Human β-Defensin 2 and 3 and Their Mouse Orthologs Induce Chemotaxis through Interaction with CCR2

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Abstract

β-defensins play a dual role during immune response. Their direct antimicrobial properties contribute to the local innate immune response by combating microbial invasions. Furthermore, previous studies revealed the capacity of certain β-defensin family members to chemoattract immature dendritic cells and CD45RO$^+$ CD4$^+$ T cells through chemokine receptor CCR6. However, because β-defensins also chemoattract macrophages and monocytes, which do not express CCR6, efforts have been made to identify other receptors for these polypeptides. In this study, we demonstrate the capacity of human β-defensin (hBD)2 and 3 and their mouse orthologs, β-defensin 4 and 14, to interact with CCR2, a chemokine receptor expressed on monocytes, macrophages, and neutrophils. These β-defensins, fused to the Fc region of human IgG$_1$, showed binding to CCR2-transfected HEK293 cells, as revealed by flow cytometry. The β-defensin fusion proteins also induced CCR2-specific chemotaxis of transfected HEK293 cells, human peripheral blood monocytes, and mouse peritoneal exudate cells in a dose-dependent manner. Preincubation of human monocytes with CCL2/MCP-1, the chemokine ligand for CCR2, abolished migration induced by β-defensins. Conversely, pre-incubation with hBD2:Ig or hBD3:Ig inhibited MCP-1 induced migration. Peritoneal exudate cells from CCR2-deficient mice failed to migrate toward these fusion proteins. In conclusion, the β-defensins used in this study contribute to the innate and adaptive immune response in their role as chemoattractants. Our data indicate that hBD2 and hBD3, together with their mouse orthologs (β-defensin 4 and 14), are chemotactic for a broad spectrum of leukocytes in a CCR6- and CCR2-dependent manner.

The family of β-defensins consists of a variety of cationic antimicrobial polypeptides that contribute to the immune response against bacterial, fungal, and viral infections (1). Their tertiary structure is composed of a N-terminal α helix, followed by a β-sheet, and contains three family-specific disulfide connectivities between cysteines 1–5, 2–4, and 3–6 (2–4). The expression of β-defensins is induced by different pathogen-associated molecular patterns,

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such as LPS, bacterial lipoprotein, and other TLR and NODR ligands (5–7). Furthermore, distinct cytokines, such as IFN-γ, TNF, and IL-1β, IL-17, and IL-22, can also trigger β-defensin expression (8–11).

In addition to their antimicrobial effects within the innate immune system, β-defensins contribute to the adaptive immune response. It was shown that mouse β-defensin (mBD)2 activates immature dendritic cells through TLR4, triggering a Th1 milieu(12). Human β-defensin (hBD)3 activates APCs via TLR1 and TLR2 in a NF-κB–dependent manner (13). In earlier reports, we and other investigators described the capacity of hBDs (e.g., 1–3) (14, 15) or mBDs (e.g., 2 and 14) (16, 17) to induce chemotaxis through CCR6. Recently, it became evident that Cys5 plays a crucial role in CCR6-dependent chemotaxis (18). However, there is also clear evidence for the chemotactic interaction of β-defensins with another pertussis toxin sensitive G_i protein-coupled receptor expressed on monocytes and macrophages or mast cells, which do not express CCR6 (15, 19–21).

Tumor-derived hBD3 uses CCR2 to attract tumor-infiltrating monocytes (G. Jin, personal communication). It was reported that hBD3 inhibits CXCR4-dependent chemotaxis and calcium mobilization induced by CXCL12/stromal cell-derived factor-1α (SDF-1α), and it induces the internalization of CXCR4, indicating an interaction of hBD3 with this chemokine receptor (22).

In this study, however, we demonstrated the capability of βdefensins to bind to the chemokine receptor CCR2, rather than CXCR4, and to subsequently induce chemotaxis. CCR2 is expressed on monocytes, dendritic cells, and macrophage subsets (23, 24), and two of its well-characterized ligands are CCL2/MCP-1 and CCL7/MCP-3 (25–27). The interaction of MCP-1 and −3 with CCR2 plays a pivotal role during the immune reaction against bacterial or viral infections, attracting CCR2-expressing inflammatory cells to the sites of infection (23, 26, 28).

In this article, we provide data that substantiate the role of bdefensins as chemoattractants recruiting CCR6^{+} and CCR2^{+} cells.

Materials and Methods

Animals

Wild-type C57BL/6 mice were purchased from Charles River Laboratories (Frederick, MD). CCR2-deficient mice (29) on a C57BL/6 background were bred in the Animal Production Area of the National Cancer Institute. All mice were kept under specific pathogen-free conditions, with water and food given ad libitum. All experiments with mice were performed in compliance with the principles and procedures outlined in the National Institutes of Health Guide for the Care and Use of Animals and were approved by the National Cancer Institute Frederick Animal Care and Use Committee.

Expression and purification of β-defensin fusion proteins

rβ-defensin fusion proteins were generated and purified as described earlier (17). In brief, cDNA encoding for the mature polypeptides of hBD2, hBD3, mBD4, or mBD14 was cloned

into the Signal Ig plus vector (R&D Systems, Wiesbaden, Germany). All cDNAs fused to the human IgG-Fc sequence were subcloned into the pMTBiP/V5-His A expression vector (Invitrogen, Karlsruhe, Germany), containing a hygromycin cassette, by PCR amplification of the hBD2:Ig, hBD3:Ig, mBD4:Ig, and mBD14:Ig cDNAs, using the following primers: 5′-CCC AGA TCT GTT ACG TGC CTG AAA AGC GG-3′ for hBD2–5′; 5′-CCC AGA TCT CAG AAA TAC TAC TGC CGT G-3′ for hBD3–5′; 5′-CCC AGA TCT AAT CCA ATA ACA TGC ATG-3′ for mBD4–5′; 5′-CCC AAG CTT CGA AAA TTT TTC TGC AGA-3′ for mBD14–5′; and 5′-CG CGG CCG CCA TCA TTT ACC CGG AGA CAG G-3′ for human IgG-Fc-3′. After transfection, stable expressing *Drosophila*-S2 cells were selected and maintained in hygromycin (0.3 mg/ml; Invitrogen). The β-defensin fusion proteins were purified from the culture medium using HiTrap Protein G HP columns (GE Healthcare, Munich, Germany), according to the manufacturer's instructions. Expression and purification of the fusion proteins were confirmed by Western blotting using peroxidase-conjugated donkey anti-human IgG mAb (Dianova, Hamburg, Germany) and polyclonal Abs against hBD2, hBD3, and mBD4 (Santa Cruz Biotechnology, Heidelberg, Germany).

Cell isolation and culture

HEK293 cells expressing human CCR2 variant B (hCCR2/HEK293) and HEK293 cells expressing human CXCR4 (hCXCR4/HEK293) were maintained in DMEM (Mediatech, Herndon, VA) containing 10% FCS (HyClone, Thermo Scientific, Logan, UT; inactivated for 30 min at 56°C) and 800 μg/ml G418 and were harvested when they reached 70–80% confluence.

Human peripheral blood enriched in mononuclear cells was obtained from healthy donors by leukapheresis (Transfusion Medicine Department, Clinical Center, National Institutes of Health, with approved human subject agreement). The blood was centrifuged through Histopaque-1077 (Sigma-Aldrich, St. Louis, MO), and PBMCs collected at the interface were washed with PBS. After centrifugation through an iso-osmotic Percoll (GE Healthcare, Pittsburgh, PA) gradient, the enriched monocytes (peripheral blood monocytes) were obtained from the top of the gradient.

Mouse peritoneal exudate cells (PECs) were elicited by i.p. injection of 2 ml 3% thioglycollate into 8–12-wk-old C57BL/6 wild-type or CCR2-deficient mice. After 3 d, PECs were isolated by lavage of the peritoneum with 5 ml ice-cold PBS containing heparin (20 U/ml) and EDTA (5 mM).

Chemotaxis assay

Cells were suspended in chemotaxis medium (RPMI 1640 containing 1% BSA, 20 mM HEPES, 2 mM L-glutamine, 100 U/ml penicillin, and 100 mg/ml streptomycin) at 1×10^6 cells/ml for human monocytes, 5×10^5 cells/ml for PECs, and 2×10^6 cells/ml for human CCR2+, human CXCR4+, or wild-type HEK293 cells. In certain experiments, primary cells were preincubated with 10–100 ng/ml chemoattractant (as indicated) for 30 min at 37°C in humidified air containing 5% CO_2. Synthetic hBD2 and hBD3 were purchased from Peptide Institute (Osaka, Japan). Human or mouse CCL2/MCP-1 and human CXCL12/SDF-1α were purchased from PeproTech (Rocky Hill, NJ) and used as positive control. The migration of

cells in response to chemoattractants (β-defensins or control chemotactic factors) was determined using the 48-well microchemotaxis chamber assay, as previously described (30). In brief, chemoattractants diluted in chemotaxis medium at various concentrations were put into the lower wells of a 48-well microchemotaxis chamber (NeuroProbe, Gaithersburg, MD), and cell suspension was added to the upper wells. The lower and upper compartments were separated by an uncoated polycarbonate filter membrane (5 μm pore size) for primary cells or a collagen-coated polycarbonate filter membrane (10 mm pore size) for human CCR2^{+}, human CXCR4^{+}, or wild-type HEK293 cells (both from NeuroP-robe). After incubation at 37°C for 1.5 h for primary cells and 5 h for HEK293 cells in humidified air with 5% CO_2, the filters were removed, scraped, and stained. The cells that migrated across the filter were counted under a light microscope using Bioquant Life Science software (Bioquant Image Analysis, Nashville, TN). The results (mean ± SD of triplicate wells) are presented as the number of cells per high-power field.

Flow cytometry

All staining steps were performed for 20 min on ice in PBS containing 2% FCS and 0.05% Azide. HEK293 cells were incubated with 10% goat serum to prevent nonspecific binding. Primary cells were blocked with 10% goat or rat serum. HEK293 cells were labeled with 1 μg/ml β-defensin fusion protein. After washing, cells were stained with a PE-conjugated anti-human IgG Ab (Jackson ImmunoResearch Laboratories, West Grove, PA). Expression of CCR2 or CCR6 on human monocytes was analyzed using an anti-human CCR2-PE Ab (clone 48607) or an anti-human CCR6-FITC Ab (clone 53103) purchased from R&D Systems (Minneapolis, MN). Detection of CCR2 or CCR6 expression on PECs was performed using an anti-mouse CCR2 Ab from goat (GeneTex, Irvine, CA) and an anti-goat IgG-FITC secondary Ab (Jackson ImmunoResearch Laboratories) or an anti-mouse CCR6 Ab from rat (clone 140706; R&D Systems) and an anti-rat IgG-Alexa 488 Ab (Molecular Probes, Eugene, OR). Flow cytometry was performed on a FACScan cytometer using Cell Quest software (BD Biosciences, San Jose, CA). Flow cytometry data were analyzed with FlowJo software (version 8.1.0) (Tree Star, Ashland, OR).

Statistical analysis

Statistical analyses of chemotaxis data were performed using one-way ANOVA with the Dunnett posttest (GraphPad Prism, version 4.0c; Graph-Pad, San Diego, CA); medium control served as the reference value. Statistical significance was considered at $p < 0.01$.

Results

Binding of β-defensins to CCR2 but not CXCR4

The β-defensins used in this study were expressed as fusion proteins, fused to the Fc region of human IgG_1 (Fig. 1A), allowing easy detection of binding. Expression of fusion proteins was detected by Western blot analysis using an anti-human IgG Ab (Fig. 1B, *lower panels*). The β-defensin domain of the fusion proteins was detected by polyclonal Abs against hBD2, hBD3, and mBD4 (Fig 1B, *upper panels*). Both detection methods revealed an apparent m.w. of 37 kDa for the β-defensin fusion proteins (Fig. 1). There is no commercially available Ab

against mBD14. In earlier studies, we showed that these β-defensin fusion proteins retained antimicrobial activity comparable to unfused β-defensins (17, 31).

In this study, hBD2:Ig and hBD3:Ig, as well as their mouse orthologs mBD4:Ig and mBD14:Ig, were used to examine their interaction with CCR2 and CXCR4. FACS analysis revealed that hBD2:Ig, hBD3:Ig, mBD4:Ig, and mBD14:Ig bound to human CCR2-expressing HEK293 cells independently of species specificity (Fig. 2A). In contrast, no binding of β-defensin fusion proteins was observed using human CXCR4-expressing HEK293 cells (Fig. 2B). Unfused human IgG_1 did not bind to wild-type or transfected HEK293 cells (Fig. 2).

The β-defensins induced chemotaxis of human CCR2-expressing HEK293 cells

Chemotaxis assays using human CCR2-expressing HEK293 cells were performed to elucidate whether the binding of hBD2:Ig and hBD3:Ig, as well as mBD4:Ig and mBD14:Ig, to human CCR2 has functional consequences. Human MCP-1 induced dose-dependent migration of human CCR2-expressing HEK293 cells in the typical bimodal manner, reaching a maximum at a concentration of 10 ng/ml (Fig. 3A). Human SDF-1α, the ligand for human CXCR4, induced dose-dependent migration of human CXCR4-expressing HEK293 cells, with maximal migration at 100 ng/ml (Fig. 3B). The β-defensin fusion proteins induced chemotaxis of human CCR2-expressing HEK293 cells in a dose-dependent manner, which peaked at a concentration of 100 ng/ml (Fig. 3C–F). In agreement with the inability of the β-defensin fusion proteins to bind to human CXCR4^{+} HEK293 cells, they also failed to induce chemo-taxis of these cells (Fig. 3C–F). To exclude effects related to the human IgG_1-Fc part of the fusion proteins, we tested unfused human IgG_1 and showed that it did not induce cell migration (Fig. 3G).

The β-defensin fusion proteins induced chemotaxis of human peripheral blood monocytes

Human monocytes isolated from peripheral blood, which express endogenous CCR2 but not CCR6 (Fig. 4), were used to determine whether the β-defensin fusion proteins were also chemotactic for primary monocytes. Similar to the migration induced by human MCP-1 (Fig. 5A), the chemotactic effect of hBD2:Ig and hBD3:Ig, as well as mBD4:Ig and mBD14:Ig, peaked at a concentration of 10 ng/ml (Fig. 5C–F). In contrast, monocytes preincubated with 10 ng/ml human MCP-1 failed to migrate toward the β-defensin fusion proteins (Fig. 5C–F). Conversely, preincubation of monocytes with 10 ng/ml hBD2:Ig or hBD3:Ig similarly induced desensitization and inhibited subsequent chemotactic responses toward human MCP-1 (Fig. 5A). However, these preincubated cells retained their ability to migrate toward human SDF1-a (data not shown). Human monocytes failed to migrate toward human IgG_1 (Figs. 5B, 6). The chemotactic effect of hBD2:Ig and hBD3:Ig, at concentrations of 10 ng/ml, was comparable to that of synthetic hBD (shBD)2 and shBD3 for human monocytes (Fig. 6). Pretreatment of monocytes with 10 ng/ml shBD2 inhibited hBD2:Ig-induced migration (Fig. 6A). Similarly, pre-treatment with 10 ng/ml shBD3 abolished hBD3:Ig-induced chemotaxis (Fig 6B). Synthetic mBD4 and mBD14 were not commercially available for further analysis.

CCR2-dependent migration of PECs

Thioglycollate-elicited PECs from C57BL/6 wild-type mice are a heterogeneous cell population. The majority of PECs expressed CCR2 (Fig. 7A). CCR6-expressing cells were not detected (Fig. 7B). Mouse MCP-1 induced maximal migration at a concentration of 1 ng/ml (Fig. 8A). However, the PECs also showed chemotactic responses to mBD4:Ig, mBD14:Ig, and hBD2:Ig, peaking at a concentration of 100 ng/ml (Fig. 8C–E). Furthermore, hBD3:Ig induced maximum migration at a concentration of 10 ng/ml (Fig. 8F). The capacity to migrate toward β-defensin fusion proteins or mouse MCP-1 was abolished in PECs from CCR2-deficient C57BL/6 mice (Fig. 8A, 8C–F). Unfused human IgG$_1$ did not induce migration of PECs (Fig. 8B).

Discussion

In earlier reports, we described the production and characterization of β-defensin fusion proteins. These fusion proteins retained their antibacterial and chemotactic activities (17, 31) and were chemo-tactic for CCR6-expressing HEK293 cells, as well as for monocytes that do not express CCR6. The constant human IgG$_1$ domain of the fusion proteins facilitated efficient purification and detection in Western blot analysis, as well as flow cytometric analysis. Polyclonal Abs against hBD2, hBD3, and mBD4 allowed specific detection of the β-defensin domains of these fusion proteins. Abs against mBD14 are not commercially available.

Observations suggested that CCR2 may be another G$_i$ protein-coupled receptor for hBD3 (G. Jin, personal communication). Therefore, we investigated whether hBD2, as well as hBD3 and their mouse orthologs mBD4 and mBD14, uses CCR2 in addition to CCR6. We highlight the effects of these β-defensins, fused to the Fc region of human IgG$_1$, with CCR2. As we reported earlier, the interaction of mBD4:Ig and mBD14:Ig with CCR6 is species specific, but hBD2:Ig and hBD3:Ig were able to bind to human and mouse CCR6. In contrast, the binding of the mBD fusion proteins, as well as their human orthologs, to CCR2 is not species specific, and they interact with human or mouse receptors. Recombinant human CCR2-expressing HEK293 cells showed directional migration induced by the β-defensin fusion proteins, indicating functional interaction of hBD2, hBD3, mBD4, and mBD14 with CCR2. Primary human peripheral blood monocytes expressing CCR2, but not CCR6, also demonstrated consistent migration induced by all of the β-defensin fusion proteins used in this study.

In an effort to ascertain whether the β-defensins and MCP-1 share the same receptor, their mutual desensitizing effects were investigated. Preincubation of monocytes with human MCP-1 abolished the migration induced by hBD fusion proteins. Conversely, preincubation with hBD fusion proteins abolished migration induced by human MCP-1, indicating that these proteins share the same chemotaxis-inducing receptor. In contrast, the chemotactic response to human SDF-1α was not desensitized by preincubation with hBDs or human MCP-1 (data not shown). The hBD fusion proteins induced chemotaxis of human monocytes similarly to synthetic hBDs. Furthermore, pretreatment of monocytes with these synthetic peptides inhibited migration induced by hBD2:Ig and hBD3:Ig, demonstrating the capacity of synthetic peptides to desensitize the interaction of recombinantly expressed fusion

proteins with CCR2. The effects of synthetic mBD4 or mBD14 could not be tested, because these peptides are not commercially available. Additionally, chemoattraction of PECs from C57BL/6 wild-type mice by human and mBD fusion proteins was abrogated using cells from CCR2-deficient C57BL/6 mice. Because the lack of CCR2 in these gene-targeted mice is specific and irreversible, we were able to evaluate CCR2-dependent chemotaxis without concern for residual receptor function and, thus, ruled out the involvement of another chemotaxis-inducing receptor. These results clearly demonstrate specific interaction of hBD2:Ig, hBD3:Ig, mBD4:Ig, and mBD14:Ig with human and mouse CCR2.

A recent study indicated a possible interaction of hBD3 with the chemokine receptor CXCR4. The investigators described the competition of hBD3 with SDF-1α binding to CXCR4 and further reported hBD3-induced internalization of the chemokine receptor. However, direct interaction with CXCR4 was not shown (22). None of the β-defensin fusion proteins used in the present study demonstrated direct binding to human CXCR4 or chemoattraction of human CXCR4-expressing HEK293 cells. This argues against any direct interaction of these β-defensin fusion proteins with CXCR4.

Selective interaction of β-defensins with CCR2 and CCR6 raises the question of the underlying common binding motif. Although β-defensins and CC-chemokines, such as CCL2/MCP-1 or CCL20/MIP-3α, do not share significant sequence similarities, they do share structural similarities, such as the α helix, the abundance of cationic residues, and disulfide linkages, responsible for the distinct tertiary structure. Thus, common structural motifs and electrostatic overlap may be responsible for the CC-chemokine receptor-dependent chemotaxis-inducing activity of certain β-defensins (32). Although some chemokines also interact with multiple receptors, and because no chemokines interact with both CCR2 and CCR6, further studies are necessary to understand how β-defensins interact with several distinct chemokine receptors.

CCR2 and CCR6 were shown to be crucial for the recruitment of professional APCs to inflamed tissue (33). In particular, CCR2^{+} monocytes are recruited to sites of tissue inflammation, contributing to clearing pathogens and triggering adaptive immune response, whereas CCR2^{−} monocytes infiltrate noninflamed tissues, where they may be involved in tissue homeostasis (24). Our findings support a potential role for β-defensins as mediators of enhanced immune reaction during inflammation. CCR6 is expressed by different lymphocyte subsets, such as CD45RO^{+} CD4^{+} T cells (34) and Th17 cells (35), as well as immature dendritic cells (14). Chemoattraction of these cells to sites of infection is necessary to initiate the adaptive immune response. However, CCR2 is largely expressed on myeloid cells, such as monocytes, macrophages (36, 37), and neutrophils (38), which are crucial for inflammation and phagocytosis. Thus, our data suggest that β-defensins contribute to the recruitment of a broad spectrum of leukocytes to sites of infection and inflammation.

In addition to their chemotactic and antimicrobial activity, there is evidence suggesting that β-defensins participate in the regulation of host innate and adaptive immune responses. A recent study provided data showing the capacity of hBD1 to induce the expression of maturation markers (e.g., MHC class II and CD83), costimulatory markers (e.g., CD80,

CD86, and CD40), and proinflammatory cytokines (e.g., TNF, IL-6, and IL-12p70) in human monocyte-derived dendritic cells (39). Funderburg et al.(13) described TLR1- and TLR2-mediated expression of costimulatory molecules on monocytes and myeloid dendritic cells induced by hBD3. Furthermore, mBD2 is able to induce TLR4-mediated maturation of dendritic cells (12). Thus, we hypothesize that β-defensins, expressed by epithelial cells and leukocytes at sites of infection and inflammation, promote chemotactic recruitment and activation of leukocytes, such as monocytes, macrophages, and dendritic cells. Consequently, β-defensins produced at nanomolar concentrations by epithelial cells and leukocytes in response to infectious and injurious challenges form a haptotactic gradient in the tissue and along blood vessel walls to attract more APCs and monocytes to the endangered site. In addition, micro-molar concentrations of β-defensins exert their antimicrobial activity against invasive microorganisms.

Recent studies indicated that CCR2^{+} macrophages are crucial mediators of ulcerative colitis-associated colon carcinogenesis(40). In oral carcinoma, hBD3 was shown to be overexpressed by tumor cells, correlating with the recruitment and infiltration of macrophages (41). Furthermore, endothelial progenitor cells were reported to be recruited into tumors in a CCR2- and CCR5-dependent manner, contributing to neovascularization(42). These findings, together with our results, make it plausible for β-defensins to contribute to tumorigenesis and tumor development, as suggested by G. Jin (personal communication). Further studies are required to identify potentially different cellular-recruitment patterns in tissues following infection, inflammation, or tumorigenesis, depending on the type of β-defensin expressed.

CCR2 seems to be the unidentified G_i protein-coupled receptor responsible for CCR6-independent β-defensin–mediated chemo-taxis of monocytes. The β-defensin fusion proteins are able to recruit a broad spectrum of leukocytes in a CCR6- and CCR2-dependent manner. Based on our data, we conclude that the recruitment capabilities of β-defensins contribute to the innate and adaptive immune response in their role as chemoattractants.

Acknowledgments

Abbreviations used in this paper:

|	
hBD	human β-defensin
mBD	mouse β-defensin
PEC	peritoneal exudate cell
SDF-1α	stromal cell-derived factor-1α

| **shBD** | synthetic human β-defensin |

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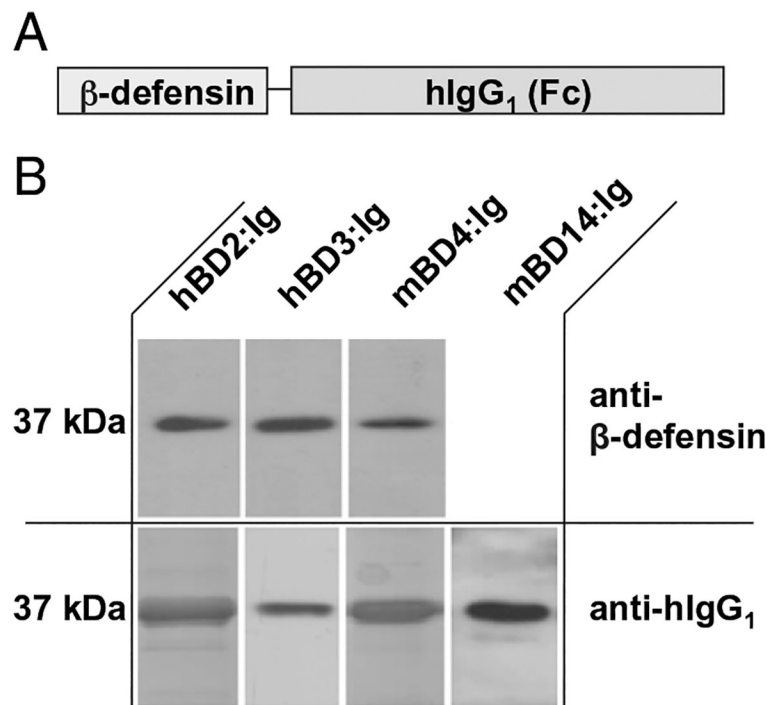
FIGURE 1.
Expression of hBD2, hBD3, and their mouse orthologs (mBD4 and mBD14) as human
IgG$_1$-Fc fusion proteins. A, Schematic representation of the β-defensin fusion protein (not to
scale). B, Western blot analysis of recombinantly expressed hBD2:Ig, hBD3:Ig, mBD4:Ig,
and mBD14:Ig using polyclonal Abs against hBD2, hBD3, and mBD4 (*upper panels*) or
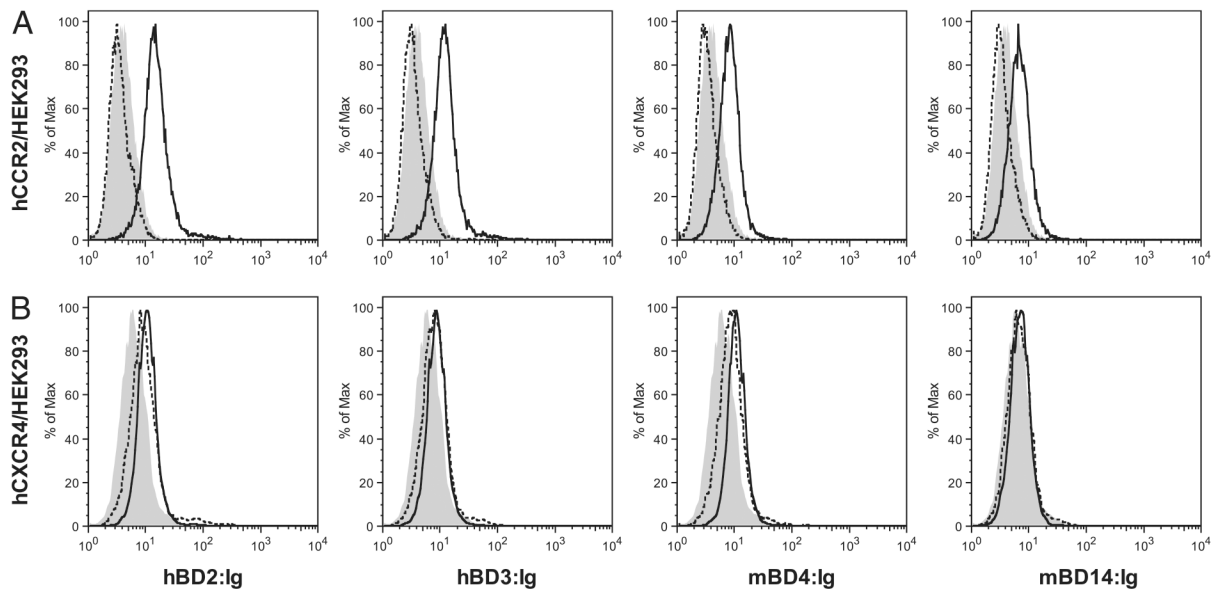anti-human IgG$_1$ Ab (*lower panels*). Data are representative of at least three independent
experiments.

FIGURE 2.
Selective binding of hBD2:Ig, hBD3:Ig, mBD4:Ig, and mB14:Ig to human CCR2. For binding analysis, HEK293 cells stably transfected with human CCR2 (*A*) or human CXCR4 (*B*) were incubated with 1 μg/ml of β-defensin fusion protein (solid line) or 1 mg/ml human IgG$_1$ (shaded graph). Wild-type HEK293 cells were incubated with 1 mg/ml of the respective β-defensin fusion protein (dashed line). Binding was detected using a PE-conjugated anti-human IgG Ab. Data are representative of two or three independent experiments.
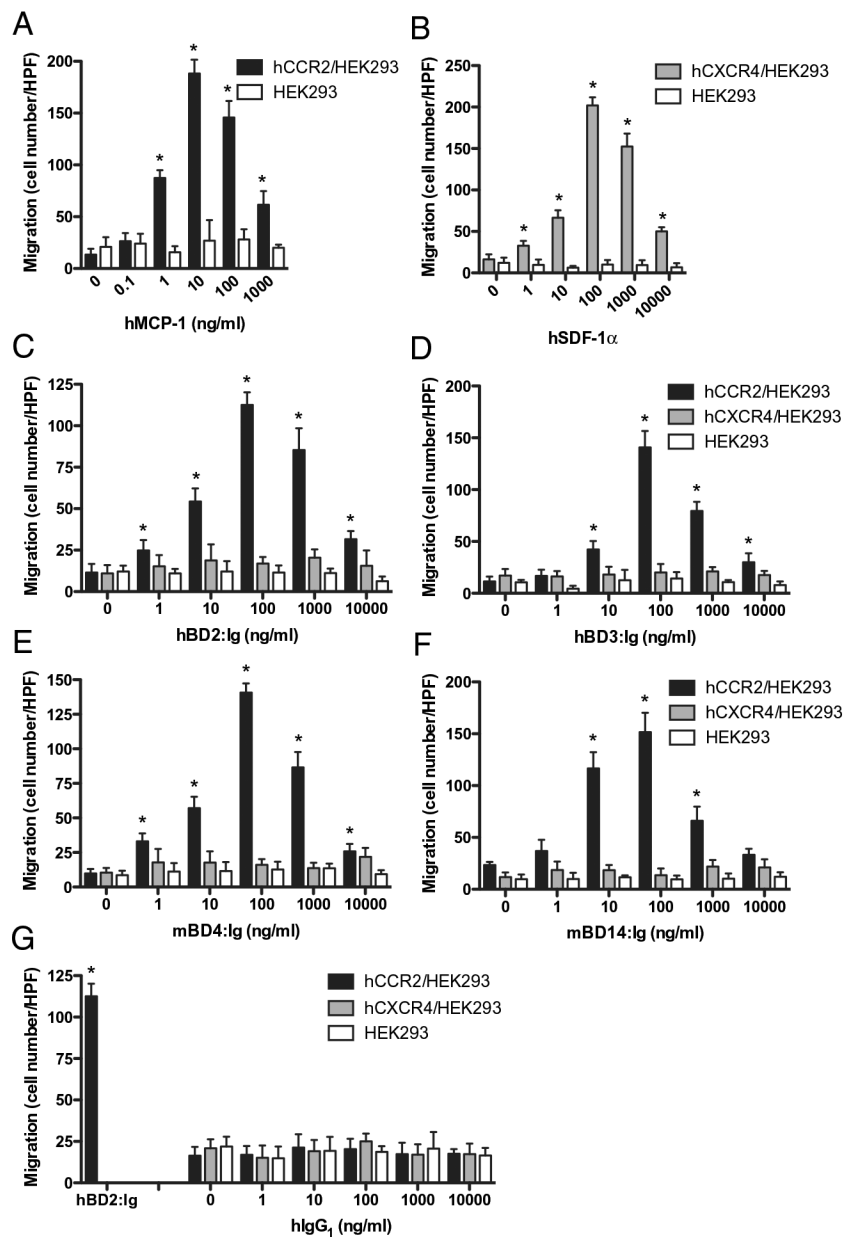
FIGURE 3.

Comparison of the chemotactic effect of β-defensin fusion proteins and human MCP-1 on human CCR2/HEK293 cells. Migration of human CCR2/HEK293 cells, human CXCR4/ HEK293 cells, or wild-type HEK293 cells induced by human MCP-1 (*A*) or human SDF-1α (*B*). Chemotaxis of chemokine receptor-expressing or wild-type HEK293 cells induced by hBD2:Ig (*C*), hBD3:Ig (*D*), mBD4:Ig (*E*), or mBD14:Ig (*F*). *G*, Migration induced by human IgG₁. Data are representative of three independent experiments. *$p < 0.01$, one-way ANOVA with the Dunnett posttest versus media control.
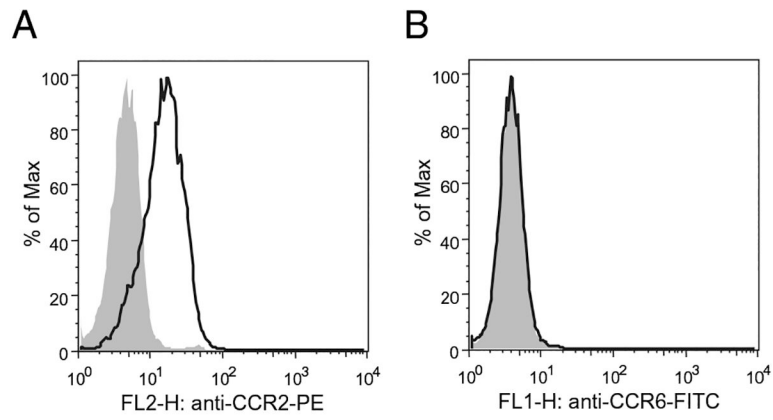
FIGURE 4.
Expression of CCR2, but not CCR6, on human peripheral blood monocytes. Monocytes, isolated from human blood, were labeled with anti-hCCR2-PEAb(*A*, opengraph)oranti-CCR6-FITCAb(*B*, opengraph)andisotype control (shaded graph). Data are representative of three independent experiments.
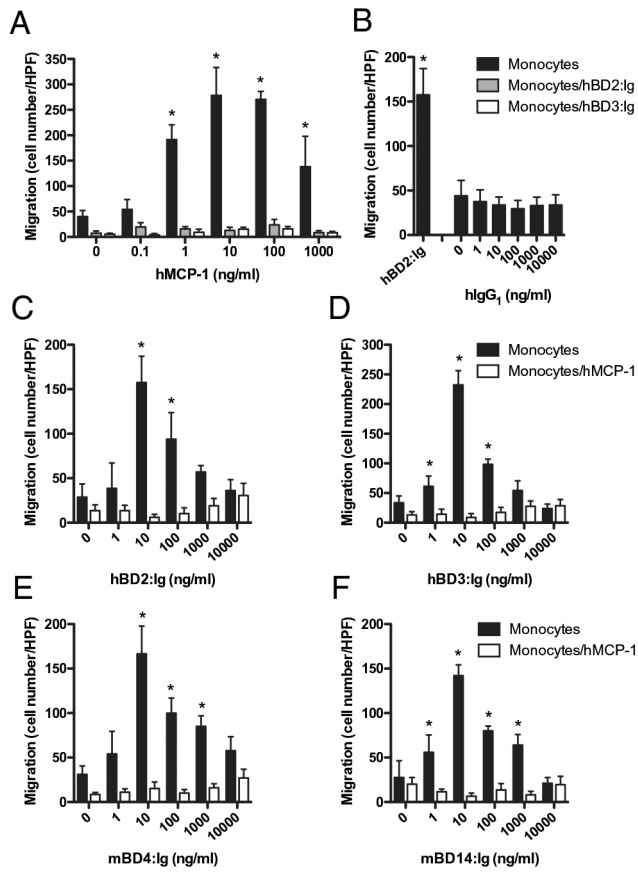
FIGURE 5.

β-defensin fusion protein-induced chemotaxis of human peripheral blood monocytes. A, Dose-dependent migration of human monocytes induced by human MCP-1. Cell migration of monocytes preincubated for 30 min with 10 ng/ml hBD2:Ig or hBD3:Ig. B, Migration of monocytes induced by unfused human IgG$_1$. Chemotaxis of untreated monocytes or monocytes preincubated for 30 min with 10 ng/ml human MCP-1 induced by hBD2:Ig (*C*), hBD3:Ig (*D*), mBD4:Ig (*E*), or mBD14:Ig (*F*). Data are representative of three or four independent experiments. *$p < 0.01$, oneway ANOVA with the Dunnett posttest versus media control.
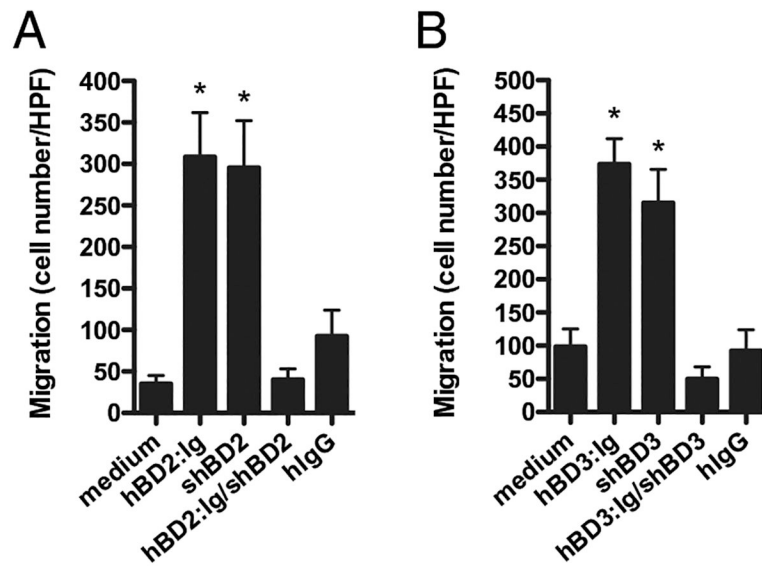
FIGURE 6.
Inhibition of β-defensin fusion protein-induced chemotaxis of human peripheral blood monocytes by pretreatment with synthetic β-defen-sins. Migration of human monocytes induced by 10 ng/ml hBD2:Ig, shBD2, or unfused human IgG (*A*) and 10 ng/ml hBD3:Ig, shBD3, or unfused human IgG (*B*). Abrogated chemotaxis of monocytes after pretreatment for 30 min with 10 ng/ml shBD2 in response to hBD2:Ig (*A*) and 10 ng/ml shBD3 in response to hBD3:Ig (*B*). Data are representative of three independent experiments. *$p < 0.01$, one-way ANOVA with the Dunnett posttest versus media control.
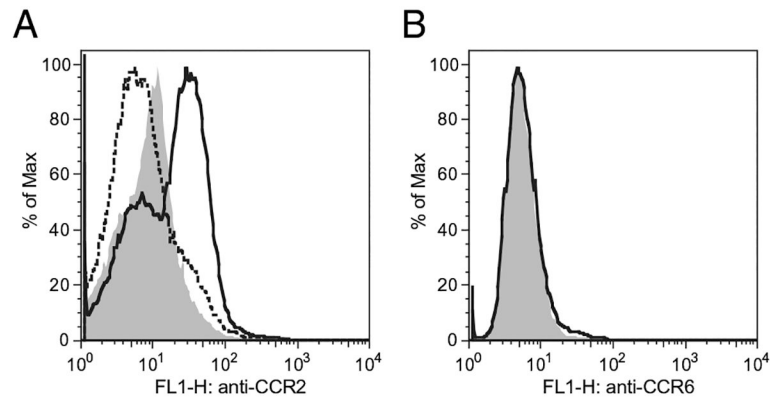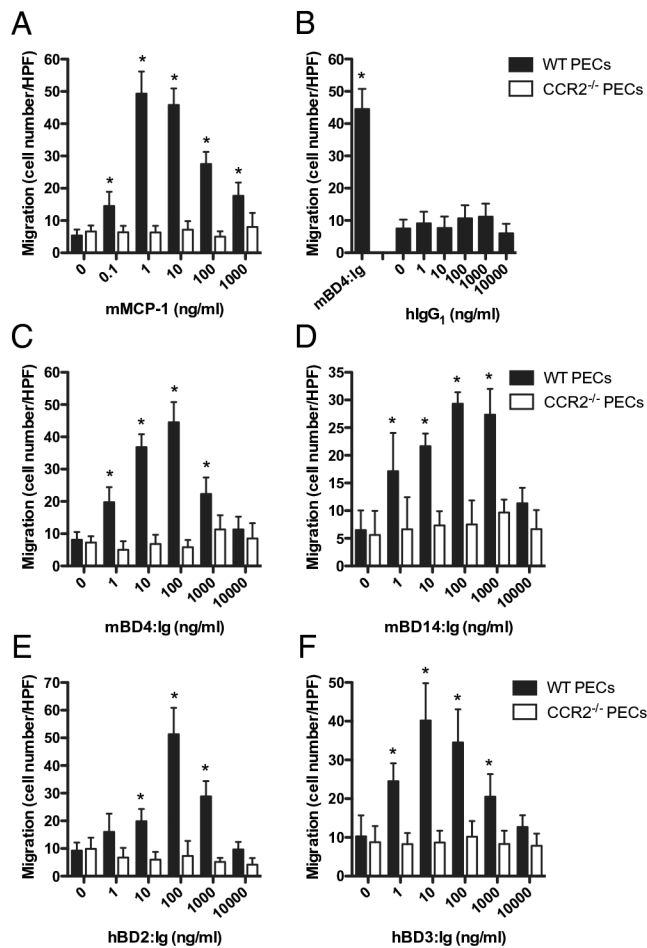
FIGURE 7.
Comparison of expression of CCR2 on PECs from wild-type and CCR2-deficient C57BL/6 mice and expression of CCR6. A, Thioglycollate-elicited mouse PECs from wild-type mice (solid line) or CCR2-deficient mice (dashed line) labeled with anti-mCCR2 polyclonal Ab from goat and secondary Ab anti-goat IgG-FITC and PECs incubated with secondary Ab alone (shaded graph). *B*, PECs from wild-type mice labeled with anti-mCCR6 (solid line) or isotype control (shaded graph). Data are representative of three independent experiments.

FIGURE 8.

Comparison of migration of wild-type PECs and CCR2-deficient PECs toward β-defensin fusion proteins. *A*, Chemotaxis of thioglycol-late-elicited mouse PECs from wild-type mice or CCR2-deficient mice induced by human MCP-1. *B*, Migration of wild-type PECs induced by unfused human IgG$_1$. Chemotaxis induced by mBD4:Ig (*C*), mBD14:Ig (*D*), hBD2:Ig (*E*), or hBD3:Ig (*F*). Data are representative of three or four independent experiments. *$p <$ 0.01, one-way ANOVA with the Dunnett posttest versus media control.