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Down-Regulation of Basophil Function by Human CD200 and Human Herpesvirus-8 CD200

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Human and rodent CD200 are recognized by the inhibitory CD200R, and these molecules play an important role in the regulation of the immune system. Several viruses, such as human herpesvirus-6 (HHV-6), HHV-7, and HHV-8, possess a CD200 homologue, suggesting that these viruses regulate the immune response via CD200R. In this study, we analyzed the effect of human CD200 and the viral CD200 homologues on human CD200R-expressing cells. We found that human CD200R is predominantly expressed on basophils in amounts higher than on other human peripheral blood leukocytes. Furthermore, the viral CD200 homologues as well as human CD200 were recognized by human CD200R, and the activation of basophils was down-regulated by these CD200 proteins. These results suggested that CD200R is an important regulatory molecule of basophil activation. In addition, the presence of CD200 homologues on several viruses suggests a potentially unique relationship between basophil function and viral infection. The Journal of Immunology, 2005, 175: 4441–4449.

Immune cells express inhibitory receptors to prevent damage to self. Many of the inhibitory receptors recognize broadly expressed self-proteins, such as MHC class I (1, 2). CD200R (3) is an inhibitory receptor that possesses a tyrosine phosphatase-recruiting inhibitory motif in its cytoplasmic domain (3, 4). CD200R is primarily expressed on leukocytes of the myeloid lineage, and it recognizes CD200, which is broadly expressed on a variety of cell types (3, 5, 6). In mice, disruption of the gene encoding CD200 led to expansion of the macrophage and granulocyte populations in the spleen and the macrophage population in mesenteric lymph nodes (7). Furthermore, CD200-deficient mice showed rapid onset of experimental autoimmune encephalomyelitis (7). Administration of CD200-Ig fusion protein, which functions as an agonist for the inhibitory CD200R, suppressed allograft rejection and collagen-induced arthritis in animal models (8, 9). These data suggested that recognition of CD200 by an inhibitory CD200R plays an important role in regulating immune responses.

Several viruses that persistently infect the host have acquired ligands for inhibitory receptors, presumably to suppress an immune response against these pathogens. For example, murine CMV (MCMV) has evolved m157, which functions as a ligand for an inhibitory Ly49 receptor in certain MCMV-susceptible mouse strains (10). Similarly, CMV acquired UL18 as a ligand for the inhibitory CD85J (leukocyte inhibitory receptor/ Ig-like transport) receptor (11). These decoy ligands for inhibitory receptors may play an important role, allowing the viruses to evade the immune system and potentially cause persistent infectious diseases (12). Interestingly, herpesviruses, such as human herpesvirus-6 (HHV-6), HHV-7, and HHV-8 (13–15), and poxviruses, including myxoma virus and shope fibroma virus, possess genes encoding proteins with homology to CD200 (16, 17). Although amino acid homologies between human CD200 and these viral CD200 homologues are <40%, this level of similarity is sufficient to permit binding to the inhibitory CD200R. In fact, the CD200 homologue of HHV-8 (HHV-8 CD200) has been described as a decoy ligand for CD200R (18, 19). However, the effect of the HHV-8 CD200 on CD200R-expressing human cells has remained unclear, and there are conflicting reports in the literature (18, 19).

In the present study we show that basophils are a major CD200R-positive population in human peripheral blood. Furthermore, we show that not only the HHV-8 CD200, but also the CD200 homologues of HHV-6 (HHV-6 CD200) and HHV-7 (HHV-7 CD200), are recognized by CD200R and that activation of basophils is down-regulated by recognizing human CD200 and HHV-8 CD200. These findings suggest that CD200R regulates basophil activation, and the viral CD200 homologues are involved in the regulation of basophil-dependent immune responses.

Materials and Methods
cDNA preparation

CD200 homologues of HHV-7 (U85) and HHV-8 (K14) were cloned using PCR from genomic DNA of HHV-7 and HHV-8 (provided by Dr. L. Coscoy, University of California, Berkeley, CA). The CD200 homologue of HHV-6 (U85) was cloned using PCR directly from supernatant containing HHV-6 virus (strain SF; provided by Dr. L. Coscoy). The GenBank/EMBL/DDBJ accession numbers of HHV-6, HHV-7, and HHV-8 are AB190768, HHU43400, and AF367765, respectively. Human CD200 and CD200R were cloned using PCR from cDNA generated from human PBMC-derived poly(A) RNA. Mouse CD200R was cloned from cDNA generated from mouse spleen-derived poly(A) RNA.
Ig fusion proteins
cDNA fragments corresponding to the extracellular domains of human CD200R (aa residues 25–267), mouse CD200R (aa residues 26–238), human CD200 (aa residues 56–263), and HHV-8 CD200R (aa residues 25–267) were cloned into a unique XhoI site of a modified pBE18S expression vector carrying the human CD150 leader segment and the 5′-portion of a human IgG1 (20). COS-7 cells were transiently transfected by using 293fectin (Invitrogen) with these expression vectors to generate Ig fusion proteins. After 72 h, the culture supernatants were collected, and the amounts of Ig fusion protein were measured using standard ELISA methods. For some experiments, human CD200-Ig and HHV-8-CD200-Ig fusion proteins were further purified using protein A-coupled Sepharose columns (Amersham Biosciences) by standard methods.

Cells and transfectants
FLAG-tagged human CD200, HHV-6-CD200, HHV7-CD200, and HHV-8-CD200 were transfected into Ba/F3 cells using the pMX-neo retrovirus vector, which contains a human CD8α signal sequence and a FLAG tag at the N terminus of the mature protein (20). These transfectants were stained using anti-FLAG mAb (clone M2; Sigma-Aldrich), and FLAG-positive cells were isolated using a MACS purification system (Miltenyi Biotec). Human CD200- and HHV-8-CD200-expressing cells were transfected into 721.221 cells using the pMX-ires-GFP retrovirus vector, and GFP-positive cells were purified using a flow cytometer (FACSVantage). BC1, a human primary effusion lymphoma cell line harboring HHV-8, was provided by Dr. K. Ueda (Osaka University, Osaka, Japan). We recloned BC1 cells and selected cells that were highly stained with human NK cell line NKL (21) using the pMx-puro retrovirus vector, and cells and GFP-positive cells were purified using a flow cytometer (FACSVantage; BD Biosciences). Human CD200R was also transfected into the human NK cell line NKL (21) using the pMX-puro retrovirus vector, and cells stained by human CD200-Ig fusion protein were purified using flow cytometry (FACSVantage). BC1, a human primary effusion lymphoma cell line harboring HHV-8, was provided by Dr. K. Ueda (Osaka University, Osaka, Japan). We recloned BC1 cells and selected cells that were highly stained with human CD200R-Ig fusion protein after induction of lytic reactivation by PMA (20 ng/ml) for 24 h. All cells were cultured in RPMI 1640 medium containing 10% FCS, except for NKL cells, in which case 400 U/ml human IL-2 (PeproTech) was added to the culture medium.

Human basophils used for the degranulation assay were purified from EDTA-treated (to prevent coagulation) venous blood using Percoll density gradient centrifugation (Amersham Biosciences) (22). Basophils were further purified using a MACS basophil isolation kit (Miltenyi Biotec) for measurement of CD200R expression. The purities of Percoll- and MACS-purified basophils determined by Alcian blue staining (23) were 8–30 and 95%, respectively. Human CD3-, CD14-, CD19-, and CD56-positive lymphocytes were purified from human PBMC using a MACS purification system (Miltenyi Biotec), and the purity of each population was >90%.

Flow cytometry
Cells were incubated with saturating concentrations of various Ig fusion proteins or mAbs for 30 min on ice, followed by incubation with F(ab′)2 of PE-conjugated goat anti-human IgG or anti-mouse IgG (Jackson ImmunoResearch Laboratories) for 30 min. For analyses of CD200R expression on human PBMC, we generated a PE-labeled Ig fusion protein complex by mixing CD200-neutralizing Ig (25) were used as templates. Real-time PCR analysis was performed using a SYBR Green PCR kit (Applied Biosystems) and an ABI PRISM 7900 HT instrument (Applied Biosystems). Primers used for amplification were as follows: β-actin: sense primer, 5′-TCTACAAAT
GAGCTGCGTGTTG-3′; antisense primer, 5′-CGTAGATGGGGCAGACAGTTGG-3′; GAPDH: sense primer, 5′-ATGCGTGGCGTCTAGTCTC-3′; antisense primer, 5′-CAGGGGTGCTAACATGTGGTGT-3′; and human CD200R: sense primer, 5′-CTTCCGTGTTCCAGTGTCGACA-3′; antisense primer, 5′-GCTCCATGCTGCTCCTTTG-3′. β-Actin and GAPDH were used to standardize the relative amounts of CD200 transcripts in the different cell populations. Comparable results were obtained when either β-actin or GAPDH was used for normalization of the data.

Histamine release assay
Histamine release from basophils was analyzed as previously described (22). Purified basophils (2.0–4.0 × 10^6) were incubated with 10 μg/ml human CD200-Ig, HHV-8-CD200-Ig, or control-Ig fusion proteins in PIPES-Albumin (PIPES-A) buffer containing 25 mM PIPES, 119 mM NaCl, 5 mM KCl, and 0.03% human serum albumin (pH 7.4) for 30 min on ice. Cells were then washed in ice-cold PIPES-A and incubated with 5 μg/ml F(ab′)2 of goat anti-human IgG Fcγ Ab (Jackson ImmunoResearch Laboratories) in PIPES-A for 30 min on ice. Basophils were then washed in ice-cold PIPES-A, resuspended at 4.0–8.0 × 10^6 basophils/ml in PIPES-A buffer containing 2 mM Ca^{2+} and 0.5 mM Mg^{2+} (PIPES-ACM), and stimulated with 1 μg/ml mouse anti-human FcεRI mAb (CRA1, Kyokuto Pharmaceutical Industry) or 300 pM human rIL-3 (donated by Kirin Brewery) for 45 min at 37°C. For coculture experiments, purified basophils (2.0–4.0 × 10^6) were suspended with 1 × 10^5 of mock-transfected or CD200-transfected 721.221 cells in PIPES-ACM and were cen-
trifuged at 4°C, followed by incubation for 30 min on ice. Cells were then resuspended at 4.0–8.0 × 10^6/ml in PIPES-ACM containing 1 μg/ml mouse anti-human FcεRI mAb or 300 pM human rIL-3 and then cen-
trifuged at 4°C, followed by incubation for 45 min at 37°C. The concentration of histamine in supernatants was measured using an automated fluorometric analyzer. Histamine release was calculated as a percentage of the total histamine content, as previously described (26).

Cytotoxic assays
51Cr-labeled, mock-transfected or CD200-transfected 721.221 cells (1 × 10^6/well) were cocultured with various numbers of mock-transfected or CD200-transfected NKL cells in 96-well, round-bottom plates for 4 h. For Ab-blocking experiments, CD200-transfected 721.221 cells were incu-
bated with anti-human CD200 mAb (MRC OX-104; BD Pharmingen) or an isotype-matched control mAb for 30 min on ice before the coculture with effector cells. Specific lysis (percentage) was analyzed by 51Cr release assays using standard methods (20).

Cytokine production
We cocultured 3 × 10^6 mock-transfected or CD200R-transfected NKL cells with various numbers of mock-transfected or CD200-transfected 721.221 cells in 96-well, round-bottom plates. Culture supernatants were collected after 24 h, and concentrations of IFN-γ in the supernatants were determined using a human IFN-γ ELISA kit (BD Pharmingen). To analyze the response against HHV-8-infected cells, BC1 cells were stimulated with 20 ng/ml PMA for 24 h. Thereafter, PMA-stimulated BC1 cells were washed four times and cocultured with 3 × 10^6 of mock-transfected or CD200R-transfected NKL in 96-well, round-bottom plates for 24 h.

Results
Specific binding of soluble CD200R to viral CD200 homologues of HHV-6, -7, and -8
Some herpesviruses and poxviruses possess CD200 homologues (13–15, 17), for example, HHV-6, -7, and -8. These viral CD200 homologues are thought to serve as decoy ligands for the inhibi-
tory receptors CD11b and CD18 (Integrin beta 2). Recent studies have indicated that CD200R and different CD200 homologues are expressed on mouse pro-B Ba/F3 cells using retroviruses. We added a FLAG epitope tag at the N terminus of the viral CD200 homologues to permit detection of these proteins. Ba/F3 cells stably expressing human CD200 or the viral CD200 homologues on the cell surface were purified using Ab-coupled magnetic beads. Fig. 1A shows the levels of FLAG-tagged human CD200 or viral CD200-like molecules expressed on these transfectants.

We then analyzed whether Ig fusion proteins of human and mouse CD200R recognize these viral CD200 homologues. As shown in Fig. 1B, human and mouse CD200R-Ig specifically
bound to cells expressing human CD200. The fluorescence intensity of cells stained with mouse CD200R-Ig was 1/10th the fluorescent intensity of cells stained with human CD200R-Ig. This result is consistent with findings of a prior study that demonstrated a lower affinity of mouse CD200R compared with human CD200R measured using a Biacore (Biacore International) (6). When cells transduced with the viral CD200 homologues were analyzed, all transductants expressing the viral CD200 homologues were stained with human CD200R-Ig. In contrast, mouse CD200R-Ig did not recognize these viral CD200 homologues despite the fact that the homologies of human and mouse CD200 to the viral CD200 homologues are almost the same (amino acid homologies of the viral CD200 proteins of HHV-6, -7, and -8 to human CD200 are 24, 24, and 31%, respectively, whereas those to mouse CD200 are 16%, 24, and 32%, respectively). In addition, mouse CD200R-Ig recognized not only mouse CD200, but also human CD200 (Fig. 1B and data not shown).

Anti-CD200 mAb recognized human CD200, but not the viral CD200 homologues (Fig. 1B). These results suggested that these human herpesviruses might have acquired CD200 homologues as ligands for the human inhibitory CD200R.

**Specific expression of CD200R on basophils in human peripheral blood**

Recent studies have suggested that CD200R is expressed on most human leukocytes, including T cells, monocytes, granulocytes, and a small subset of NK cells (6). However, the expression level of CD200R on these populations was relatively low, and the function of CD200R on these cells has not been evaluated (6). To study the cells expressing CD200R, we stained peripheral blood leukocytes with CD200-Ig and HHV-8 CD200-Ig fusion proteins in combination with various lineage-specific mAbs. As shown in Fig. 2A, ~1% of the cells were brightly stained with the CD200-Ig fusion protein. Most cells binding to the CD200-Ig fusion protein were costained with anti-CD123 mAb, but not with anti-CD3, -CD14, -CD16, -CD19, or -CD56 mAb. Similar results were obtained when cells were stained with the HHV-8 CD200-Ig fusion protein.

This suggested that T cells, B cells, NK cells, and monocytes express lower levels of CD200R than the CD123-positive population. Because basophils are the main population strongly expressing CD123 in peripheral blood leukocytes, we purified basophils and stained them with the human CD200-Ig and HHV-8 CD200-Ig fusion proteins (Fig. 2B). As predicted, all basophils were stained with the CD200-Ig fusion protein. In contrast, eosinophils and neutrophils were not stained by the CD200-Ig fusion protein (Fig. 2B). Granulocytes have been reported previously to express CD200R, as detected using an anti-human CD200R mAb (6), indicating that the CD200-Ig fusion and HHV-8 CD200-Ig fusion proteins probably stain cells with only the highest amounts of CD200R. Anti-human CD200R mAb was not available for a direct comparison with the fusion protein staining; however, the mAb almost certainly has a higher affinity than the fusion proteins and would be capable of detecting cells expressing lower levels of CD200R. Nonetheless, because CD200-Ig and HHV-8 CD200-Ig specifically bind to CD200R, these data suggested that basophils represent a major CD200R-positive cell type in human peripheral blood. Next, to confirm that CD200R is expressed on basophils, we purified each leukocyte population and analyzed the expression of CD200R transcripts by real-time PCR. As shown in Fig. 2C, the expression of CD200R transcripts in basophils was substantially higher than that in other populations. When the nucleic acid sequence of the CD200R cDNA expressed in basophils was analyzed, there was no difference in the sequence from that of the CD200R cDNA previously reported (GenBank accession no. AY284975; data not shown). These data indicated that basophils are a major population in peripheral blood expressing CD200R.

**Human CD200- and HHV-8 CD200-expressing cells suppress effector functions of basophils through inhibitory CD200R**

Degranulation of basophils is correlated with the pathogenesis of allergic disorders. Engagement of FcεRI with anti-FcεRI mAb induces CD11b up-regulation and histamine release from basophils (27). We analyzed the function of CD200R on basophils by cross-linking the receptor with human CD200-Ig and HHV-8 CD200-Ig.
As shown in Fig. 3, cross-linking CD200R with human CD200-Ig, but not with control-Ig, clearly suppressed both CD11b up-regulation and histamine release of basophils induced by the engagement of FcɛRI. Cross-linking CD200R with HHV-8 CD200-Ig also suppressed FcɛRI-dependent CD11b up-regulation and histamine release. In contrast, IL-3 induced up-regulation of CD11b expression, but not histamine release (27, 28). Human CD200-Ig and HHV-8 CD200-Ig showed negligible effects against IL-3-dependent CD11b up-regulation on basophils.

Next, we analyzed the function of CD200R on basophils using CD200 transfectants. We transfected human CD200 or the HHV-8 CD200 into human B lymphoblastoid cell line 721.221 cells. Although 721.221 cells express low amounts of endogenous CD200 on the cell surface, the level of CD200 expression was greatly
increased when these cells were transduced with CD200 (Fig. 4A). The human CD200-transfected 721.221 cells were stained brightly by both CD200R-Ig fusion protein and anti-human CD200 mAb. 721.221 cells transfected with the HHV-8 CD200 were also stained brightly by the CD200R-Ig fusion protein, but not by anti-human CD200 mAb. Basophils were cocultured with human CD200 or HHV-8 CD200-transfected 721.221 human B lymphoblastoid cells, and FceRI on basophils was cross-linked with an anti-FceRI mAb (Fig. 4B). Both CD11b up-regulation and histamine release induced by engagement of FceRI were significantly down-regulated in the presence of human CD200-transfected 721.221 cells and HHV-8 CD200-transfected 721.221 cells, but were not down-regulated in the presence of mock-transfected 721.221 cells. These data suggested that the CD200 homologue of HHV-8 as well as human CD200 suppress FceRI-dependent activation of basophils through CD200R.

**HHV-8 CD200 down-regulates activation of CD200R-transfected NK cells**

To analyze the function of CD200R, we used a human NK cell line, NKL, as a model system to validate the inhibitory function of the CD200R when it is engaged by its human CD200 or viral CD200 ligands. We transduced human CD200R into NKL, which lacks endogenous expression of CD200R (Fig. 5A). We then analyzed the cytolytic activity of NKL or human CD200R-transfected NKL cells against CD200-transfected 721.221 target cells. Mock-transfected NKL cells showed high and equivalent levels of cytotoxicity against 721.221 and the CD200 transfectants (Fig. 5B). By contrast, human CD200R-transfected NKL cells showed diminished cytotoxic activity against parental 721.221 cells (probably due to expression of endogenous CD200) and negligible cytotoxicity against 721.221 cells transfected to express high amounts of human CD200 or the HHV-8 CD200 (Fig. 5B). Cytotoxicity mediated by human CD200R-expressing NKL cells against parental 721.221 cells was augmented when anti-CD200 mAb was added to the assay, indicating that endogenous CD200 expressed by 721.221 is functional (Fig. 5C). Moreover, CD200R-transfected NKL cells did not kill human CD200-transfected 721.221 cells expressing high amounts of CD200 (Fig. 5B); however, cytotoxicity was restored when anti-CD200 mAb was added to the assay (Fig. 5C). This indicated that interactions between CD200R on the effector NK cells and CD200 on the target cells down-regulated the lytic function of NKL cells. Similarly, CD200R-transfected NKL cells showed negligible cytotoxicity against 721.221 cells transfected with HHV-8 CD200 (Fig. 5B). However, addition of anti-human CD200 mAb did not restore the cytotoxicity mediated by human CD200R-transfected NKL cells because the anti-human CD200 mAb does not recognize HHV-8 CD200 (Fig. 5C). Essentially identical data were obtained when IFN-γ production by NKL was analyzed (Fig. 5D). Human CD200R-expressing NKL cells did not express substantial amounts of cytokine in response to human CD200 or HHV-8 CD200-transfected target cells. These data indicated that human CD200 and the HHV-8-CD200 expressed on the target cells down-regulated the effector functions of CD200R-expressing effector cells.

**HHV-8 infected cells down-regulate activation of CD200R-transfected NK cells**

We analyzed the function of HHV-8 CD200 using the HHV-8 persistently infected cell line, BC1. BC1 cells express only limited HHV-8 viral gene products. However, BC1 cells express several viral proteins when lytic reactivation is induced by PMA stimulation (29). As shown in Fig. 6A, nonstimulated BC1 cells were only weakly stained by human CD200R-Ig, and BC1 cells were not stained by anti-human CD200 mAb. By contrast, when BC1 cells were activated with PMA, BC1 cells expressed high levels of CD200, which was recognized by both anti-human CD200 mAb and human CD200R-Ig fusion protein (Fig. 6B). These data suggested that the HHV-8 CD200 homologue down-regulates the effector functions of CD200R-expressing effector cells.
were stimulated with PMA, most BC1 cells were stained with CD200R-Ig, but not with anti-human CD200 mAb, 1 d after stimulation. The K3 and K5 proteins of HHV-8 are well known to down-regulate cell surface expression of MHC class I proteins (30, 31). Indeed, the amount of MHC class I expression on BC1 cells was significantly decreased 2 d after stimulation. These data suggested that HHV-8 CD200 was expressed on the cell surface after PMA-induced reactivation.

We cocultured parental (i.e., CD200R-negative) NKL cells with nonstimulated or PMA-stimulated BC1 cells and analyzed IFN-γ production. Because lytic reactivation was observed after 1-d stimulation with PMA (Fig. 6A), we stimulated BC1 cells with PMA for 1 d, washed the cells extensively, and cocultured them with parental NKL cells for an additional day. NKL did not produce IFN-γ when these cells were cocultured with unstimulated BC1 cells (Fig. 6B); however, NKL produced a large amount of IFN-γ when they were cocultured with PMA-stimulated BC1 cells. Culture supernatants of PMA-stimulated BC1 cells did not activate NKL cells. In addition, BC1 itself did not produce IFN-γ upon PMA stimulation (data not shown). These data suggested that PMA-stimulated BC1 cells directly activated NKL cells. However, the activation of NKL was not specific to HHV-8-infected BC1 cells, because other B lymphoblastoid cell lines, such as the EBV-transformed 721.221 and C1R cell lines or the EBV-negative BJAB cell line, also stimulated NKL after PMA stimulation of these B cell lines (data not shown). Nevertheless, this system is suitable to analyze the function of the HHV-8 CD200 expressed on HHV-8-infected cells, because NKL seemed to be activated by direct recognition of PMA-stimulated BC1 cells.

We analyzed IFN-γ production by mock-transduced or CD200R-transduced NKL cells upon coculture with PMA-stimulated BC1 cells. Mock-transduced NKL cells produced significant
amounts of IFN-γ after coculture with PMA-stimulated BC1 cells, but not after culture with unstimulated BC1 cells (Fig. 6C). Production of IFN-γ by CD200R-expressing NKL cells after coculture with PMA-stimulated BC1 cells was significantly lower than that by mock-transfected NKL cells. There were no significant differences in cytokine production between the mock and CD200R transfectants when these transfectants were directly stimulated with PMA (data not shown). Because BC1 cells do not express CD200, these data suggested that the CD200 homologue of HHV-8 expressed on PMA-stimulated BC1 cells down-regulates the activation of CD200R-expressing effector cells.

Discussion
In the present study we found that basophils are a major population expressing CD200R in human peripheral blood. Recently, CD200R has been reported to be expressed on various populations, including granulocytes, macrophages, and dendritic cells, as detected using an anti-CD200R mAb (6, 32, 33). However, we could not detect CD200R expression on peripheral blood cells other than basophils using CD200-Ig fusion protein, most likely because the fusion protein has a lower affinity than the anti-CD200R mAb and therefore detects only cells with the highest amounts of CD200R. Although our studies indicate that basophils express higher levels of CD200R than other PBL, the broad distribution of CD200R indicates that CD200R may regulate the immune response of many different hemopoietic cell populations. Furthermore, the amounts of CD200R on leukocytes in tissues and organs other than peripheral blood need to be evaluated to determine the potential role for this inhibitory receptor in regulating immune responses.

We showed that engagement of CD200R by its ligand resulted in the down-regulation of FcεRI-dependent activation of basophils. These findings suggest that CD200R may play an important role in the regulation of basophil function. Recently, the inhibitory CD200R has been reported to regulate the activation of mouse bone marrow-derived mast cells and human cultured mast cells (4, 33). Although mast cells are similar to basophils in their array of inflammatory mediators and cell surface expression of FcεRI, their tissue distributions and developmental pathways are quite different. Indeed, mature mast cells do not exist in blood leukocytes, in contrast to basophils. Although basophils bear a closer developmental relationship to eosinophils than to mast cells, eosinophils do not stain with CD200-Ig or HHV-8 CD200-Ig fusion proteins (Fig. 4B). Therefore, basophils are a unique leukocyte subpopulation in human peripheral blood brightly expressing CD200R. In contrast, because basophils can migrate into tissues during an allergic response, CD200R expressed on basophils as well as on resident mast cells might function as a negative regulator of an allergic response in local tissue sites. Therefore, analysis of the function of CD200R expressed on cells at the site of an allergic response would be informative.
Not only herpesviruses, but also several other viruses, such as poxviruses, possess viral CD200 homologues. However, the functions of the CD200 homologues of most viruses have not been established. In the present study, we demonstrate that the CD200 homologues of HHV-6, -7, and -8 are ligands for the human inhibitory CD200R. Furthermore, we show that viral CD200 can act as a CD200R-downregulator of basophils and CD200R-transduced NKL cells. We have shown that not only HHV-8 CD200-transfected cells but also HHV-8-infected cells down-regulate the activation of CD200R-bearing effector cells. These functional analyses suggested that these viral CD200 homologues might play an important role in down-regulating an antiviral immune response. In other words, these data suggested that HHV-6, -7, and -8 might have acquired CD200 viral homologues to regulate the function of basophils in addition to other CD200R-bearing immune cells.

Regarding the function of the CD200 homologue of HHV-8, two conflicting papers have been published. Chung et al. (18) prepared soluble HHV-8 CD200 homologue as a GST fusion protein and showed that the soluble HHV-8 CD200 homologue activated human monocytes. In contrast, Foster-Cuevas et al. (19) reported that transfectants expressing the HHV-8 CD200 suppressed the activation of monocytes, similar to our findings presented in this study. This discrepancy might be due to the manner in which the HHV-8 CD200 protein was prepared in the different studies. Production of HHV-8 CD200 by transfection in mammalian cells may be better mimic the structure of the protein in virus-infected cells, because it will be properly folded and glycosylated. In contrast, the GST fusion protein would not be glycosylated and could be contaminated with bacterial products, which might inadvertently activate macrophages.

A number of recent reports have suggested that drug-induced hypersensitivity syndrome, characterized by serious adverse systemic reactions in addition to skin rash, is associated with the reactivation of human herpesviruses, mainly HHV-6 (34, 35). Indeed, in this case, viral infection exacerbates allergic diseases. However, because basophils highly express inhibitory CD200R and are actively involved in the pathogenesis of allergic disorders in concert with eosinophils and mast cells, at least in part, herpesvirus might have acquired CD200 homologues to suppress the inflammatory response mediated by these effector cells. Therefore, CD200R might be used as a unique therapeutic target to manage allergic disorders, including pathologies caused by herpesvirus infections.

We have recently proposed a hypothesis that paired receptors, which consist of related activating and inhibitory receptors, play an important role in the regulation of viral infections (10, 36). The m157 protein of MCMV, which is structurally related to MHC class I proteins, is recognized by the inhibitory Ly49I receptor in certain MCMV-susceptible mouse strains, whereas m157 is recognized by the activating Ly49H receptor in an MCMV-resistant mouse strain (10). This suggested that MCMV acquired m157 as a ligand for inhibitory receptors, and the immune system evolved the activating Ly49H to protect against viral infection. These observations suggest that the virus and the paired receptors evolved together, allowing for coexistence of host and virus. Interestingly, the mouse CD200R family consists of one inhibitory receptor and three activating receptors (6). These activating CD200R are expressed on various populations, including mouse basophils and activated NK cells (37) (I. Shiratori, M. Yamaguchi, M. Suzukiwaka, K. Yamamoto, L. L. Lanier, T. Saito, and H. Arase, unpublished observation). According to our hypothesis, the activating mouse CD200R may have evolved to protect against virulent viruses that had acquired CD200 homologues. However, as yet, we have not identified a mouse virus encoding a CD200-like protein to test this concept. In contrast, although the human genome contains a linked gene that encodes a putative activating CD200R, the activating human CD200R is not expressed on the cell surface due to the lack of a cysteine residue that is required to form its Ig-like domain (6). Whether this activating CD200R is still in the stages of being selected in the human population or is being eliminated because it is no longer necessary for protection against an extinct viral pathogen encoding a CD200 homologue is not known. However, it is interesting to note that HHV-6, -7, and -8 do not cause lethal infectious diseases in healthy individuals, although they show persistent infection. Nonetheless, the presence of a CD200R in the human genome with the capacity to generate an activating receptor by mutation might be important in the future to acquire resistance to an emerging virulent virus that possesses a CD200 homologue. Individuals with a polymorphism generating a functional activating CD200R may, in theory, survive such a viral infection. Therefore, identification of viruses that are recognized by paired activating and inhibiting receptors may serve to support a physiological role of paired receptors in host defense.

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Disclosures

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