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Anti-infective peptide IDR-1002 augments monocyte chemotaxis towards CCR5 chemokines

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ABSTRACT

Innate defense regulator (IDR) peptides are a class of immunomodulators which enhance and modulate host innate immune responses against microbial pathogens. While IDR-mediated protection against a range of bacterial pathogens is dependent on enhanced monocyte recruitment to the site of infection, the mechanisms through which they increase monocyte trafficking remain unclear. In this study, anti-infective peptide IDR-1002 was shown to enhance monocyte chemotaxis towards chemokines CCL3 and CCL5. This enhancement correlated with the selective upregulation of CCR5 surface expression by peptide-treated monocytes. It was found that IDR-1002 enhancement of monocyte chemotaxis was fully dependent on CCR5 function. Furthermore, IDR-1002 enhanced chemokine-induced monocyte p38 MAPK phosphorylation in a CCR5-dependent fashion. Overall, these results indicate that peptide IDR-1002 can selectively influence monocyte recruitment by host chemokines through the regulation of chemokine receptors.

1. Introduction

As the incidence of antibiotic-resistant pathogens continues to grow, so does the need for new therapeutic agents. Innate Defense Regulator (IDR)-peptides are a class of complex immunomodulators derived from endogenous host defense peptides [1] with the potent ability to combat bacterial pathogens through the complex regulation of the innate immune response. IDR-1, a peptide with no direct antimicrobial activity, confers prophylactic and therapeutic protection against methicillin-resistant *Staphylococcus aureus*, vancomycin-resistant *Enterococcus*, and *Salmonella enterica* serovar Typhimurium in murine infection studies [2]. This protection correlates with an increase in monocyte/macrophage numbers, promotion of chemokine induction, and a suppression of inflammatory cytokine production at the site of infection. Similarly, IDR-1002, a derivative of bovine bactenecin, enhances host protection against *S. aureus* and *Escherichia coli* in murine infection models, again correlating with increased leukocyte recruitment and increased chemokine production at the infectious site [3]. Furthermore, IDR-1018, promotes wound healing and closure in porcine models of *S. aureus*-infected skin wounds [4]. These studies demonstrate the antimicrobial properties of IDR-peptides and highlight their potential as anti-infective therapeutics in situations of acute bacterial infection. While IDR-peptides show promise as novel antimicrobial agents, the mechanisms through which they enhance protection remain inadequately understood and must be further investigated.

The enhanced mobilization of monocytes during bacterial infection appears to be paramount in the anti-infective effects of IDR-peptides. This is further supported by the abolishment of peptide-mediated protection in animal models in which monocytes/macrophages were depleted [2,3]. Thus to understand the anti-infective nature of IDR-peptides, we sought to elucidate the mechanisms through which they enhance monocyte recruitment. In a previous study, we determined that IDR-1002, a peptide with no direct chemotactic or chemokinetic effects, augments monocyte migration through a fibronectin network via the promotion of β1-integrin-mediated adhesion [5]. Observations in this study also implicated an adhesion-independent, chemokine-specific mechanism utilized by IDR-1002 to promote monocyte recruitment. Thus, we hypothesized that IDR-1002 could regulate monocyte responses towards chemokines, and in this manner promote monocyte mobilization.

In this study, IDR-1002 was found to enhance human monocyte chemotaxis towards CCL3 and CCL5, but not CCL2 or CCL7. This

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selective effect correlated with the increased surface expression of chemokine receptor (CCR)5, but not CCR1 or CCR2, by peptide-stimulated monocytes. Inhibition of CCR5 function eliminated the augmentation of monocyte chemotaxis by IDR-1002, demonstrating a chemokine-receptor-specific mechanism. Furthermore, IDR-1002 enhanced chemokine-induced phosphorylation of p38 MAP kinase in a CCR5-dependent manner. Overall, these results show an enhancement of human monocyte sensitivity to CCR5-chemokines through the selective upregulation of their receptor. These findings reveal a novel mechanism through which IDR-peptides enhance monocyte recruitment and thus enhance host protection against bacterial infection.

2. Materials and methods

2.1. Reagents

Peptide IDR-1002 (VQRWLIVWRIRK-NH_2) was synthesized by solid phase F-moc chemistry by CPC Scientific (Sunnyvale, CA). Chemokines CCL2, CCL3, CCL5, and CCL7 were obtained from R&D Systems (Minneapolis, MN).

2.2. Cell isolation and culture

PBMCs were isolated as previously reported [6]. Briefly, venous blood was collected from healthy volunteers using heparin-containing Vacutainer tubes (BD Bioscience, San Jose, CA) in accordance with UBC ethical guidelines. Blood was layered over Ficoll–Paque Plus (Amershan, Piscataway, NJ) prior to separation by density-gradient centrifugation. The mononuclear cell layer was extracted and washed twice with PBS (Invitrogen, Carlsbad, CA). The monocyte population was enriched by negative selection magnetic bead purification from PBMCs using the Easy-Sep Human Monocyte Enrichment Kit (Stemcell Technologies, Vancouver, BC, Canada) as per manufacturer's instructions. Monocytes were then resuspended in RPMI 1640 with 1% (v/v) heat-inactivated FBS, 2 mM L-glutamine, and 1 mM sodium pyruvate (all from Invitrogen), placed in a polypropylene tube (BD Falcon, San Jose, CA) and cultured in a humidified incubator at 37 °C with 5% CO_2.

2.3. Monocyte chemotaxis assay

Migration was performed using a 48-well microchemotaxis chamber (Neuro Probe, Gaithersburg, MD). Monocytes (5×10^4 per well) were stimulated with 20 μg/ml of IDR-1002 and added to the upper wells of the chamber. Indicated concentrations of chemokines in RPMI 1640 with 1% FBS were added to the lower wells. Lower wells containing only RPMI 1640 with 1% FBS were used as negative media-only controls. Wells were separated by a polycarbonate membrane with 5-μm-diameter pores (Neuro Probe). After 1 h of incubation, non-migrated monocytes were removed by PBS washing and scraping with a rubber blade. Adhered cells on the underside of the membrane were stained with the Diff-Quik Staining Kit (VWR Scientific Products, Radnor, PA). Migration was measured by averaging the number of cells per 200x magnification high powered fields (HPF) over five fields, with each treatment done in duplicate. Fold-change-over-control values were calculated by dividing the average migrated cells per HPF of each treatment by the average of the controls.

In inhibitor experiments, monocytes were pre-treated for 1 h with 20 μg/ml of an anti-human CCR1-blocking mouse IgG1 Ab (D063-3, MBL International, Woburn, MA), an anti-human CCR5-blocking mouse IgG2b Ab (45531, R&D Systems, Minneapolis, MN), or an isotype control mouse IgG2b Ab (MG2b-57, Biolegend, San Diego, CA) prior to chemotaxis. Fold-change-over-control values were calculated as above, with comparisons to isotype-treated monocytes as controls.

2.4. Flow cytometry to measure chemokine receptor surface expression

Flow cytometry studies were performed using a FACSCalibur cytometer in conjunction with CellQuest Pro (BD Biosciences). Human PBMCs (4×10^5 cells per condition), seeded on polystyrene tissue-culture plates (BD Falcon), were stimulated with 20 μg/ml of IDR-1002 and incubated for the indicated periods of time in a humidified incubator at 37 °C and 5% CO_2. Following treatment, PBMCs were fixed with 2% (w/v) formaldehyde (Fisher Scientific, Pittsburgh, PA) at 23 °C for 20 min. PBMCs were washed with PBS and 0.5% (w/v) BSA and stained at 23 °C for 1 h with an anti-human-CCR1 mouse IgG2b- AlexaFluor®647 Ab (TG4/CCR1), an anti-human-CCR5 rat IgG2a-AlexaFluor®647 Ab (HEK/1/85a), an anti-human-CCR2 mouse IgG2b- AlexaFluor®647 Ab (TG5/CCR2), or an isotype mouse IgG1- AlexaFluor®647 Ab (MOPC-21) (all from Biolegend). Also, PBMCs were stained with an anti-human-CD14 mouse AlexaFluor®488 Ab (M5E2, Biolegend). PBMCs were then washed with PBS and resuspended in PBS with 0.5% BSA and 0.5% formaldehyde for analysis. Monocytes were investigated by gating on the CD14-expressing population, and the CCR expression was determined by measuring the geometric mean fluorescence intensity (MFI) levels of 1000 monocytes. The displayed fold-change-over-basal values were calculated by dividing the MFI values of each treatment by the MFI values of the untreated sample of their respective time-points.

2.5. Measurement of p38 MAPK phosphorylation by flow cytometry

PBMCs (4×10^5 cells per condition), seeded on polystyrene tissue-culture plates, were pre-stimulated with 20 μg/ml of IDR-1002 for 15 min and then stimulated with 12.5 ng/ml of CCL3 or CCL5 for 5 min. Following treatment, PBMCs were fixed with 2% formaldehyde for 20 min at room temperature. PBMCs were washed with 0.5% BSA in PBS and permeabilized in 90% (v/v) methanol at 4 °C for 30 min. Cells were washed and stained at room temperature for 1 h with anti-phospho-p38 MAPK (Thr180/Tyr182) 3D7 rabbit mAb (Cell Signalling Technology, Danvers, MA). Cells were then stained with a goat anti-rabbit IgG-AlexaFluor®647 (H + L, Invitrogen) and with an anti-human-CD14 mouse AlexaFluor®488 M5E2 mAb for 30 min at room temperature. Cells were washed and resuspended in PBS with 0.5% BSA and 0.5% formaldehyde. Monocytes were investigated by gating on the CD14 expressing cells within the PBMC population. Phosphorylated p38 MAPK was determined by measuring the geometric MFI of 1000 cells, subtracted by the MFI levels of unstimulated monocytes. In inhibitor studies, PBMCs were pre-treated for 1 h with 20 μg/ml of an anti-human CCR5-blocking mouse IgG2b Ab or an isotype control mouse IgG2b Ab prior to peptide and chemokine stimulation. Phosphorylated p38 MAPK was determined by measuring the MFI levels of 1000 cells, subtracted by the MFI levels of unstimulated monocytes in the respective inhibitor conditions.

3. Results

3.1. IDR-1002 enhances human monocyte chemotaxis towards CCL3 and CCL5

To investigate whether IDR-1002 regulated monocyte responses to endogenous chemoattractants, human monocyte

chemotaxis assays were performed using a panel of human chemokines. The concentrations of chemokines used was based on their estimated maximal active dose range. Chemokines CCL2, CCL3, CCL5, and CCL7 were able to induce monocyte migration as expected (Fig. 1). Monocytes stimulated with IDR-1002 displayed no significant difference in migration towards CCL2 and CCL7. In contrast, peptide-stimulated monocytes exhibited significantly stronger chemotactic activity towards CCL3 and CCL5 at nearly all chemokine concentrations tested. IDR-1002 stimulation in the absence of chemokines had no effect on baseline monocyte migration, confirming a previous study that showed the lack of direct chemokinetic properties of this peptide on monocytes [5]. Overall, these results demonstrated that IDR-1002 could selectively enhance monocyte migration towards chemokines CCL3 and CCL5.

3.2. IDR-1002 upregulates human monocyte surface expression of CCR5

To determine whether the enhancement of chemotaxis stemmed from receptor regulation, IDR-1002-stimulated monocytes were investigated for their surface expression of chemokine receptors. Over the course of 1 h, IDR-1002 stimulation of monocytes had no significant effects on the surface expression of CCR1 or CCR2 (Fig. 2). However, monocyte surface expression of CCR5 was increased by approximately 50%. A significant increase in CCR5 expression was observable as early as 5 min after peptide stimulation. These results demonstrated a selective regulation of CCR surface expression by monocytes which correlated with the selective regulation of chemokine-induced migration.

3.3. IDR-1002 enhancement of monocyte chemotaxis is CCR5-dependent

The promotion of monocyte CCR5 surface expression by IDR-1002 correlated with an enhancement of monocyte chemotaxis towards CCR5-chemokines, CCL3 and CCL5. To investigate whether CCR expression was responsible for the promotion of migration towards these chemokines, chemotaxis experiments were repeated in a CCR-inhibited system. Human monocytes pre-treated with an isotype Ab control retained their ability to migrate towards CCL3 and CCL5 (Fig. 3). This effect was significantly enhanced in IDR-1002-stimulated monocytes. After pretreatment with a CCR1-inhibitor Ab, IDR-1002 augmentation of migration remained evident, suggesting CCR1 played a minimal role in peptide effects. CCR5-inhibitor Ab pre-treatment resulted in a small, non-significant, decrease in monocyte chemotaxis towards CCL3 and CCL5. However, CCR5-inhibition eliminated any effects of IDR-1002 on monocyte chemotaxis, suggesting a CCR5-dependent mechanism of enhancement. Antibody pre-treatment of monocytes had no significant effects on baseline migration towards media only controls (data not shown). Additionally, IDR-1002 stimulation alone had minimal effects on baseline monocyte migration in all antibody-treatment conditions (data not shown). The minimal effects of CCR1 or CCR5 inhibition alone on monocyte chemotaxis must be noted. Through the redundancy inherent in the host chemokine system, namely the ability of chemokines to utilize multiple receptors, inhibition of a single receptor can be compensated for by the use of another [7]. In agreement with this, monocytes pre-treated with both a CCR1-and CCR5-inhibiting antibody exhibited no chemotaxis towards CCL3 or CCL5 (Fig. S1), demonstrating that these inhibitors are indeed functional and that a compensation

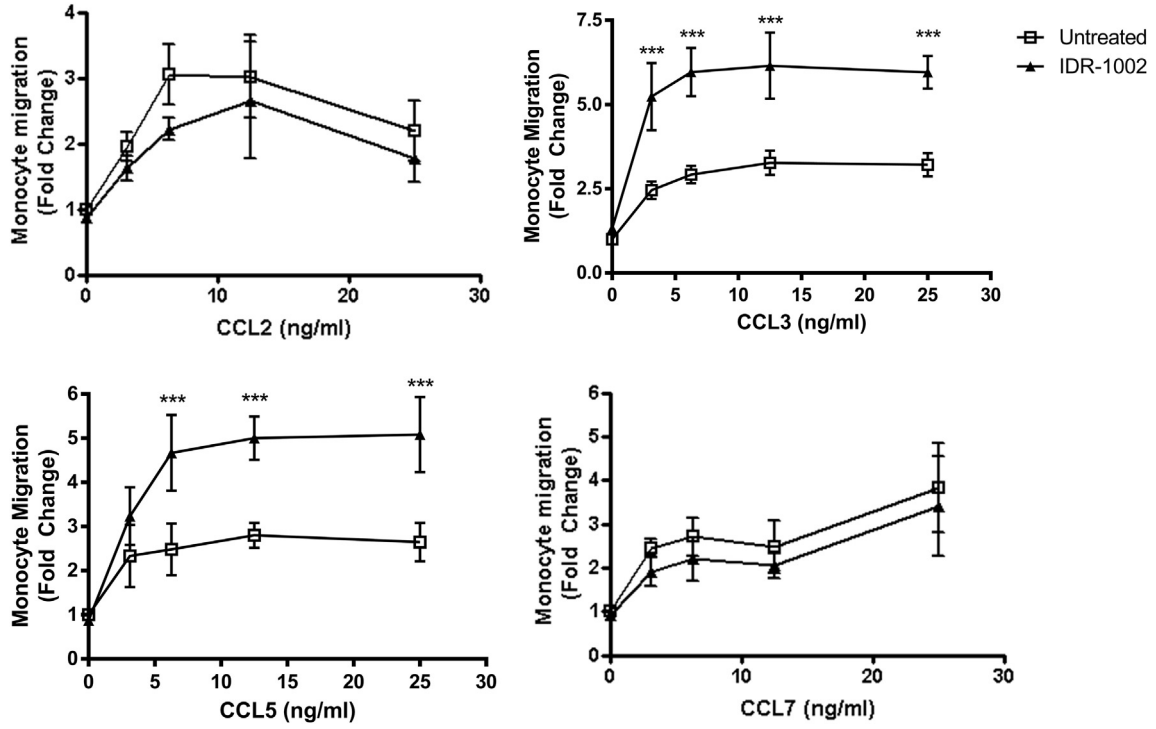

Fig. 1. IDR-1002 enhances monocyte chemotaxis towards CCL3 and CCL5. Effects of IDR-1002 stimulation (20 μg/ml) on monocyte chemotaxis towards chemokines after 1 h. Data are presented as the mean fold-increases in monocyte migration over baseline migration towards media alone (±SE) of at least 4 independent experiments, each from independent donors. Statistical comparisons between IDR-1002-stimulated monocytes and untreated monocytes were done by two-way ANOVA followed by Bonferroni's multiple comparisons test. ***$p < 0.001$.

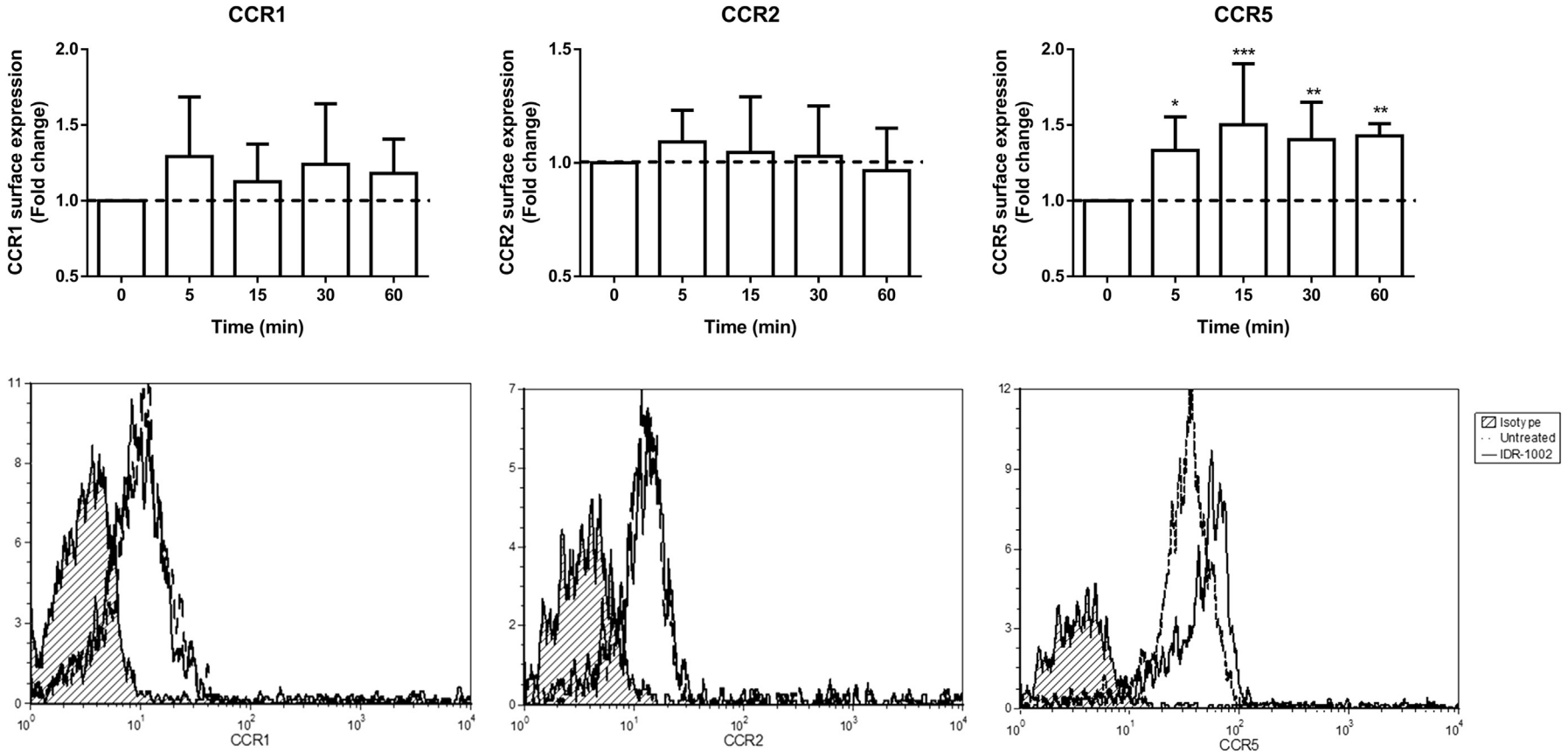

Fig. 2. IDR-1002 upregulates human monocyte surface expression of CCR5. Time course determination of monocyte CCR levels after treatment with IDR-1002 (20 μg/ml) was done by flow cytometric detection of anti-CCR antibodies, gating on the $CD14^+$ monocyte population. Data are presented as the mean fold-increases of CCR expression over unstimulated cells (±SE) of at least 4 independent experiments, each from an independent donor. Representative histograms display surface expression of chemokine receptors by monocytes stimulated with IDR-1002 (20 μg/ml) for 1 h. Statistical comparisons to unstimulated cells were done by two-way ANOVA followed by Bonferroni's multiple comparisons test.*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

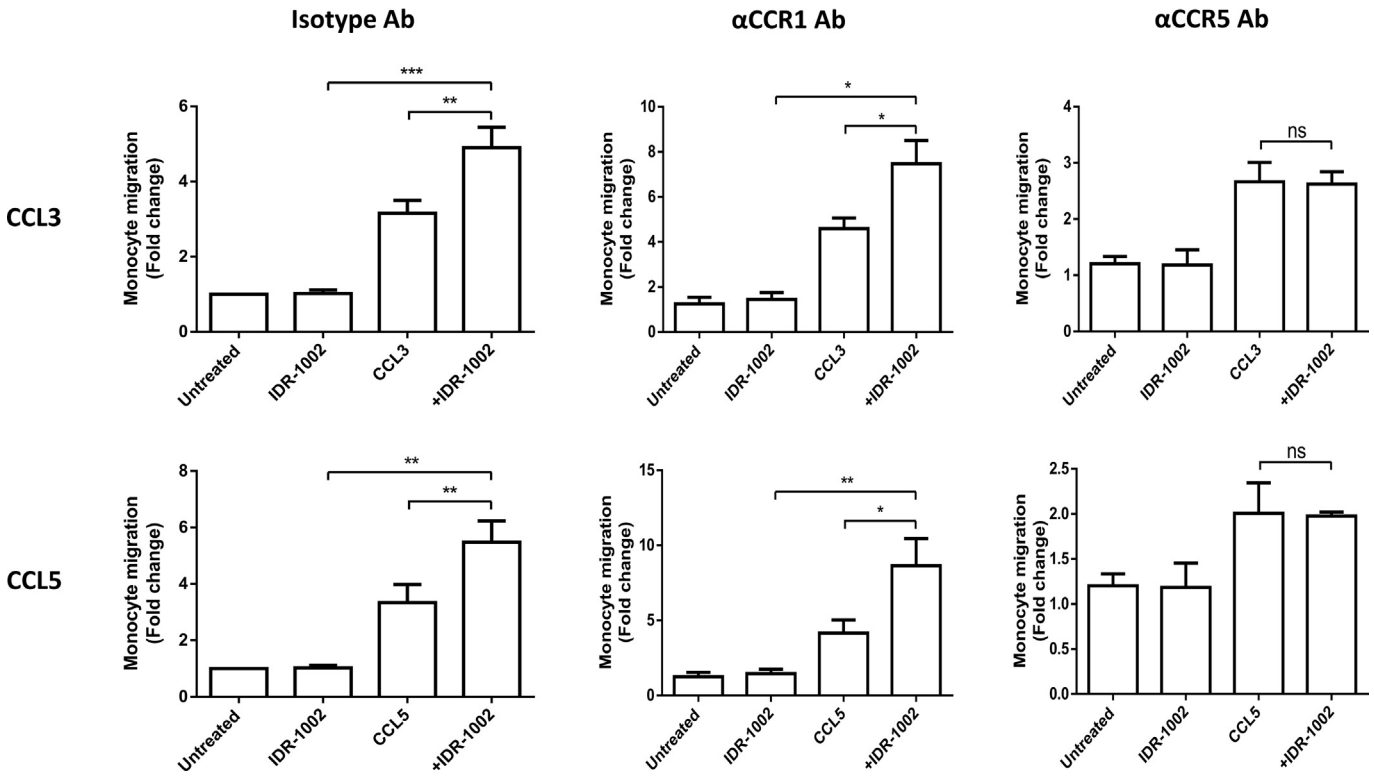

Fig. 3. IDR-1002 enhancement of monocyte chemotaxis is CCR5-dependent. Monocytes were pre-treated with an anti-CCR1 inhibiting mAb, an anti-CCR5 inhibiting mAb, or an isotype mAb (all 20 μg/ml) for 1 h. The effects of IDR-1002 stimulation (20 μg/ml) on monocyte chemotaxis towards chemokines (12.5 ng/ml) after 1 h of migration were assessed. Data are presented as the mean fold-increases in monocyte migration over baseline migration towards media alone (±SE) of at least 4 independent experiments, each from independent donors. Statistical comparisons between IDR-1002-stimulated monocytes and untreated monocytes were done by one-way ANOVA followed by Bonferroni's multiple comparisons test. *$p < 0.05$; **$p < 0.01$. ***$p < 0.001$.

mechanism was active in single antibody inhibition studies. Overall, these results demonstrated that the enhancement of monocyte migration by IDR-1002 was linked to its effects on CCR surface expression.

3.4. IDR-1002 augments CCL3-and CCL5-induced p38 MAPK phosphorylation in human monocytes

As IDR-1002 was shown to upregulate monocyte CCR5 expression, correlating with an enhancement in CCR5-mediated chemotaxis, it was hypothesized that IDR-1002 may potentiate CCR5-mediated signal transduction activity in human monocytes. G-protein-mediated activation of the MAPK signaling axes is a common downstream effect of chemokine-CCR binding and is essential for numerous chemokine-mediated effects [8]. Thus, we investigated the effects of IDR-1002 on CCR5-mediated p38 MAPK phosphorylation as an indicator of p38 MAPK activation. Human PBMCs were pre-treated with IDR-1002 prior to stimulation with CCL3 or CCL5, and p38 MAPK phosphorylation in monocytes was determined by flow cytometry gating on the $CD14^+$ population. Monocytes stimulated with IDR-1002 alone exhibited elevated p38 MAPK phosphorylation levels compared to untreated controls (Fig. 4A), consistent with many reports of p38 MAPK utilization by IDR-peptides [2,3]. Similarly, monocytes stimulated with either CCL3 or CCL5 demonstrated rapid elevation of p38 MAPK phosphorylation as expected. Pre-treatment of monocytes with IDR-1002 followed by the addition of chemokines resulted in p38 MAPK phosphorylation levels significantly greater than those seen in chemokine- or peptide-stimulated monocytes alone. This result suggests a potential enhancement of chemokine-induced signal transduction activity by IDR-1002.

To investigate whether this enhancement in MAPK activation stemmed from the regulation of CCR5 by IDR-1002, this experiment was repeated under CCR5-inhibitory conditions. In monocytes pre-treated with an isotype antibody, stimulation with CCL3, CCL5, or IDR-1002 alone resulted in increased p38 MAPK phosphorylation, as observed previously (Fig. 4B). The combination of IDR-1002 and chemokine stimulation resulted in levels of p38 phosphorylation greater than those seen for the chemokine-alone or peptide-alone treatments. Inhibition of CCR5 alone via a blocking antibody did not significantly affect CCL3-, CCL5-, or IDR-1002-induced p38 MAPK phosphorylation. Only in monocytes stimulated with both IDR-1002 and chemokines, did inhibition of CCR5 result in a reduction of p38 activation, with p38 phosphorylation levels not exceeding that of IDR-1002 alone. Levels of phosphorylated p38 MAPK in this scenario were comparable to those seen in monocytes singly-treated with chemokines or IDR-1002. These results suggests that the elevation of chemokine-induced p38 MAPK activation by IDR-1002 is at least in part dependent on CCR5.

4. Discussion

It was demonstrated in this study that IDR-1002 enhanced human monocyte chemotaxis towards chemokines CCL3 and CCL5. The synergistic effect on monocyte migration between IDR-1002 and certain endogenous chemokines has significant implications in the role of IDR-peptides during an anti-infective immune response. The initial phase of microbial infection is in part characterized by the rapid production of endogenous immune-regulating mediators, including a diverse range of inflammatory cytokines. CCL3 and CCL5, and others belonging to the subset of inducible chemokines, are rapidly produced by immune cells in

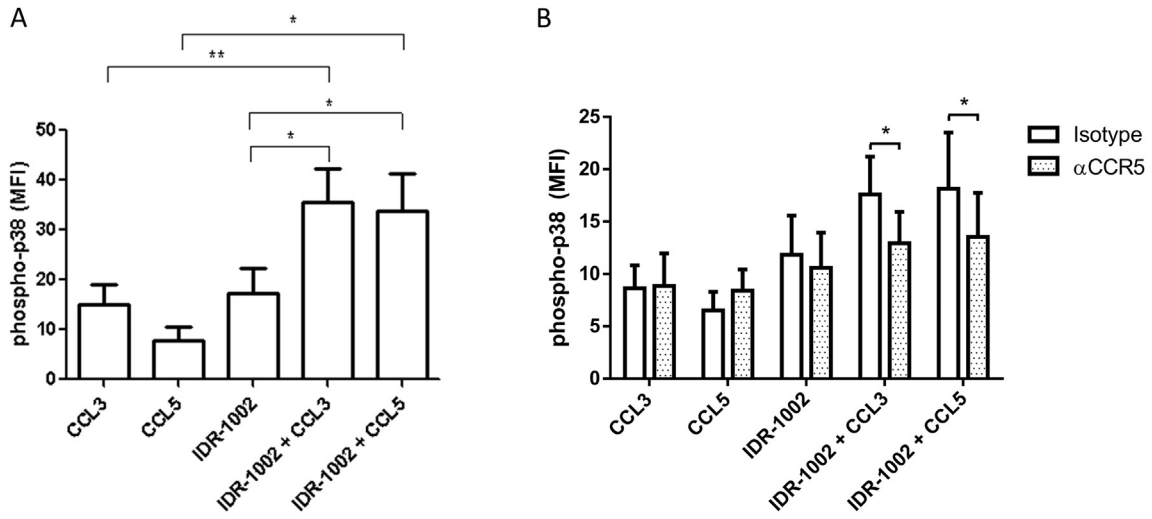

Fig. 4. IDR-1002 promotes CCR5-chemokine-induced activation of p38 MAPK in human monocytes. (A) PBMCs were pre-treated with IDR-1002 (20 μg/ml) for 15 min, prior to stimulation with CCL3 or CCL5 (both 12.5 ng/ml) for 5 min (B) PBMCs were pre-treated with an anti-CCR5 inhibiting antibody or an isotype mAb for 1 h prior to treatment with IDR-1002 and chemokines. Monocyte p38 MAPK activation was measured by flow cytometric detection of intracellular phosphorylated p38 MAPK, gating on the $CD14^+$ monocyte population. Data are presented as the mean MFI values over unstimulated monocytes of 5 independent experiments, each from independent donors. two-way ANOVA followed by Bonferroni's multiple comparisons test. $^*p < 0.05$; $^{**}p < 0.01$.

response to microbial signatures (also termed pathogen-associated molecular patterns) and endogenous inflammatory mediators [9]. These effects result in the creation of chemokine gradients that are responsible for the targeting of monocytes and other immune cells in circulation to the local site of infection. Ultimately, sensitivity of these immune cells to these chemokine gradients dictates how efficiently cells are recruited. It is feasible that the priming of monocytes by IDR-1002, for enhanced migration within a CCL3 and CCL5 gradient, contributes to the promotion of monocyte recruitment that was observed in previous *in vivo* studies [2,3]. Thus, this investigation has revealed a novel mechanism through which IDR-1002 enhances monocyte migratory behavior and potentially enhances host anti-infective responses.

The enhancement of monocyte migration to CCL3 and CCL5, but not CCL2 or CCL7, merited further investigation. In terms of function, members of the sub-family of chemoattractive cytokines are highly similar. Despite the high similarity between the general functions of chemokines and the similar consequences of their actions, different chemokines play distinct roles in immunity, directing specific cell populations to various locales at different temporal stages of an anti-infective response [9]. The method by which the immune system orchestrates these highly similar cytokines as complex multi-stage directors of cellular trafficking relies in large part on differential expression of chemokine receptors on host cell surfaces [7,9]. Increased surface expression of specific receptors can enhance cellular responses to their specific chemokine ligands, whereas internalization or downregulation of chemokine receptors is a common method of desensitizing cells to specific chemokines [7]. In this study, IDR-1002-stimulated monocytes were shown to upregulate surface expression of CCR5, while CCR1 and CCR2 expression remained unchanged. An upregulation of CCR5 expression, and subsequent enhancement of downstream receptor-induced signal transduction, seems likely to account for the selective enhancement by IDR-1002 of monocyte migration towards CCR5-chemokines. Consistent with this interpretation, IDR-1002 augmentation of chemotaxis towards CCL3 and CCL5 was eliminated in the presence of a CCR5-inhibiting antibody, whereas CCR1-inhibition had no effect on the enhancing effect of IDR-1002, further supporting the hypothesis that IDR regulation of chemokine receptors leads to this observed enhancement. It is interesting to note that although monocyte chemotaxis towards CCL3 and CCL5 is mediated by multiple chemokine receptors, enhancement of chemotaxis towards these chemokines by IDR-1002 was primarily dependent on CCR5.

Although this study demonstrates the regulation of CCR5 surface expression by IDR-1002, potentially leading to a promotion of chemotaxis towards CCR5-chemokines, the mechanism of regulation is currently unknown. Regulation of chemokine receptor mRNA expression can lead to the alteration of the levels of receptor proteins synthesized, an effect utilized by many endogenous cytokines to coordinate cell sensitivity to chemokines. While the modulation of chemokine receptor gene transcription and protein synthesis by IDR-1002 is certainly possible, the rapid effect on receptor expression and enhancement of chemotaxis are consistent with a postulated post-translational mode of regulation. The level of chemokine receptors on cell surfaces is largely dependent on the balance between internalization and receptor recycling [9]. Chemokine receptors undergo basal levels of internalization mediated by multiple endocytic pathways, an effect which is greatly increased by ligand binding and is the major mode of chemokine desensitization. Internalized chemokine receptors are then either sent into degradative pathways or trafficked back to the plasma membrane in a re-sensitized state. It is possible that IDR-1002 promotes rapid receptor expression by acting on this process, whether by limiting chemokine receptor uptake or promoting recycling receptors to the membrane. Actin polymerization, an essential process for CCR5 movement and recycling, is also essential for IDR-mediated chemokine induction [3,10]. There is a possibility that IDR-1002 modulates monocyte actin polymerization and actin-mediated cytoskeletal rearrangement. This would then likely result in downstream effects on chemokine receptor steady-state levels. The mode by which IDR-1002 influences CCR5 expression, without affecting CCR1 or CCR2 expression, also remains unknown. This selective regulation may originate from differences in the trafficking behavior between the multiple receptors. CCR5 seems to be predominantly recycled, accumulating in early endosomes that are recycled in a perinuclear location following internalization. CCR5 ligand binding does not affect the basal rate of

receptor degradation, and indeed agonist-bound CCR5 can be repeatedly internalized and quickly recycled to the plasma membrane instead of being directed towards degradative lysosomal compartments [10,11]. In contrast, CCR2 can be found within lysosomal compartments as early as 30 min following ligand binding and restoration of CCR2 surface levels occurs at a relatively slower pace [12]. Sub-cellular localization of chemokine receptors may also contribute to the selective regulation by peptide. CCR1 and CCR2, pre-dominantly found on the surface membrane of monocytes cells [13,12], seem to be uninfluenced by the early-stage effects of IDR-1002 on receptor mobilization. In contrast, CCR5 is found in abundance in the intracellular compartment of monocytes and T-lymphocytes where it could be rapidly mobilized to the surface in response to exogenous signals [14]. It is possible that differences in localization, trafficking mechanisms, and degradative behavior between these chemokine receptors, coupled with possible effects on receptor compartment mobilization by IDR-1002, resulted in the observed upregulation of CCR5 but not CCR1 or CCR2.

This study focused on the ability of IDR-1002 to regulate chemokine receptor surface expression as a mechanism for enhancing monocyte chemotaxis. However, regulation of chemokine/chemokine receptor function can occur on many levels. Direct receptor modifications, such as through phosphorylation by G protein-coupled receptor kinases, can impact on receptor sensitivity and activity. In addition, modulation of receptor-mediated downstream signal transduction might result in changes to chemokine-mediated behavior, such as chemotaxis. The signal transduction cascades regulating these processes overlap with those impacted by IDR-1002, including calcium flux-mediated signal transduction and those regulated by the PI3K-Akt, MAPK, and G protein pathways [3]. It seems likely that the modulation of these networks by IDR-1002, in addition to modulation of receptor expression, impacts on chemokine-mediated functions. The finding that IDR-1002 enhanced chemokine-induced p38 MAPK phosphorylation suggests IDR-1002 may be reinforcing chemokine-mediated signaling events. While this enhancement may stem partially from the upregulation of CCR5 expression by IDR-1002, the enhancement of downstream signaling events through distinct signal transduction mechanisms is certainly possible. Further investigations regarding the possible cross-talk between IDR-mediated signaling and chemokine-induced pathways are needed to determine the extent of regulation that IDR-1002 exerts on chemokine function. A synergistic enhancement of chemokine signal transduction might however account for the observed increase in monocyte chemotaxis.

In summary, this study revealed a novel mechanism by which IDR-1002 enhanced monocyte recruitment; namely the enhancement of CCR5-mediated chemotaxis towards host chemokines CCL3 and CCL5. We propose that this effect stems from the selective promotion of CCR5 expression on monocyte surfaces and a potential reinforcement of chemokine-mediated signal transduction pathways. This study not only reveals a novel regulatory axis of IDR-1002 on monocyte mobilization, in addition to the known enhancement of β1-integrin-mediated monocyte adhesion and induction of chemokine production [5], but also presents a novel avenue through which IDR-peptides might regulate anti-infective host defences. Synergy between IDR-1002 and chemokines, may have noteworthy ramifications on the anti-infective immune response. Chemokines, in addition to mediating recruitment, are highly involved, directly and indirectly, in many aspects of immunity, including cellular growth, wound healing, and differentiation, processes that are also influenced by HDPs. It would be of great interest to determine the effects of IDR-peptides on cell growth and differentiation, and whether this stems from their ability to enhance chemokine-mediated responses. Overall, this study further characterizes the augmenting effects of IDR-1002 on monocyte recruitment and hints at the ability of IDR-peptides to modulate an array of immune processes through cooperation with host chemokines.

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Transparency document

Transparency document related to this article can be found online at http://dx.doi.org/10.1016/j.bbrc.2015.07.038.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.bbrc.2015.07.038.

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