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Regulation of Myeloid Cell Function through the CD200 Receptor

Maria C. Jenmalm, Holly Cherwinski, Edward P. Bowman, Joseph H. Phillips, and Jonathon D. Sedgwick

Myeloid cells play pivotal roles in chronic inflammatory diseases through their broad proinflammatory, destructive, and remodeling capacities. CD200 is widely expressed on a variety of cell types, while the recently identified CD200R is expressed on myeloid cells and T cells. CD200 deletion in vivo results in myeloid cell dysregulation and enhanced susceptibility to autoimmune inflammation, suggesting that the CD200-CD200R interaction is involved in immune suppression. We demonstrate in this study that CD200R agonists suppress mouse and human myeloid cell function in vitro, and also define a dose relationship between receptor expression and cellular inhibition. IFN-γ- and IL-17-stimulated cytokine secretion from mouse peritoneal macrophages was inhibited by CD200R engagement. Inhibitory effects were not universal, as LPS-stimulated responses were unaffected. Inhibition of U937 cell cytokine production correlated with CD200R expression levels, and inhibition was only observed in low CD200R expressing cells, if the CD200R agonists were further cross-linked. Tetanus toxoid-induced human PBMC IL-5 and IL-13 secretion was inhibited by CD200R agonists. This inhibition was dependent upon cross-linking the CD200R on monocytes, but not on cross-linking the CD200R on CD4+ T cells. In all, we provide direct evidence that the CD200-CD200R interaction controls monocyte/macrophage function in both murine and human systems, further supporting the potential clinical application of CD200R agonists for the treatment of chronic inflammatory diseases. The Journal of Immunology, 2006, 176: 191–199.

Myeloid cells (i.e., macrophages, dendritic cells (DC), neutrophils, mast cells, and eosinophils) play important roles in maintaining chronic inflammation (1–5). They can be regulated through cell-cell interactions that trigger matched sets of activating and inhibitory receptors, in addition to being regulated by secreted factors (6). The regulation of myeloid cell activity by direct cell-cell contact allows a more localized control than that mediated by cytokines. The CD200-CD200R interaction also provides a cell-cell contact regulatory interaction for myeloid cells. The widely expressed glycoprotein CD200 is closely related structurally to the T cell costimulatory molecules CD80 and CD86 and is genetically linked to them on human chromosome 3 and mouse chromosome 16 (7, 8). Structurally, CD200 contains two Ig superfamily (IgSF) domains in a typical V/C2 arrangement (9).

Deletion of CD200 resulted in myeloid cell dysregulation and enhanced susceptibility to autoimmune inflammation such as experimental autoimmune encephalitis (EAE) and collagen-induced arthritis (CIA) (10), suggesting that CD200 normally induces immune suppression through CD200R. Consistent with this was the demonstration that a soluble form of CD200 (mouse (m) CD200lg) administered to mice blocks autoimmune inflammation in vivo (11). In terms of mechanism of action, endogenous CD200 (and soluble CD200lg) could send a signal that deviates the immune response away from a damaging effector pathway, as proposed (12), and/or it could directly inhibit effector cell function (10). As in vivo studies cannot discriminate between these possibilities, a mast cell model was established to test whether CD200R directly mediates cellular inhibition. IgE-mediated activation of mast cells was inhibited by mCD200lg or an agonist rat-anti-mCD200R (DX109) when the cells were transfected with mCD200R, and by human (h) CD200lg or agonist rat-anti-hCD200R (DX136 and DX153) when the cells were transfected with hCD200R (13). These agonists directly blocked mast cell degranulation as well as TNF and IL-13 secretion, with a typical IC50 of 0.2–1 nM. Thus, CD200 directly triggered cellular inhibition through CD200R engagement.

The CD200R is expressed at the surface of human and mouse myeloid cells, such as macrophages, DCs, neutrophils, and mast cells, and also on T cells (14, 15). CD200R is closely structurally related to CD200, located on the same chromosome, the genes probably evolved by gene duplication (15). However, the receptor is distinct from CD200 by virtue of a longer cytoplasmic tail containing three conserved tyrosine residues, one of which is contained within an NPXY motif (14, 15). Phosphorylated NPXY motifs bind phosphotyrosine-binding domains that are present in signaling adaptor molecules such as Shc (16), suggesting that the CD200R can signal after ligation by CD200. CD200R lacks an ITIM motif present in almost all immune inhibitory receptors (17). Using the mast cell system, we have shown that the NPXY motif is critical for receptor activity, and that signaling and cellular inhibition are linked to Dok1 and Dok2 phosphorylation and subsequent Ras/MAPK pathway inhibition (18).
The aim of the present study was to further characterize the regulation of myeloid cell function through the CD200R in vitro, focusing especially on the effects of the CD200R on macrophage and DC function in human and mouse.

Materials and Methods

CD200R-binding reagents

Rat mAbs that were agonistic for mouse and human CD200R were generated in rats as previously described (15). Used were DX109 (ratIgG1-anti-mCD200R), DX136 (ratIgG2a-anti-hCD200R), and DX153 (ratIgG1-anti-hCD200R). DX109 does not bind the activating DNA-binding protein 12 (DAP-12)-paired mCD200RLa and mCD200RLb (15). hCD200RLa is not expressed and is probably nonfunctional (15).

Ligand (CD200)-Ig fusion proteins were generated as described (13) by fusion of the extracellular domain of human or mouse CD200 to the Fc domain of mlgG1 mutated in the CH2 domain (D265A) to inhibit binding to FcRs (19). mCD200L does not bind the activating DAP-12-paired mCD200RLa and mCD200RLb (15, 20).

All mAb and protein preparations used contained <0.1 ng of endotoxin/mg protein, as determined by the Limulus Amebocyte Lysate Pyrogen Testing kit, QCL-1000 (BioWhittaker).

Cells and cell culture

Mouse peritoneal cells. Resident peritoneal cells were obtained by peritoneal lavage of 8- to 12-wk-old C57BL/6 mice (The Jackson Laboratory) with 5 mL of DMEM (Meditech). A total of 1 × 10^6 cells were cultured in 1 mL of DMEM with 10% heat-inactivated FCS (HyClone) and 1% penicillin and streptomycin (Sigma-Aldrich) in Falcon 24-well plates (BD Biosciences Discovery Labware) at 37°C in a humidified incubator containing 5% CO₂. In some experiments, nonadherent cells were removed after 2 h to obtain a more pure macrophage population. After a 30-min incubation with mCD200Lg, control Ig, rat-anti-mCD200R (DX109), or rat IgG1 isotype control (BD Pharmingen), cells were stimulated with recombinant murine IFN-γ (R&D Systems) and rIFN-γ for 24 and 48 h. In some experiments, cells were washed after the 30-min incubation, and cell-bound hCD200Lg or rat-anti-hCD200R were cross-linked with 5 μg/mL endotoxin-depleted Fab(’2) goat anti-mouse/rat IgG, and incubated for another 30 min before addition of TNF.

Supernatants were collected and analyzed for IL-6, IL-10, IL-12p70, MCP-1/CCL2, MIP-1α/CCL3, RANTES/CCL5, and TNF secretion by ELISA or cytometric bead array (CBA) as described below.

Human monocyte cell line. Human U937 monocytes (American Type Culture Collection) were transfected with hCD200R. cDNA encoding full-length hCD200R was subcloned into the pMX-pie retrovirus expression vector (21) containing a puromycin resistance gene, an internal ribosomal entry site (IRES) element, and an enhanced GFP gene. Plasmid DNA was then transfected into the Phoenix ecotropic virus packaging cell line (a gift from G. Nolan, Stanford University, Palo Alto, CA). The U937 cells were infected by coculture with the transfected packaging cell line. After drug selection, the resulting hCD200R or control vector transfectants were expanded by FACs sorting for hCD200R expression and assayed for CD1a, CD32, CD80, CD83, CD86, and HLA-DR expressing cells or 10% heat-inactivated normal human serum for human cells. In some experiments, cells were washed after the 30-min incubation and the cell-bound hCD200Lg or rat-anti-hCD200R were cross-linked with 5 μg/mL endotoxin-depleted Fab(’2), goat anti-mouse/rat IgG (Jackson ImmunoResearch Laboratories), and 0.1 ng/mL recombinant murine IFN-γ (Invitrogen Life Technologies) at 37°C in a humidified incubator containing 5% CO₂ and split every 3–4 days.

A total of 1 × 10⁶ hCD200R or vector control-transfected U937 cells were cultured in 1 mL of RPMI 1640 with the supplements described above in Falcon 24-well plates at 37°C in a humidified incubator containing 5% CO₂. After a 30-min incubation with hCD200Lg, control Ig, rat-anti-hCD200R (DX153), or rat IgG1 isotype control, hCD200R or vector control-transfected U937 cells were stimulated with 100 U/mL recombinant human IFN-γ (R&D Systems) for 18 h. In some experiments, cells were washed after the 30-min incubation and the cell-bound hCD200Lg or rat-anti-hCD200R were cross-linked with 5 μg/mL endotoxin-depleted Fab(’2), goat anti-mouse/rat IgG (Jackson ImmunoResearch Laboratories), and 0.1 ng/mL recombinant murine IFN-γ (Invitrogen Life Technologies), and incubated for another 30 min before addition of TNF. Supernatants were collected and analyzed for IL-1β, IL-6, IL-8/CXCL8, IL-10, IL-12p70, IFN-inducible protein 10 (IP-10)/CXCL10, MCP-1/CCL2, MIP-1α/CCL3, RANTES/CCL5, and TNF secretion by ELISA or CBA as described below.

Flow cytometry

Cells were stained and washed in PBS containing 0.1% NaN₃, (Sigma-Aldrich) and either 1% heat-inactivated FCS for mouse cells or 1% heat-inactivated normal human serum (Jackson ImmunoResearch Laboratories) for human cells. To block FcRs, 1 × 10⁶ cells/tube were incubated with 0.1% Fc block (anti-mouse, clone 2G12; Caltag Laboratories) or 0.1% Fc block (anti-rat, clone 2G12; Caltag Laboratories) for 20 min on ice. To block CD11c on mouse cells or 10% heat-inactivated normal human serum for human cells. The cells were then stained with FITC-, PE-, PerCP-, allophycocyanin-, or biotin-conjugated Abs (BD Pharmingen, except as indicated) for 30 min 4°C in the dark. Biotinylated Abs were secondary labeled with streptavidin-allophycocyanin (BD Pharmingen). Cells were then stained with FITC-, PE-, PerCP-, allophycocyanin-, or biotin-conjugated Abs (BD Pharmingen, except as indicated) for 30 min 4°C in the dark. Biotinylated Abs were secondary labeled with streptavidin-allophycocyanin (BD Pharmingen). Cells were then stained with FITC-, PE-, PerCP-, allophycocyanin-, or biotin-conjugated Abs (BD Pharmingen, except as indicated) for 30 min 4°C in the dark. Biotinylated Abs were secondary labeled with streptavidin-allophycocyanin (BD Pharmingen). Cells were then stained with FITC-, PE-, PerCP-, allophycocyanin-, or biotin-conjugated Abs (BD Pharmingen, except as indicated) for 30 min 4°C in the dark. Biotinylated Abs were secondary labeled with streptavidin-allophycocyanin (BD Pharmingen). Cells were then stained with FITC-, PE-, PerCP-, allophycocyanin-, or biotin-conjugated Abs (BD Pharmingen, except as indicated) for 30 min 4°C in the dark. Biotinylated Abs were secondary labeled with streptavidin-allophycocyanin (BD Pharmingen). Cells were then stained with FITC-, PE-, PerCP-, allophycocyanin-, or biotin-conjugated Abs (BD Pharmingen, except as indicated) for 30 min 4°C in the dark. Biotinylated Abs were secondary labeled with streptavidin-allophycocyanin (BD Pharmingen). Cells were then stained with FITC-, PE-, PerCP-, allophycocyanin-, or biotin-conjugated Abs (BD Pharmingen, except as indicated) for 30 min 4°C in the dark. Biotinylated Abs were secondary labeled with streptavidin-allophy
rat IgG2a isotype controls. Flow cytometry was performed on a FACS-Calibur flow cytometer (BD Biosciences) or an LSR II flow cytometer (BD Biosciences). Data was analyzed using CellQuest Pro Software (BD Biosciences).

Cytokine and chemokine assays

The levels of mouse TNF and IL-6, and human IFN-γ, IL-5, IL-8/CXCL8, IL-13, and TNF were determined by ELISA, as described in (22), except that Ultra-3, 3, 5, 5′-tetramethylbenzidine substrate (Pierce) was used as substrate. The following coating Abs were used: polyclonal goat anti-human IL-6 (clone MP5-20F3; BD Pharmingen), mouse-anti-human IFN-γ (clone NIB42; BD Pharmingen), mouse anti-human IL-5 (clone TRFK 5; BD Pharmingen), mouse anti-human IL-8 (clone 3IL8-H10; Endogen), mouse anti-human IL-13 (clone JES10-5A2; BD Pharmingen), and mouse anti-human TNF (clone 28401; R&D Systems). Recombinant mouse TNF and IL-6 and human IL-5 (BD Pharmingen), recombinant human IFN-γ, IL-13, and TNF (R&D Systems), and recombinant human IL-10 (Endogen) were used for standards. Biotinylated polyclonal goat anti-mouse TNF (R&D Systems), rat anti-mouse IL-6 (clone MP5-32C11; BD Pharmingen), mouse-anti-human IFN-γ (clone 4S.B3; BD Pharmingen), mouse anti-human IL-5 (clone JES1-5A10; BD Pharmingen), mouse anti-human IL-8 (clone 18-S2; Endogen), mouse anti-human IL-13 (clone B69-2; BD Pharmingen), and polyclonal goat anti-human TNF (R&D Systems) were used for detection. The sensitivity limits for quantitative determinations were 2 pg/ml for human TNF, 3 pg/ml for mouse IL-6, and human IL-5, IL-8/CXCL8 and IL-13, 6 pg/ml for human IFN-γ, and 10 pg/ml for mouse TNF.

The CBA system (BD Pharmingen) was also used for simultaneous detection of cytokines and chemokines, according to the manufacturer’s instructions. The CD200R regulates mouse peritoneal macrophage cytokine production. Resident murine peritoneal macrophages express the CD200R (Fig. 1a) and agonistic mCD200Ig or anti-mCD200R Ab (DX109) treatment inhibited IFN-γ-induced TNF secretion by these cells (Fig. 1b). Similar effects were observed when macrophages were enriched by removing nonadherent cells. In contrast, comparable LPS-stimulated TNF secretion was not affected by either agonist (Fig. 1b). Elevated TNF responses induced by higher LPS doses were also not inhibited by CD200R agonists (data not shown).

Human cytokines and chemokines (IL-1α, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-8/CXCL8, IL-10, IL-12p70, IL-13, IP-10/CXCL10, MCP-1/CCL2, MIP-1α/CCL3, RANTES/CCL5, TNF, and IFN-γ) were also analyzed using the LINCOpex System (Linco Research), according to the manufacturer’s instructions. Cytokine levels were quantitated using the LumineX 100 system. Values were expressed as picograms per milliliter deduced from the mean fluorescent intensity of the standard curves after subtracting the blanks, using a 5-parameter logistic algorithm with MasterPlex QT Quantitation Software version 2.0 (MiraiBio).

Gene expression analysis using TaqMan real-time quantitative PCR

Methods were essentially as described in Ref. 23.

Statistical methods

Comparisons between groups were assessed with the two-tailed unpaired t test. A p value of <0.05 was considered as statistically significant. Calculations were performed with a statistical package, StatView 5.0 for Macintosh (Abacus Concepts).

Results

The CD200R regulates mouse peritoneal macrophage cytokine production.

Resident murine peritoneal macrophages express the CD200R (Fig. 1a) and agonistic mCD200Ig or anti-mCD200R Ab (DX109) treatment inhibited IFN-γ-induced TNF secretion by these cells (Fig. 1b). Similar effects were observed when macrophages were enriched by removing nonadherent cells. In contrast, comparable LPS-stimulated TNF secretion was not affected by either agonist (Fig. 1b). Elevated TNF responses induced by higher LPS doses were also not inhibited by CD200R agonists (data not shown).

FIGURE 1. mCD200R agonists inhibit IFN-γ and IL-17-induced peritoneal cell cytokine secretion. a, Expression of mCD200R on resident peritoneal F4/80+ macrophages. Resident peritoneal cells were stained with PE-conjugated anti-F4/80 and biotinylated anti-mCD200R (DX109, solid line) or biotinylated isotype control Ab (dotted line), followed by allophycocyanin-conjugated streptavidin. Similar results were seen when macrophages were phenotyped as CD11b+ Gr-1low cells (data not shown). b, mCD200R agonists inhibit IFN-γ, but not LPS-induced, peritoneal cell TNF secretion (p < 0.01 for both agonists, unpaired t test). After a 30-min incubation with 3 μg/ml control Ig (gray striped bars), mCD200Ig (black striped bars), rat IgG1 isotype control (gray bars), or 0.02 ng/ml recombinant murine IFN-γ or 0.02 ng/ml Salmonella typhimurium LPS for 18 h, TNF secretion in supernatants was determined by ELISA. Results are presented as mean ± SEM of triplicate cultures and are representative of six independent experiments. <., Below limit of detection (10 pg/ml). c, mCD200R agonists inhibit IFN-γ and IL-17-induced peritoneal cell IL-6 secretion (p < 0.01 for both stimuli and agonists, unpaired t test). Cells were cultured as in b and IL-6 secretion determined by CBA. Results are presented as mean ± SEM of triplicate cultures and are representative of two independent experiments. <., Below limit of detection (20 pg/ml).
IFN-γ- and IL-17-induced IL-6 secretion was also inhibited by mCD200lg and anti-mCD200R Ab (Fig. 1c). CD200R inhibition of IFN-γ-induced activation was not universal, as MCP-1/CCL2 production was not affected (Fig. 1d). IL-17 did not induce detectable TNF and MCP-1/CCL2 production.

CD200R expression on human peripheral blood leukocytes

We then explored the role of CD200R in the human system. CD200R expression was first investigated on human blood cells. Fresh ex vivo CD11c⁺lin⁻ DCs expressed the highest CD200R levels, followed by CD14⁻ monocytes, CD4⁺ T cells, and CD8⁺ T cells (Fig. 2). B cells did not express CD200R (15). Monocyte-derived DCs also expressed high CD200R levels (Fig. 2), and slightly down-regulated CD200R expression after further maturation with CD40L or TNF and IL-1β (data not shown).

Inhibition of TT-induced cytokine secretion from PBMC by CD200R triggering

We next investigated whether Ag-induced cytokine responses, requiring cooperation between Ag-presenting monocytes and T cells, were modulated by CD200R agonists. Thus, PBMC from recently TT-vaccinated donors were pretreated with CD200R agonists before stimulation with TT. TT dose-dependently induced IL-5, IL-13, IFN-γ, and TNF (data not shown), but not IL-2, IL-4, IL-10, and IL-12p70 secretion (Fig. 3). CD200R agonists inhibited production of IL-5, IL-13, and IL-13, but only when the agonists were further cross-linked by F(ab')₂ goat anti-mouse/rat IgG (Fig. 3 and Table I). The effects on IFN-γ (Fig. 3c) and TNF (data not shown) were lesser and more variable. No effect of CD200R stimulation was observed at suboptimal TT doses. Data from four independent donors are summarized in Table I.

The inhibitory activity of CD200R agonists could be due to a direct or indirect effect on T cell activation, since both PBMC and monocyte-derived DCs expressed the highest CD200R levels. DCs were activated with LPS, CD40L and IFN-γ, or TNF and IL-1β. We analyzed expression of the cell surface markers CD1a, CD32, CD80, CD83, CD86, and HLA-DR, as well as secretion of IL-1α, IL-1β, IL-6, IL-8/CXCL8, IL-10, IL-12p70, IP-10/CXCL10, MCP-1/CCL2, MIG/CXCL9, MIP-1α/CCL3, RANTES/CCL5, and TNF. We did not observe any effect of CD200R agonists on these parameters (data not shown). CD200R agonists also had no effect on allo- or homologous DC-induced CD4⁺ T cell proliferation or CD4⁺ T cell cytokine production using TT-loaded DC (data not shown). It is likely that human DC will also be modulated through CD200R given the recent data showing effects of CD200lg on mouse DC function (24). However, to date, we have not identified the relevant in vitro conditions needed to observe CD200R-mediated modulation of human DC function.

Inhibition of hCD200R-transfected U937 cell cytokine production by CD200R agonists

Human monocytic U937 cells were transfected with hCD200R to further explore CD200R regulation of human myeloid cell activation. U937 cells did not express the receptor endogenously, but when infected with a retrovirus encoding hCD200R, they express CD200R at high levels (Fig. 5a). IFN-γ stimulation resulted in increased IL-8/CXCL8, IP-10/CXCL10, and MIG/CXCL9 secretion, whereas TNF, IL-1β, IL-6, IL-10, and IL-12p70 production was not detectable (data not shown). CD200R agonists dose-dependently inhibited IFN-γ-induced IL-8/CXCL8 (Fig. 5b), MIG/CXCL9, and IP-10/CXCL10 (data not shown) secretion from the
FIGURE 3. hCD200R agonists inhibit TT-induced PBMC cell IL-5 and IL-13 secretion. After a 30-min incubation with 10 μg/ml control Ig (gray striped bars), hCD200Ig (black striped bars), rat IgG1 isotype control (gray bars), or rat-anti-human CD200R (black bars), 1 × 10^6 PBMC were washed and the hCD200Ig or rat-anti-human CD200R were cross-linked with 5 μg/ml endotoxin-depleted F(ab')2 goat anti-mouse/rat IgG, and incubated for another 30 min before addition of 1, 10, or 100 ng/ml TT. Supernatants were harvested after 6 days. IL-5 (a), IL-13 (b), and IFN-γ (c) secretion was determined by ELISA. Results are presented as mean ± SEM of triplicate cultures and are representative of three to four independent experiments. Statistically significant inhibition of IL-5 and IL-13 secretion by hCD200R agonists was observed (p < 0.01 for both agonists and cytokines, unpaired t test).

Table I. hCD200R agonists inhibit tetanus toxoid-induced PBMC IL-5 and IL-13 secretion^a

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^a A total of 1 × 10^6 PBMCs were cultured in 1 ml of RPMI 1640 with 10% heat-inactivated FCS, 1% penicillin and streptomycin at 37°C in a humidified incubator containing 5% CO2. After a 30-min incubation with control-Ig, hCD200Ig, rat IgG1 isotype control, or rat-anti-human CD200R, cells were washed and the hCD200Ig or rat-anti-human CD200R were cross-linked with 5 μg/ml endotoxin-cleaned F(ab')2 goat anti-mouse/rat IgG, and incubated for another 30 min before addition of tetanus toxoid. Supernatants were harvested after 6 days. IL-5, IL-13, IFN-γ, or TNF secretion was determined by ELISA, CBA, or Luminex. Data from the optimal tetanus toxoid dose (10 ng/ml for donor 1 and 100 ng/ml for donors 2, 3, and 4) are presented as mean values picograms per milliliter of triplicate cultures.
As macrophages play pivotal roles in chronic inflammatory diseases through their broad proinflammatory, destructive, and remodeling capacities (1–3, 5), regulation of their function is critical for treatment of these disorders. One way of controlling inflammation is through blocking proinflammatory cytokines produced by macrophages, such as via anti-TNF therapy for rheumatoid arthritis, Crohn’s disease, and psoriasis (25). Triggering inhibitory receptors represents an alternative way of regulating myeloid cells (6), potentially superior to focused anti-cytokine therapies given the ability to affect a number of effector pathways via cellular

FIGURE 4. TT-induced IL-5 and IL-13 secretion is inhibited when the hCD200R is cross-linked on monocytes, but not when the hCD200R is cross-linked on CD4+ T cells. Monocytes and CD4+ T cells were isolated by MACS to >95% purity. After separate incubation of 250,000 monocytes/well and 750,000 CD4+ T cells/well with 10 μg/ml rat IgG1 isotype control (gray bars) or rat-anti-human CD200R (black bars), cells were washed and the rat-anti-human CD200R was cross-linked with 5 μg/ml endotoxin-cleaned F(ab’)2 goat anti-mouse/rat IgG, and incubated for another 30 min before addition of 100 ng/ml TT. IL-5 (a) and IL-13 (b), mean ± SEM of triplicate cultures, was determined by the LINCplex Simultaneous Multianalyte Detection System using a Luminex. <, Below limit of detection (3 pg/ml). Inhibition was only observed when hCD200R was cross-linked on monocytes, or on both monocytes and T cells or in PBMC (p < 0.01 for both agonists and cytokines in all three cultures, unpaired t test).

FIGURE 5. hCD200R agonists dose-dependently inhibit IFN-γ-induced hCD200R-transfected U937 cell IL-8/CXCL8 secretion. a, Expression of hCD200R on hCD200R- and vector control-transfected U937 cells. The transfected U937 cells were stained with biotinylated anti-hCD200R (DX136, solid line) or biotinylated isotype control Ab (dotted line), followed by allophycocyanin-conjugated streptavidin. b, hCD200R agonists inhibit IFN-γ-induced hCD200R-transfected U937 cell IL-8/CXCL8 secretion. After a 30-min incubation with control Ig (circles with no connecting line), hCD200lg (circles with connecting lines), rat IgG1 isotype control (squares with no connecting line), rat-anti-human CD200R (squares with connecting lines), rat IgG1 isotype control (squares with no connecting line), rat-anti-human CD200R (squares with connecting lines), 106 hCD200R-transfected U937 cells were stimulated with 100 U/ml recombinant human IFN-γ (filled symbols with solid connecting lines) or cultured in medium alone (open symbols with dashed connecting lines) for 18 h. IL-8/CXCL8 secretion in supernatants was determined by ELISA and is presented as mean ± SEM of triplicate cultures. Statistically significant inhibition (unpaired t test) was observed from 0.3 μg/ml hCD200lg and 0.1 μg/ml rat-anti-hCD200R.
targets. Many of these inhibitory receptors are members of the IgSF of integral membrane proteins. Data is now emerging that signaling through these receptors is essential for normal myeloid cell regulation in the peripheral immune system. Thus, disruption of signaling through the myeloid inhibitory receptors SIRPα and FcγRIIB leads to potentiation of cellular function with consequent effects on inflammatory disease processes (17, 26).

The CD200-CD200R interaction provides a new regulatory mechanism for myeloid cells. The IgSF glycoprotein CD200, formerly known as OX-2, is expressed widely, including on thymocytes, B cells, endothelial cells, smooth muscle cells, and neurons (6, 27). CD200R is more restricted and is predominantly expressed on myeloid cells and T cells (14, 15). CD200 deletion resulted in myeloid cell dysregulation and enhanced susceptibility to autoimmune inflammation such as EAE and CIA (10), suggesting that CD200 normally induces immune suppression through CD200R. Soluble CD200R protein, binding CD200 and preventing CD200-CD200R interactions, increased the incidence of CIA in normally resistant mice (10). Mice receiving soluble CD200 were conversely resistant to CIA induction (11).

One potential mechanism through which CD200 may control inflammation in vivo is via inhibition of TNF and IL-6 production following stimulation of mouse macrophages with IFN-γ, a very important activator of macrophage function (28–30). TNF plays a central role in autoimmune disease (25). TNF has several proinflammatory activities such as up-regulation of adhesion molecule expression, induction of cytokines and chemokines, angiogenesis and bone resorption (1, 25). IL-6 is the most strikingly elevated cytokine in human rheumatoid arthritis (1, 31), and it is required for experimentally induced autoimmune diseases, including CIA (32) and EAE (31, 33). Clinical trials with a humanized anti-IL-6R-antibody for treatment of rheumatoid arthritis and Crohn’s disease are ongoing (34). Proinflammatory effects of IL-6 include enhancing autoantibody production, bone absorption and triggering of acute phase responses (31), and blocking regulatory T cell suppression (35).

The observation that IL-17-induced IL-6 production was suppressed by CD200R agonists is also relevant to the in vivo mechanism of CD200R. This is the first report that IL-17 activates mouse macrophages, although enhancement of IL-6 production has been observed in human rheumatoid arthritis synovial cultures and human macrophages after IL-17 stimulation (36, 37). IL-17 is a T cell-derived cytokine that exhibits pleiotropic biological activities on various types of cells, such as fibroblasts, endothelial
monocytes, but not on CD4+ T cells. The inhibition of IL-5 and chemokine secretion, however, after stimulation of the cells with TT. It is possible that other aspects of DC activation may be affected (e.g., via indoleamine 2,3-dioxygenase-based effects (24)), or that the in vitro conditions do not permit effects of CD200R triggering (e.g., contact with other cells and extracellular matrix proteins present in the in vivo environment are absent).

Human hCD200R-expressing U937 cells were used to further explore the CD200R regulation of human myeloid cell activation. CD200R engagement inhibited IFN-γ-stimulated secretion of IL-8/CXCL8, IP-10/CXCL10, and MIG/CXCL9. Inhibition of cytokine production positively correlated with CD200R expression levels. Inhibition of low CD200R-expressing cells was only observed if the CD200R agonists were further cross-linked. This may explain why inhibition of TT-induced secretion required cross-linking of the CD200R. Inhibition of secretion of the chemotactic factors IL-8, IP-10, and MIG would reduce recruitment of neutrophils and Th1 cells to sites of inflammation (46).

In conclusion, we provide direct evidence in vitro that the CD200/CD200R interaction controls monocyte/macrophage function in both mice and humans and that the efficiency of CD200R-mediated inhibition of cellular functions is proportional to the receptor density at the cell surface. Our data solidify the concept that CD200R agonism may provide a novel approach to the treatment of chronic inflammatory diseases.

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References