receptor responsible for the recruitment of eosinophils, the role of CCR1 is controversial, as different expression profiles on eosinophils have been reported (14–16). Moreover, *in vitro* studies demonstrated that eosinophils respond differentially to CCL3 (17–20) and *in vivo* studies in dogs and human subjects suggest that CCL3 is not (dog) or only partially (human) responsible for the recruitment of eosinophils to the site of CCL3 application (28, 29). However, recent studies indicate that eosinophils from 15–20% of donors respond well to CCL3 and that eosinophils from these donors express CCR1 (21, 22). Two evidences support the role of CCR1 in allergic diseases. The CCL3 expression is increased in the human asthmatic lung (23) and eosinophils from donors responding highly to CCL3 gave a history of atopy-associated diseases (21). The aim of this study was to investigate the surface expression and regulation of CCR1 on human eosinophils. For this purpose we focused on its modulation by cytokines and chemokines as well as its pattern of recycling.

Stimulated by unsatisfactory studies dealing with the expression of CCR1 on the surface of human eosinophils (14–16), we investigated the expression level of CCR1 on the surface of 73 healthy donors. In the recruited collective we found that 17.8% of the donors ($n = 13$) highly express CCR1 on the surface of human eosinophils, whereas 82.2% of the donors ($n = 60$) did not. In comparison with the consistently high expression levels of CCR3, CCR1 is weakly expressed on eosinophils. For both chemokine receptors, we could not detect a significant difference between atopic and nonatopic donors. The latter observation is in contrast to a previous study in which the authors found more donors displaying high values for CCR1 expression in the atopic group compared with the nonatopic group (21). On the other hand the authors found neither correlation between serum IgE and eosinophils CCR1 expression nor could they identify that CCR1 expression has a predictive value for the diagnosis of atopy. Moreover, the study presented here

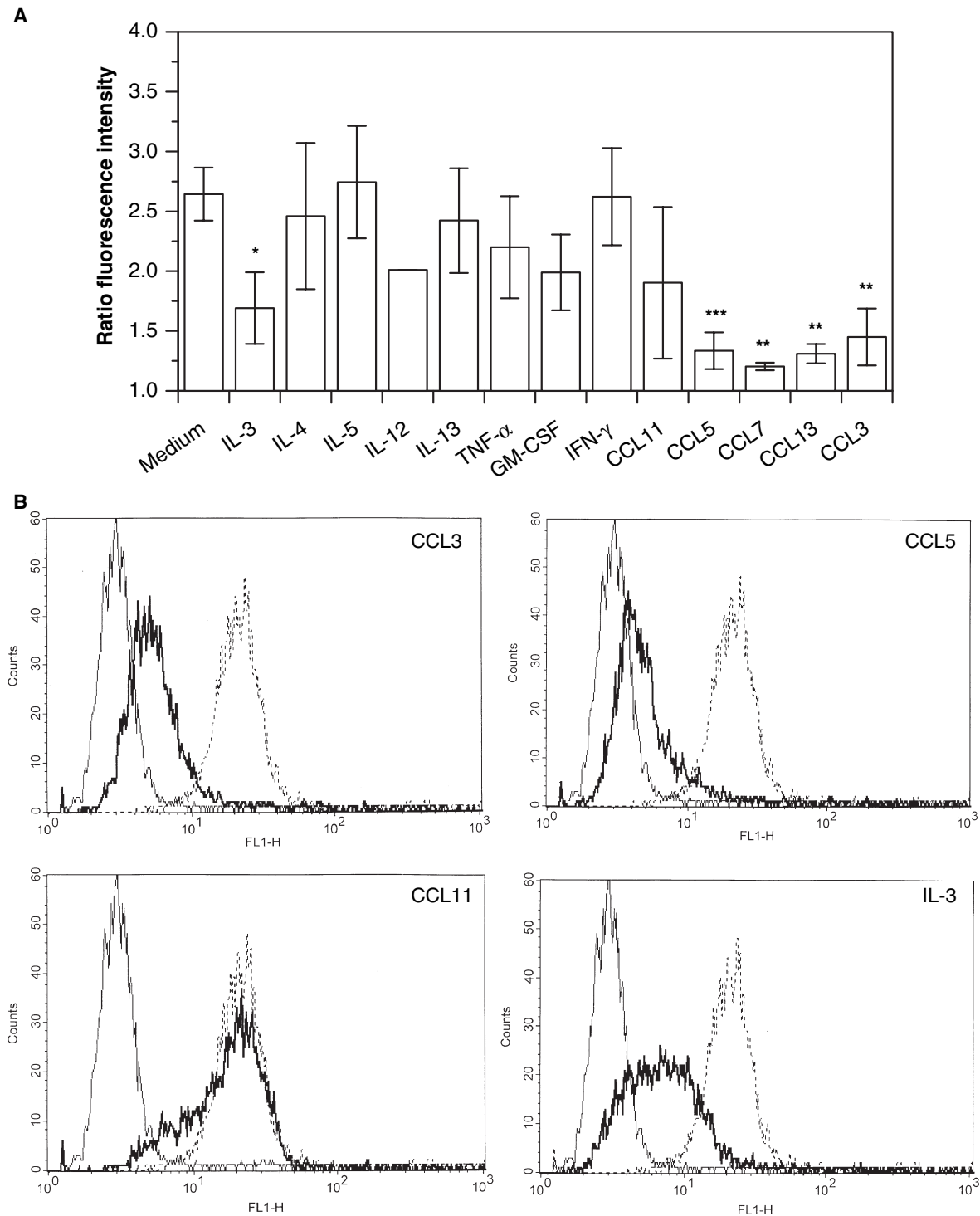


Figure 2. CCR1 down-regulation from the surface of human eosinophils by IL-3 and CCR1 ligands. (A) Highly purified CD16-negative selected eosinophils from CCR1^{high} donors were incubated for 24 h with medium, IL-3 (5.7×10^{-9} M), IL-5 (1.5×10^{-9} M), GM-CSF (7.1×10^{-9} M), IL-4 (7.1×10^{-9} M), IL-12 (5.4×10^{-9} M), IL-13 (8.3×10^{-9} M), TNF- α (8.3×10^{-9} M), IFN- γ (5.9×10^{-9} M), CCL11 (10^{-7} M), CCL5 (10^{-7} M), CCL13 (10^{-7} M), or CCL3 (10^{-7} M). Data are expressed as the ratio of fluorescence intensity between anti-CCR1 mAb and isotype control: * $P < 0.05$, ** $P \leq 0.01$, and *** $P \leq 0.001$ using Student's *t*-test. (B) Effect of CCL3, CCL5, CCL11, and IL-3 on CCR1 expression, which is shown as multiple histogram analysis of one representative experiment shown in Fig. 2A. Broken line, CCR1 expression after 24 h treatment with medium; bold line, CCR1 expression after 24 h treatment with the indicated stimulus; thin line, isotype control.

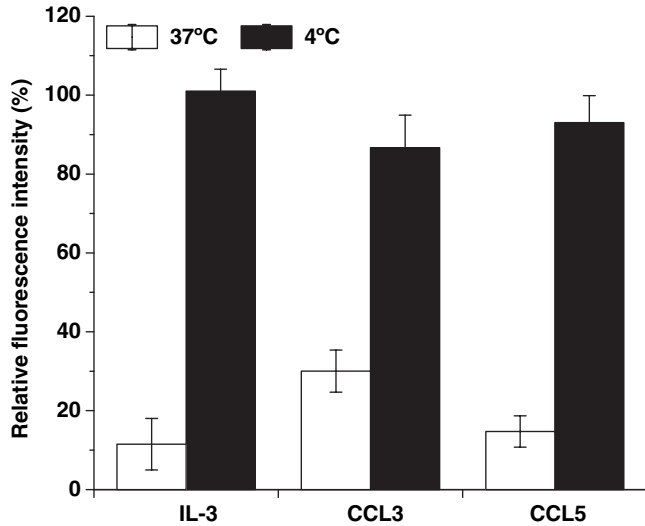


Figure 3. IL-3, CCL3 and CCL5 induce internalization of CCR1 in human eosinophils. Highly purified CD16-negative selected eosinophils from CCR1^{high} donors ($n = 5$) were incubated for 24 h at 37 and 4°C with IL-3 (5.7×10^{-9} M), CCL3 (10^{-7} M) and CCL5 (10^{-7} M). Data are expressed as relative fluorescence intensity (%) as described in 'Materials and methods'.

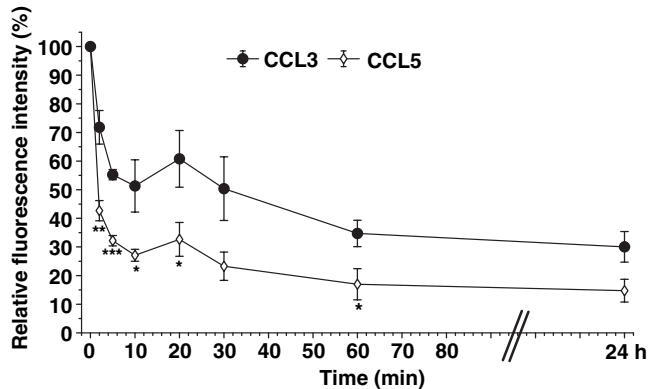


Figure 4. Time kinetic of CCR1 internalization in human eosinophils in response to CCL3 and CCL5. Highly purified CD16-negative selected eosinophils from CCR1^{high} donors ($n = 5$) were incubated with medium, CCL3 (10^{-7} M) and CCL5 (10^{-7} M), respectively, at 37°C for different periods of time as described in 'Materials and methods'. Data are expressed as relative fluorescence intensity (%) as described in 'Materials and methods': * $P < 0.05$, ** $P \leq 0.01$, and *** $P \leq 0.001$ using Student's t -test.

clearly demonstrates a donor-to-donor variety of CCR1 on the surface of human eosinophils, which is not dependent on the status of atopy.

To get more insight into the regulation of CCR1, we investigated the regulation of CCR1 on the surface of human eosinophils from CCR1^{high} donors in response to several cytokines and chemokines. From the chemokines

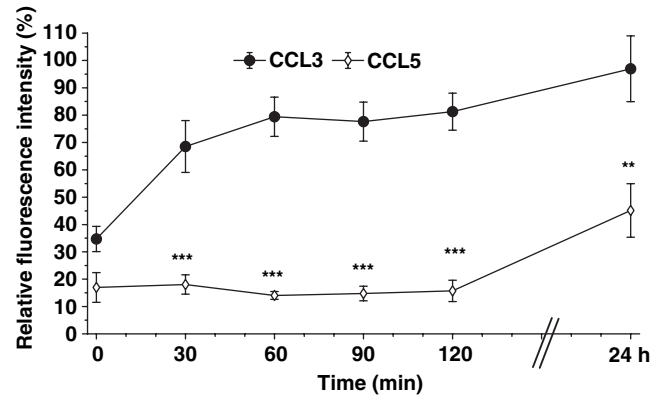


Figure 5. CCL5, but not CCL3, induces a prolonged internalization of CCR1 in human eosinophils. Highly purified CD16-negative selected eosinophils from CCR1^{high} donors ($n = 5$) were incubated with medium, CCL3 (10^{-7} M) and CCL5 (10^{-7} M), respectively, at 37°C for 60 min to induce maximal CCR1 internalization (indicated as '0 min' value). Thereafter, cells were resuspended in chemokine-free medium for different periods of time and analyzed as described in 'Materials and methods': ** $P \leq 0.01$ and *** $P \leq 0.001$ using Student's t -test.

tested, we found that CCL3, CCL5, CCL7, and CCL13 induced a down-regulation of CCR1 from the surface of eosinophils in contrast to CCL11. Of all the cytokines tested, IL-3 exclusively induced a significant down-regulation of CCR1. It is noteworthy that none of the cytokine tested induced an up-regulation of CCR1 on CCR1^{low} donors (data not shown). These data point out an extraordinary role of IL-3, which has recently been described for its effect on CCR3 expression on human eosinophils (24). In that study, the IL-3 related cytokines, IL-5 and GM-CSF, did not affect CCR3 expression whereas IL-3 was able to induce a down-regulation of CCR3 (24). Since IL-3-induced CCR1 down-regulation could only be detected at 37°C but not at 4°C, it can be concluded that IL-3 induces internalization of CCR1 (24). Moreover, the effect of IL-3 on CCR1 internalization was in the same range as for CCL3 and CCL5. Previous studies also found that cytokines are able to induce chemokine receptor internalization: IL-3 induces an internalization of CCR3 (24) and TNF- α and IFN- γ induce an internalization of CXCR4 (30). Therefore, besides its extraordinary role in CCR3 regulation, IL-3 is also able to regulate CCR1 through internalization. From the human CCR3 it is well known that internalization of CCR3 for a time period of 3 h is accompanied by receptor degradation and that *de novo* synthesis of CCR3 protein significantly contributes to the total level of CCR3 protein following chemokine binding (31). Therefore, it is likely that after a time period of 24 h CCR1 total protein would also be degraded which could be accompanied by a decrease in total CCR1 protein or mRNA.

In search of new therapies for allergic, inflammatory and infectious diseases chemokine receptors moved into the focus as possible targets for chemokine receptor antagonists (32). A novel concept have been proposed by Mack et al. who described a ligand, AOP-RANTES that induces a prolonged internalization of CCR5 and inhibits HIV entry due to the lack of CCR5 co-receptor surface expression (25, 33). In view of the fact that CCL5 is able to induce a prolonged internalization of CCR1 on whole blood monocytes (27) the effect of CCL5 and CCL3 on CCR1 recycling on human eosinophils from CCR1^{high} donors was analyzed. In comparison with CCL3, CCL5 is more effective to induce the internalization of CCR1 from the surface of human eosinophils. More remarkable however, was the distinct recycling pattern of CCR1 to the cell surface in response to CCL5. Whereas CCL3-stimulated eosinophils re-express most CCR1 receptors on their surface already after 30 min, CCL5-stimulated eosinophils re-express few CCR1 within 120 min. Even after 24 h, CCL5-stimulated eosinophils express substantially reduced levels of CCR1, whereas CCL3-stimulated eosinophils reach basic levels.

Taken together, this study demonstrates that a sub-population of human donors is able to express CCR1 on the surface of eosinophils, which helps to explain the different responses of eosinophils to CCL3 in previous

studies. If CCR1 is expressed on eosinophils it can be down-regulated by IL-3, CCL3, CCL5, CCL7, and CCL13, which is related to internalization of CCR1. It is tempting to speculate whether the higher positive charge of CCL5 – which is accompanied by high affinity binding to glycosaminoglycans (GAGs) – is responsible for the prolonged CCR1 internalization in comparison to CCL3 being overall negatively charged (34). Since data on other chemokines strongly support that receptor internalization, particularly the prolonged internalization, plays a central role in chemokine-mediated inhibition of receptor function (25, 27, 35), CCR1 besides CCR3 seems to be a relevant target on human eosinophils. In this context, we have recently published data on a modified chemokine, NNY-CCL14 that is able to induce an internalization and desensitization of chemokine receptors leading to the prevention of eosinophil recruitment into the lung and airway hyperresponsiveness in a murine model of allergic airway inflammation (36).

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