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*J Immunol* 2004; 173:6786-6793; ;
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CD200 and its receptor CD200R are both type I membrane glycoproteins that contain two Ig-like domains. Engagement of CD200R by CD200 inhibits activation of myeloid cells. Unlike the majority of immune inhibitory receptors, CD200R lacks an ITIM in the cytoplasmic domain. The molecular mechanism of CD200R inhibition of myeloid cell activation is unknown. In this study, we examined the CD200R signaling pathways that control degranulation of mouse bone marrow-derived mast cells. We found that upon ligand binding, CD200R is phosphorylated on tyrosine and subsequently binds to adapter proteins Dok1 and Dok2. Upon phosphorylation, Dok1 binds to SHIP and both Dok1 and Dok2 recruit RasGAP, which mediates the inhibition of the Ras/MAPK pathways. Activation of ERK, JNK, and p38 MAPK are all inhibited by CD200R engagement. The reduced activation of these MAPKs is responsible for the observed inhibition of mast cell degranulation and cytokine production. Similar signaling events were also observed upon CD200R engagement in mouse peritoneal cells. These data define a novel inhibitory pathway used by CD200R in modulating mast cell function and help to explain how engagement of this receptor in vivo regulates myeloid cell function.

generated from a rat immunized with a fusion protein consisting of the extracellular domain of mouse CD200Rα fused to the Fe region of human IgG1 as previously described (4). Cross-linking CD200Rα with DX89 induces a strong dose-dependent degranulation response in mouse mast cells (data not shown). In the present study, mAb DX89 was used to activate mouse mast cells because the IgM isotype (Iso) did not require additional cross-linking reagents to activate the receptor and did not bind FcRs for IgG.

Cell culture, gene transduction, and flow cytometry

Mouse BMMs were generated from bone marrow of 2- to 3-week-old C57BL/6 mice as previously described (4). Mast cells overexpressing CD200R and CD200Rα (DT733) were generated by retroviral transduction of BMsCs. A cDNA containing the CD8 leader segment followed by the Flag epitope tag (DYKDDDDK) and joined to the extracellular, transmembrane and cytoplasmic domains of mouse CD200Rα was cloned into the retroviral vector pMXneo. The resultant construct was then introduced into the mast cell line H9262 by transfection as described above. Cells were sorted for cell surface CD200R expression using the anti-Flag Ab M2 (Sigma-Aldrich, St. Louis, MO). A cDNA containing the CD8 leader segment followed by the ε7-myosin epitope tag (EQQKLISEEDL) and joined to the extracellular, transmembrane and cytoplasmic regions of mouse CD200Rα were subcloned into the retroviral vector pMXneo. The resultant construct was then introduced into the mast cell line H11003 by retroviral transduction as described above. Cells were sorted for cell surface CD200R expression using the anti-Myc Ab 9E10 and subsequently verified for gene expression using anti-CD200R mAb. Cells ≥95% positive and having similar levels of expression for both CD200R and CD200Rα were used for the biochemical analyses and degranulation assays as described below.

NIH3T3 mouse fibroblast cells expressing the membrane form of mouse CD200Rα tagged by a retargeting sequence. The surface expression of CD200 was determined by flow cytometry using PE-conjugated rat anti-mouse CD200 (clone MR6:OX90; Serotec, Oxford, U.K.) Abs. The negative expressing cells were sorted and used as negative control. The parental NIH3T3 and transductants were cultured in six-well plates and incubated with either BMSCs or DT733 cells upon confluence. The activation of CD200R was determined as described below.

Mouse peritoneal cells were isolated from 8- to 10-week-old C57BL/6 mice by standard protocol (17). Cells (4 × 10^7 cells/ml) were stimulated with control mIg or CD200-mIg (10 μg/ml) for 10 min, and then immunoprecipitation and Western blot were done as described below.

Surface expression of CD200R and CD200Rα was analyzed by standard flow cytometry techniques. In brief, mast cells were washed once with PBS and stained with either FITC-conjugated anti-CD200R (DX109, rat IgG2b,κ), anti-CD200Rα (DX157, rat IgG2a,κ) Abs. After incubation at 4°C for 20 min, cells were washed twice in PBS with 0.5% BSA and analyzed on a FACScan flow cytometer (BD Biosciences, San Jose, CA).

Degranulation assays and cytokine ELISA

Mast cell degranulation was determined using a β-hexosaminidase release assay as previously described. The degranulation was triggered by incubating mast cells with indicated amounts of DX89 Ab for 1 h in RPMI 1640 medium in 96-well plates. Supernatants were assayed for β-hexosaminidase activity. For CD200-mediated inhibition, the cells were pretreated with indicated amounts of control mIg or CD200-mIg for 30 min before activation. For FcR blockage experiments, cells were pretreated with the FcR-blocking Ab DT733 for 10 min and then incubated with 50 μl of avidin-agarose beads at 4°C for 1 h, followed by incubation with 50 μl of avidin-agarose beads at 4°C for 30 min. The protein complexes were washed extensively and subjected to PAGE and Western blotting as described above.

Ras activation assay

Ras activation was measured using a Ras activation assay kit (Upstate Biotechnology) according to the manufacturer’s instructions. Briefly, after stimulation, cells were lysed and GTP-bound Ras was pulled down by GST fusion protein containing the Ras-binding domain of Raf-1 bound to glutathione-agarose. The precipitated Ras-GTP was detected by Western blot.

Results

CD200R engagement inhibits mast cell activation

CD200R is highly expressed on myeloid cells such as mast cells, macrophage, and neutrophils (4). As reported elsewhere, engagement of the mouse CD200R by agonist Abs or ligand results in a potent inhibition of mast cell degranulation and cytokine secretion in mouse mast cells overexpressing CD200R. To study the molecular mechanisms of CD200 inhibition, mouse mast cells were generated from C57BL/6 bone marrow. As shown in Fig. 1A, these BMSCs expressed relatively high level of CD200R and CD200Rα. Engagement of the CD200R by soluble CD200-mIg, however, did not inhibit mast cell degranulation induced by the activating Ab DX89 (Fig. 1B). To increase the sensitivity of the assay, mast cells were induced to overexpress CD200R and CD200Rα by retroviral gene transfer. As shown in Fig. 1, DT733 cells expressed higher levels of both CD200R and CD200Rα. Engagement of the CD200R by soluble CD200-mIg, however, did not inhibit mast cell degranulation induced by the activating Ab DX89 (Fig. 1B). To increase the sensitivity of the assay, mast cells were induced to overexpress CD200R and CD200Rα by retroviral gene transfer. As shown in Fig. 1, DT733 cells expressed higher levels of both CD200R and CD200Rα. Engagement of the CD200R by soluble CD200-mIg, however, did not inhibit mast cell degranulation induced by the activating Ab DX89 (Fig. 1B). To increase the sensitivity of the assay, mast cells were induced to overexpress CD200R and CD200Rα by retroviral gene transfer. As shown in Fig. 1, DT733 cells expressed higher levels of both CD200R and CD200Rα. Engagement of the CD200R by soluble CD200-mIg, however, did not inhibit mast cell degranulation induced by the activating Ab DX89 (Fig. 1B).
were used subsequently in all studies to dissect the molecular mechanisms of CD200R signaling.

**CD200R is tyrosine phosphorylated upon CD200 binding**

Unlike the majority of inhibitory receptors, CD200R lacks an ITIM, but contains three tyrosine residues Y286, Y289, and Y297 in the cytoplasmic domain of mouse receptor. Sodium pervanadate pretreatment of cells expressing CD200R has been shown to result in CD200R tyrosine phosphorylation (2). To determine whether engagement of CD200R by its ligand induced CD200R phosphorylation, we treated DT733 cells with CD200-mIg and examined the phosphorylation of CD200R. As shown in Fig. 2A, CD200 stimulation induced strong tyrosine phosphorylation of CD200R, which was detected as early as 1 min after receptor engagement, and CD200R remained phosphorylated for >30 min. The same treatment in BMMCs did not result in significant phosphorylation of CD200R. However, cross-linking the CD200-mIg with a secondary goat anti-mouse Ab induced the receptor phosphorylation (data not shown).

Since CD200 is a transmembrane protein, we then examined whether the membrane form of CD200 could induce the receptor phosphorylation in both BMMCs and mast cells overexpressing CD200R. NIH3T3 cells expressing the membrane form of mouse CD200 were generated by retroviral transduction. The CD200-negative expressing cells were sorted and used as negative control. As shown in Fig. 2B, NIH3T3 cells expressed low levels of endogenous CD200, and CD200 was highly expressed on the transductants. Incubation of mast cells overexpressing CD200R with CD200-expressing NIH3T3 cells resulted in strong tyrosine phosphorylation of CD200R (Fig. 2C). The weak phosphorylation of CD200R induced by NIH3T3 cells was due to the low level expression of CD200 in these cells. Although soluble CD200-mIg fusion protein did not induce tyrosine phosphorylation of CD200R in low-expressing BMMCs, the membrane form of CD200 expressed on NIH3T3 cells was able to trigger the CD200R phosphorylation in BMMCs. These results suggest that membrane CD200 would achieve natural cross-linking of the CD200R.

**Dok1 and Dok2 are tyrosine phosphorylated upon CD200R engagement and associate with Ras-GAP, SHIP, and CD200R**

CD200R cytoplasmic domain has a potential PTB motif, NPXY297. To test which proteins may bind to CD200R, we made synthetic biotinylated peptides encompassing the three tyrosine residues: Y286, Y289, and Y297, which were phosphorylated in all possible combinations (Fig. 3A). After stimulation with control mIg or CD200-mIg, the cells were lysed and cell lysates were precleared with avidin-agarose beads, then incubated with each peptide. The protein complexes were pulled down by avidin-agarose beads and subjected to PAGE and Western blotting analysis. As shown in Fig. 3B, peptides 4 and 6 pulled down two clearly phosphorylated proteins with a molecular weight of 60 and 50

**FIGURE 1.** Expression of CD200R and CD200RLa and degranulation in mouse mast cells. A, Expression of CD200R and CD200RLa on mouse mast cell BMMCs and DT733 as analyzed by flow cytometry. B, Inhibition of mast cell degranulation by CD200-mIg (CD200), as compared with control mlg fusion protein (C). The degranulation was measured as described in Materials and Methods. Results are expressed as the mean of triplicates from one of three experiments.

**FIGURE 2.** CD200 induced its receptor tyrosine phosphorylation. A, DT733 cells were stimulated with control mlg (C) at 3 µg/ml for 1 min or CD200-mlg (CD200) at 3 µg/ml for indicated time periods. CD200R was immunoprecipitated from cell lysates using a rat anti-CD200R Ab and immunoblotted with anti-phosphotyrosine Ab (pY). The same membrane was stripped and reblotted with anti-CD200R Ab. B, Surface expression of CD200 on NIH3T3 (3T3), CD200-negative NIH3T3 (CD200−), and CD200-overexpressing NIH3T3 (CD200+) cells was analyzed by flow cytometry. The cells were stained for anti-CD200 Ab (bold line) or an Iso control Ab (dotted line). C, NIH3T3 (3T3), CD200-negative NIH3T3 (CD200−), or CD200-overexpressing NIH3T3 (CD200+) cells were incubated with either BMMCs or DT733 cells at 37°C for 10 min. The phosphorylation of CD200R was determined as described above.

**FIGURE 3.** Peptides containing tyrosine residues Y286, Y289, and Y297 were phosphorylated in all possible combinations (A). After stimulation with control mIg or CD200-mIg, the cells were lysed and cell lysates were precleared with avidin-agarose beads, then incubated with each peptide. The protein complexes were pulled down by avidin-agarose beads and subjected to PAGE and Western blotting analysis (B).
were not detected, suggesting that the binding to these peptides is specific for Dok and Shc proteins. Interestingly, unlike Dok1, Dok2 weakly bound to peptide 7, whereas Shc bound equally to peptides 4, 6, and 7. The binding to peptides 4 and 6 did not require phosphorylation of Doks and Shc since peptide 4 pulled down equal levels of Dok1, Dok2, and Shc from control mlg and CD200-mlg stimulated cells. However, phosphorylation of Doks and Shc only occurred after stimulation with CD200-mlg. These results clearly indicated that phosphorylation of Y297 on CD200R was required for the binding of Dok1 and Dok2 to the receptor.

To verify the peptide binding results in intact cells, mast cells were stimulated with control mlg or CD200-mlg at 37°C for indicated time periods. Dok1, Dok2, Shc, and SHIP were then immunoprecipitated from the cell lysates and separated by 8% Nu-PAGE, transferred to polyvinylidene difluoride membranes, and blotted with anti-phosphotyrosine Ab. As shown in Fig. 4A, triggering CD200R resulted in strong tyrosine phosphorylation of both Dok1 and Dok2. The membrane was then stripped and rebotted for associated CD200R, RasGAP, and SHIP. As seen in Fig. 4A, after CD200R engagement, phosphorylated Dok1 and Dok2 were associated with CD200R and RasGAP. Interestingly, Dok1, but not Dok2, was also found to bind tyrosine-phosphorylated SHIP. The binding was transient and only induced by CD200 stimulation. CD200-triggered mast cells were also immunoprecipitated for Shc and SHIP. As shown in Fig. 4B, CD200R stimulation slightly increased the tyrosine phosphorylation of SHIP and induced the association of SHIP with phosphorylated Dok1. Unlike Dok proteins, the phosphorylation state of Shc was not changed after CD200R engagement, and we could not detect association of CD200R with Shc or SHIP. These results showed that upon CD200R engagement, phosphorylated CD200R binds to adapter proteins Dok1 and Dok2, which in turn recruit RasGAP and SHIP.

**Engagement of CD200R inhibited activation of Ras/MAPK pathways**

Dok proteins have been shown to mediate inhibitory signaling by recruiting inhibitory effectors such as RasGAP, SHIP, and Csk.
(18–23). Association of Dok-1 with RasGAP has been shown to attenuate Ras activity, leading to the inhibition of downstream MAPK pathways in B cells and mast cells (18, 23). The association of Dok proteins with CD200R and RasGAP suggested a potential inhibitory pathway for CD200 signaling. To investigate this potential inhibitory pathway, we first examined whether CD200R triggering inhibited MAPK activation. Normally growing IL-3-dependent DT733 cells were incubated with either control mlg or CD200-mlg for various periods of time. Equal amounts of cell lysates were immunoblotted with anti-phospho ERK Ab that recognizes the dual-phosphorylated ERK1 and ERK2. As shown in Fig. 5A, CD200-mlg strongly inhibited constitutively activated ERKs which were induced by IL-3 in the culture medium and the inhibition occurred within 1 min after CD200R triggering. Control mlg had no effect even after a 30-min incubation (data not shown).

Experiments were then performed to determine whether triggering CD200R would inhibit activation of Ras/MAPK pathways induced by DX89 Ab. Mast cells overexpressing CD200R were factor starved for 16 h in medium without IL-3 or serum. After pretreatment with control mlg or CD200-mlg, the cells were stimulated with DX89 and the activation of ERK, p38, JNK, and Ras was examined. Activation of mouse mast cells via the DAP12 pairing receptor CD200RLa caused a pronounced phosphorylation of ERK, p38, and JNK that was directly associated with a potent degranulation response. Preincubation of mast cells with CD200-mlg markedly reduced the activation-dependent phosphorylation of ERK, p38, and JNK (Fig. 5B). Consistent with the dephosphorylation of ERK, CD200R triggering also significantly reduced Ras activation (Fig. 5C). This was not unique to the CD200RLa activation process, because activation of ERK and p38 MAPK induced by aggregation of FceRI was also inhibited by CD200R engagement (data not shown).

Since mast cell degranulation and cytokine secretion are dependent on both ERK and p38 MAPK activation (24–26), we then tested whether inhibition of these two pathways by pharmacological inhibitors would recapitulate the inhibition seen with CD200R engagement. The cells were pretreated with U0126 (a MEK inhibitor) and SB203580 (a p38 MAPK inhibitor) and then stimulated with DX89. Activation of MAPKs, degranulation response, and cytokine production were then measured. As shown in Fig. 6A, U0126 strongly inhibited the activation of ERK, JNK, and p38 MAPK while SB203580 only inhibited the activation of p38 MAPK and JNK. Pretreatment with either U0126 or SB203580 inhibited mast cell degranulation and the secretion of TNF and IL-13 (Fig. 6B and C). Interestingly, U0126 was more potent than SB203580 to inhibit degranulation and cytokine production. This correlated with its broad and potent inhibition of MAPKs. These results are consistent with the hypothesis that the activation-dependent phosphorylation of CD200R recruits phosphorylated Dok proteins which subsequently bind RasGAP and SHIP. The incorporation of RasGAP and SHIP into the CD200R complex leads to downstream inhibition of the Ras/MAPK pathways and a functional reduction in mast cell degranulation and cytokine secretion.

**Dok1, and Dok2 are tyrosine phosphorylated upon CD200R engagement in mouse peritoneal cells**

To confirm our findings using mouse mast cells in cells expressing normal CD200R ex vivo, we examined whether CD200 stimulation induced Dok phosphorylation in primary mouse peritoneal cells. These cells mainly contain macrophages and mast cells, both of which express CD200R. Resting mouse peritoneal cells were isolated from C57BL/6 mice. After stimulation with control mlg or CD200-mlg, Dok1 and Dok2 were immunoprecipitated from the cell lysates and separated by 8% Nu-PAGE, transferred to polyvinylidene difluoride membranes, and blotted with anti-phosphotyrosine Ab. As shown in Fig. 7, triggering CD200R resulted in tyrosine phosphorylation of Dok1 and Dok2. These results provide direct evidence that CD200R engagement leads to the phosphorylation of Dok1 and Dok2 in primary peritoneal myeloid cells.

**Discussion**

CD200R is an inhibitory receptor expressed highly on myeloid cells and some T cells (4). Most inhibitory receptors on immune cells contain a consensus amino acid sequence, termed the ITIM, in the cytoplasmic domain (9–11, 27). The prototype ITIM consists of the sequence (I/V/L/S)-x-Y-x-x-(L/V), where x denotes any amino acid. Ligand-induced clustering of these ITIM-containing receptors results in tyrosine phosphorylation, often by a Src family kinase, which provides a docking site for the recruitment of tyrosine phosphatases SHP1 (and occasionally SHP2) and the inositol phosphatase SHIP. As defined by sequences in their extracellular domain, ITIM-bearing receptors belong to either the Ig
superfamily or C-type lectin superfamily. By recruiting phosphatase SHP1 and/or SHIP, ITIM-bearing inhibitory receptors suppress cell activation by promoting dephosphorylation reactions. Unlike other myeloid inhibitor receptors, such as FcγRIIB (28, 29), gp49B1 (30, 31), paired Ig-like receptor (32, 33), and mast cell function-associated Ag (34, 35), CD200R lacks an ITIM, but contains three tyrosine residues in the cytoplasmic domain. One tyrosine (Y297) is located in a NPxY motif, which may represent a potential PTB domain protein-binding motif. Engagement of CD200R by soluble CD200-mIg fusion protein resulted in rapid CD200R tyrosine phosphorylation in mouse mast cells overexpressing CD200R, but not in wild-type BMMCs. This result combined with the degranulation response as shown in Fig. 1B clearly show that the CD200R density on mast cells determines the threshold of inhibition. In BMMCs, CD200R have to be cross-linked with a secondary Ab to trigger the receptor and inhibit cell activation. Since CD200 is naturally expressed as a membrane protein, the cell surface density of the ligand and its presentation to the CD200R may be different from the soluble form in that the membrane-bound CD200 will achieve a high degree of natural cross-linking of the CD200R. Our results support this hypothesis because the membrane form of CD200 expressed on NIH3T3 cells was able to trigger the CD200R phosphorylation in both BMMCs and mast cells overexpressing the CD200R (Fig. 2C).

Because CD200R could be tyrosine phosphorylated upon ligand engagement, this suggests that it may bind to downstream target proteins, in particular, the PTB domain proteins. To determine whether the NPxY motif was functional and which PTB domain proteins may bind to CD200R, we made biotinylated peptides encompassing the three tyrosine residues (Y286, Y289, and Y297), which were phosphorylated in all possible combinations, and performed the in vitro-binding assay. Among the known PTB domain proteins, Dok1, Dok2, and Shc were found to bind to the peptide in which Y297 was phosphorylated. IRS-1, another PTB domain protein, does not bind to any peptide in the experiment, suggesting that the binding is specific for Dok and Shc proteins. We were also unable to detect the binding of SHIP and SHP1 to any of the phosphorylated peptides. Interestingly, peptide 8, in which all three tyrosines are phosphorylated, did not bind Dok1, Dok2, or Shc. Phosphorylation of all three tyrosines may create steric hindrance, thus preventing the binding of Dok and Shc. The binding of Dok1 and Dok2 to CD200R was confirmed in mouse mast cells. CD200 stimulation of mouse mast cells induced tyrosine phosphorylation of Dok1 and Dok2 and their subsequent association with CD200R. Not only do Dok1 bind to CD200R after receptor stimulation, they also recruit RasGAP. Interestingly, Dok1 but not Dok2 also binds to phosphorylated SHIP after CD200R stimulation. Unlike Dok, the phosphorylation of Shc did not change following CD200 stimulation, and we could not detect the association of Shc with CD200R in intact cells, even though Shc was found to bind phosphorylated peptides in the in vitro-binding assay. These results show that upon ligand binding CD200R specifically recruits Dok1 and Dok2 to the receptor complex which in turn binds RasGAP and SHIP. We are currently examining which kinase mediates phosphorylation of CD200R and Dok1. Considering the role of Src family kinases Lyn and Fyn in mast cell activation (36–38),
they may be involved in the phosphorylation of CD200R and Dok.

Among the PTB domain proteins, Dok proteins have been shown to mediate inhibitory signaling (18–23). The Dok family comprises five known members; Dok-1, Dok-2 (also termed Dok-R and FRIP), Dok-3 (also named Dok-L), Dok-4, and Dok-5 (22, 39–42). These molecules contain an amino-terminal pleckstrin homology domain, a central PTB domain, and a carboxy-terminal region with multiple potential tyrosine phosphorylation sites and proline-rich regions, which may serve as docking sites for Src homology 3 domains. Dok proteins undergo tyrosine phosphorylation in response to a variety of stimuli, such as immunoreceptor ligation, growth factors, and cytokines. This phosphorylation triggers Src homology 2 domain-mediated interactions with inhibitory effectors including RasGAP, SHIP, and Csk. In B cells, both Dok-1 and Dok-3 undergo rapid tyrosine phosphorylation in response to B cell receptor engagement and negatively regulate cell activation (18, 22). Dok1 has been shown to inhibit MAPK activation and cell proliferation upon coaggregation of B cell receptor and FcγRIIB (18, 21). Dok2 negatively regulates T cell development by recruiting RasGAP and Nck (43). In mast cells, co-cross-linking of FcγRIIB with FcεRI stimulates Dok1 tyrosine phosphorylation and association with SHIP and RasGAP (23). Overexpression of Dok1 in the mast cell line RBL-2H3 inhibited FcεRI-mediated Ras/Raf1/ERK signaling and the de novo synthesis of TNF-α (44). These studies have established that Dok family proteins are inhibitory adaptor molecules, presumably due to their ability to recruit inhibitory effectors RasGAP, SHIP, and Csk. Our results show that CD200R also binds to Dok1 and Dok2, which then recruit RasGAP and SHIP.

Like other inhibitory receptors such as FcγRIIB, gp49B1, paired Ig-like receptor β, and mast cell function-associated Ag in mast cells, CD200R engagement inhibits mast cell degranulation and cytokine production. This inhibition is likely mediated by the reduced activation of MAPKs: ERK, p38 MAPK, and JNK, since CD200R engagement inhibited activation of all three MAPKs. Unlike the majority of myeloid inhibitory receptors, the inhibition does not require co-cross-linking of CD200R with an activating receptor because CD200 ligand alone could inhibit the activation of ERK1/2 induced by IL-3. This suggests that CD200R is a novel inhibitory receptor that employs recruitment of RasGAP to directly inhibit Ras activation. However, other molecules may also be involved in the inhibition because CD200 also inhibits p38 MAPK and JNK activation which do not depend on Ras. Our results suggest a novel inhibitory pathway used by CD200R in modulating myeloid cell function (Fig. 8). That this pathway is triggered and active without need for co-cross-linking to an activating receptor provides a unique opportunity for the use of CD200R as an anti-inflammatory target in vivo.3

In conclusion, we have shown that Dok1 and Dok2 are mediators of CD200R inhibitory signaling. They both bind to RasGAP, leading to the inhibition of Ras and downstream ERK activation. It is possible that other molecules may also be involved in the inhibition because CD200 also inhibits p38 MAPK and JNK activation which do not depend on Ras. Our results suggest a novel inhibitory pathway used by CD200R in modulating myeloid cell function (Fig 8). That this pathway is triggered and active without need for co-cross-linking to an activating receptor provides a unique opportunity for the use of CD200R as an anti-inflammatory target in vivo.

Acknowledgments
We thank Mike Bigler and Yaoli Song for assistance and Janet Wagner and Sandra Zurawski for making the fusion proteins and mAbs. We also thank Maria Jenmalm for help and discussion.

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