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CD200 (OX2) is a broadly distributed cell surface glycoprotein that interacts with a structurally related receptor (CD200R) expressed on rodent myeloid cells and is involved in regulation of macrophage function. We report the first characterization of human CD200R (hCD200R) and define its binding characteristics to hCD200. We also report the identification of a closely related gene to hCD200R, designated hCD200RLa, and four mouse CD200R-related genes (termed mCD200RLa-d). CD200, CD200R, and CD200R-related genes were closely linked in humans and mice, suggesting that these genes arose by gene duplication. The distributions of the receptor genes were determined by quantitative RT-PCR, and protein expression was confirmed by a set of novel mAbs. The distribution of mouse and human CD200R was similar, with strongest labeling of macrophages and neutrophils, but also other leukocytes, including monocytes, mast cells, and T lymphocytes. Two mCD200 receptor-like family members, designated mCD200RLa and mCD200RLb, were shown to pair with the activatory adaptor protein, DAP12, suggesting that these receptors would transmit strong activating signals in contrast to the apparent inhibitory signal delivered by triggering the CD200R. Despite substantial sequence homology with mCD200R, mCD200RLa and mCD200RLb did not bind mCD200, and presently have unknown ligands. The CD200 receptor gene family resembles the signal regulatory proteins and killer Ig-related receptors in having receptor family members with potential activatory and inhibitory functions that may play important roles in immune regulation and balance. Because manipulation of the CD200-CD200R interaction affects the outcome of rodent disease models, targeting of this pathway may have therapeutic utility.


Materials and Methods

Cell lines and staining reagents

Human mast cells were derived from freshly isolated cord blood, as previously described (9, 10). Mouse C57BL/6 mast cells were derived from bone marrow of 2- to 3-wk-old mice. Bone marrow cells were cultured in DMEM (BioWhittaker, Walkersville, MD) supplemented with 10% FCS

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Cloning of hCD200R

The National Center for Biotechnology Information (NCBI) expressed-sequence tag (EST) database was screened using the rat cDNA sequence of CD200R (GenBank accession AF231392) utilizing the BLASTn program (12). A weak match was identified in the 3′ nontranslated region with clone IMAGE:2054703. This clone was ordered from NCBI, and further sequencing revealed an open reading frame that contained an apparent insertion that affected the reading frame before the proposed transmembrane domain. The full-length hCD200R was then amplified from cDNA generated from human lung poly(A)+ RNA (Clontech, Palo Alto, CA) by PCR using oligonucleotides ccccccttgtaggtgaggtgtagttgag (sense) and gcagagcggccgcaaacagaaatgctc (antisense) to introduce a KpnI site underlined, and corresponding genes and related genes were identified by homology search of the NCBI EST database. A full-length cDNA of hCD200R was isolated by standard PCR cloning techniques from mouse bone marrow-derived mast cells. mCD200Rl was cloned from normal bone marrow-derived mast cells described (3). Briefly, cDNA library was prepared from normal mouse mast cells in a pJEFl4 vector. cDNA cloning by transient expression in 293T/FLAG-DAP12 cells was performed, as previously described (13). Transfected cells were screened for cDNA encoding proteins that were capable of pairing with mCD200Rl and translocating FLAG-tagged DAP12 to the cell surface. Cell surface DAP12 was then visualized using the anti-FLAG M2 mAb (Kodak, Rochester, NY) and PE-conjugated goat anti-mouse second step (Caltag, Burlingame, CA). Cells expressing surface FLAG-DAP12 were sorted on a FACS cell sorter (BD Biosciences), and plasmid DNA was recovered. After the cDNA of cell-sorted clones was amplified and sequenced, plasmids were isolated. mCD200RLb was identified by homology search of the NCBI EST database and a full-length cDNA isolated by standard PCR cloning techniques from mouse bone marrow-derived mast cells. mCD200RLc has not yet been isolated; however, mCD200RLc is identical to the amino acid level with mCD200RLb.

Analysis of the human and mouse genomes

The NCBi human and mouse genome databases were searched using BLAST (12), and corresponding genes and related genes were identified.

Ability of CD200RL proteins to pair with DAP12

cDNAs encoding full-length hCD200RLa, mCD200RLa, and mCD200RLb were subcloned into the pMX-pie retrovirus expression vector. Plasmid DNAs representing the various genes were transfected into the Phoenix ecotropic virus packaging cell line (G. Nolan), and the viruses subsequently obtained were used to infect Ba/F3-mDAP12 cells, as previously described (11). In the absence of a pairing partner, FLAG-tagged DAP12 remains within the cytoplasm of Ba/F3-mDAP12; however, if the CD200RL proteins can pair with DAP12, the FLAG epitope will appear on the cell surface of Ba/F3-mDAP12. After 1–2 wk in selection conditions, the Ba/F3-mDAP12 cells infected with the various CD200RL genes were analyzed by flow cytometry for the surface expression of the FLAG epitope of DAP12 (anti-M2 Ab; Sigma-Aldrich, St. Louis, MO). These cells were also stained with mAbs specific for the various CD200RL proteins.

CD200 binding to CD200R and CD200RL proteins

Fusion proteins, consisting of the extracellular domains of mCD200R and hCD200 fused to the Fe region of hIg (mCD200R-hIg, hCD200R-hIg), were used to investigate the ability of the mouse and human CD200R and CD200RL proteins to bind a soluble form of CD200. Stable transfectants of Ba/F3 expressing hCD200R, hCD200RLa, and Ba/F3-mDAP12 expressing mCD200RLa or mCD200RLb were generated as described, except as noted above. One microgram of mCD200RL-hIg, or control-hIg was used to stain −106 cells of the various receptor transfected with PE-conjugated goat anti-Ig-hlg Ab (20 min at 4°C; Caltag), washed, and analyzed on a FACScan (BD Biosciences). For receptor Ab-blocking experiments, blocking mAbs against the CD200Rs were incubated with cells (2 μg/106 cells) for 20 min at 4°C before the addition of the mouse or human CD200-hlgs.

Construction, expression, and purification of soluble recombinant proteins

The CD200-hlg fusion proteins contain two CD200 extracellular regions, but for kinetic studies a monomeric hCD200CD4d3-hlg was expressed using pEF-BOS-CD4d3 (12). A strong match was identified in the pEF-BOS-CD4d3 clone for the various CD200RL proteins. After three rounds of cell sorter selection, single plasmid colonies were isolated.

Cloning of mCD200RL genes

mCD200RLa was cloned from normal bone marrow-derived mast cells using a DAP12 trap technique, as previously described (13). In brief, a cDNA library was prepared from normal mouse mast cells in a pJEFl4 vector. cDNA cloning by transient expression in 293T/FLAG-DAP12 cells was performed, as previously described (13). Transfected cells were screened for cDNA encoding proteins that were capable of pairing with DAP12 and translocating FLAG-tagged DAP12 to the cell surface. Cell surface DAP12 was then visualized using the anti-FLAG M2 mAb (Kodak, Rochester, NY) and PE-conjugated goat anti-mouse second step (Caltag, Burlingame, CA). Cells expressing surface FLAG-DAP12 were sorted on a FACS cell sorter (BD Biosciences), and plasmid DNA was recovered. After the cDNA of cell-sorted clones was amplified and sequenced, plasmid DNA was isolated. mCD200RLb was identified by homology search of the NCBi EST database and a full-length cDNA isolated by standard PCR cloning techniques from mouse bone marrow-derived mast cells. mCD200RLc was identified by homology search of the NCBi EST database. A full-length clone of mCD200RLc has not yet been isolated; however, mCD200RLc is identical to the amino acid level with mCD200RLb.

Cloning of hCD200R

The NCBI human and mouse genome databases were searched using BLAST (12), and corresponding genes and related genes were identified.

Affinity and kinetic data were collected using a BLAcore 2000 at 37°C, as described (3). Briefly, −5,000 response units (RU) of streptavidin were coupled to a CM5 research grade chip using amine coupling. In separate experiments, biotinylated rat, mouse, or human CD200RCD4d3-hlg proteins were immobilized at high (−1,600 RU), medium (−850 RU), and low (−400 RU) levels in three flow cells and control CD4d3-hlg (−2,200 RU) in the fourth flow cell. Serially diluted monomeric hCD200RCD4d3-hlg purified soluble chimeric proteins were then injected at the indicated active concentrations over all four flow cells connected in series. The extinction coefficient, 40,534 M−1 cm−1, was determined by amino acid analysis. The minimal fraction of purified protein able to bind the hCD200R was determined by depletion using avidin-Sepharose agarose beads (Sigma-Aldrich) coated in biotinylated rat CD200RCD4d3-hlg as compared with biotinylated CD4d3-hlg. Depleted and control fractions were resolved by SDS-PAGE and densitometrically analyzed using ImageQuant software. At least 90% of hCD200R protein could be depleted by rat CD200R, and active protein concentration was calculated by taking this into account. A binding curve was obtained by fitting a 1:1 binding model to the kinetic data using the BIA evaluation software 3.0.
software, and these data are shown in Table I. In addition, $k_{on}$ rate constants were also determined by fitting a first order exponential decay curve to normalized data over the dissociation phase (see Fig. 4c), and these were in good agreement.

**Tissue expression of CD200R and related genes by mRNA analysis**

For the determination of gene expression, previously described cDNA libraries generated from a variety of mouse and human cell types and tissues were used (15). Gene expression was determined by real-time quantitative PCR using an ABI GeneAmp 5700 sequence detection system and the reporter fluorescent dye, SYBR (PerkinElmer Applied Biosystems, Foster City, CA). In brief, 20 ng of cDNA in PCR buffer contained 200 μM dATP, dCTP, and dGTP; 400 μM dUTP; 4 mM MgCl2; 1.25 U of AmpliTaq DNA polymerase; 0.5 U Amp-Erase uracil-glycosylase; 900 nM Nf1/H9262 dATP, dCTP, and dGTP; 400 kimmobilization had negligible effects on three levels of receptor immobilization (i.e., Materials and Methods obtained by binding effects were minimal.

Analysis of the interaction between CD200 and CD200R in human, mouse, and rat

To analyze the ability of mouse and human CD200R to bind CD200, we used soluble fusion proteins consisting of the extracellular domains of mouse and human CD200 fused to the Fc binding domain of hlg and Ba/F3 cell transfectants expressing either mCD200R or hCD200R. As shown in Fig. 3, mCD200 and hCD200 bound to their respective receptors, and ligand binding could be completely blocked by specific mAb to these receptors (see below).

The interaction between human CD200R and CD200 was characterized biochemically by making recombinant proteins corresponding to their extracellular regions and quantified in real time using surface plasmon resonance in a BLAcore (14). hCD200R-CD4d3-4-biotin protein and a negative control (CD4d3-4-biotin) were immobilized in separate flow cells on a streptavidin-coated sensor chip before injecting purified soluble hCD200-CD4d3-4 protein. hCD200-CD4d3-4 bound to the hCD200R-CD4d3-4 flow cell in comparison with the control reference cell (Fig. 4A). This binding was quantified by calculating the difference in RU observed in the hCD200R and control flow cells once equilibrium had been reached and is plotted as a binding curve (Fig. 4B). The equilibrium-binding affinities ($K_a$) were calculated both by nonlinear curve fitting (Fig. 4B) and Scatchard transformations (Fig. 4B, inset) of the binding data giving similar values. The CD200 interacted with the hCD200R with an equilibrium-binding affinity of $\sim 0.5$ μM at 37°C. Kinetic analysis of the interaction yielded an off rate $\sim 0.1$ s$^{-1}$ (equivalent to a 1/2 of 7 s) at different levels of hCD200R immobilization, indicating that kinetic measurements were not grossly affected by rebinding or mass transport effects (Fig. 4C). These measurements were repeated using soluble biotinylated forms of both the rat and mouse CD200R produced in an identical manner, and the data are presented in Table I. This revealed that the hCD200R interacted with CD200R with similar kinetics to the equivalent interaction in rats and mice. hCD200 showed some cross species binding to both mouse and rat CD200R, but binding to mCD200R was weaker, with about a 10-fold faster dissociation rate that explained why hCD200-hlg did not bind mCD200R (data not shown).
Identification and structural characterization of genes related to CD200R in mice and humans

In addition to the mCD200R cloned previously (3), two cDNAs for genes closely related to CD200R were isolated from normal bone marrow-derived mouse mast cells. These two proteins, named mCD200RLa and mCD200RLb, showed extensive sequence similarity in the extracellular regions to the mCD200R, but displayed short cytoplasmic regions devoid of known signaling motifs. Unlike mCD200R, the transmembrane regions of these two genes contained a positively charged amino acid, lysine (Fig. 1). Because mCD200RLa was also isolated by DAP12 trapping (see Materials and Methods), the lysine in the transmembrane was expected to form a salt bridge with DAP12, allowing these proteins to pair. Transduction of mCD200RLa and mCD200RLb into Ba/F3 cells

**Figure 1.** The human and mouse CD200R and related sequences. The hCD200R sequences have been aligned to the rodent orthologs (accession AF231392 and AF231393), and the sequence has been split according to predicted domain organization. The NH2 terminus is based on protein data for rat CD200R. The superscript bars predict the extent of the β-strands characteristic of the Ig fold by comparison with solved structures. The sequence of the allele used in all binding experiments is shown with the three positions of allelic polymorphism shown above the sequence (accession AF283760). The form with an additional spliced exon is termed CD200Ri (accession NM 138806), and only the extra sequence is shown as other residues are identical with hCD200R. A second human gene termed CD200RLa was identified by EST and cDNA analysis. Additional mouse genes include two forms isolated through their association with DAP12 (mCD200RLa and mCD200RLb; accession XM156177 and XM148097). Residues identical in four or more sequences are boxed (three for cytoplasmic regions) apart from CD200Ri form, as this is known to be a splice variant and CD200RLc and CD200RLb (incomplete sequences). One sequence, CD200RLc, was only isolated as a partial cDNA clone, and additional residues were deduced from genomic sequence and are shown in italics. The partial sequence CD200RLd was only identified as genomic sequence and is shown in italics. Potential N-linked glycosylation sites (N) and the lysine residue (K) in the transmembrane are shown in bold.
stably expressing a FLAG-tagged murine DAP12 resulted in the cell surface expression of DAP12 and the associated CD200RL proteins (Fig. 5). Immunoprecipitation experiments using mAbs against mCD200RLa and mCD200RLb further verified that these two genes are naturally pairing with DAP12 in normal mouse mast cells (data not shown). A fourth form (mCD200RLc) was also identified from EST database analysis, which has a high degree of identity to mCD200RLa and also contains a positively charged amino acid in the transmembrane domain, again suggesting pairing with DAP12. A full-length cDNA of mCD200RLc, however, has not yet been isolated. A fifth partial gene sequence, mCD200RLd, was also identified in the mouse genome (Fig. 1), and the arrangement of the genes on chromosome 16 is shown in Fig. 6. All five members of the mCD200 receptor gene family are located on a short section of chromosome 16 (~200 kbp) and within ~400 kbp of the CD200 gene itself.

In contrast to the mouse genome, analysis of the human genome showed only two CD200R-like genes: one corresponding to the CD200R form originally described in mouse and rat (3), and a second, termed hCD200RLa, which showed the most sequence homology to the mCD200RLa. hCD200RLa contains a short cytoplasmic region, and the charged residue in the transmembrane suggests that it can also interact with DAP12. The human CD200, CD200R, and CD200RL genes are located on chromosome 3, agreeing with previous cytogenetic analysis for CD200 (20). Interestingly, although both CD200 and its receptor are transcribed in the same direction in humans, this is not the case in mice (Fig. 6). Given the similarities in sequence, it seems likely that the CD200RL genes evolved from recent gene duplications and that these and CD200 evolved from a common ancestral precursor.

Distribution of CD200R and CD200RL gene products by mRNA analysis

The distribution of CD200R was determined by a highly sensitive quantitative RT-PCR method in a panel of cDNA libraries from a variety of purified cell types and normal tissues from both mice and humans (Figs. 7 and 8). Using PCR primers that specifically identify CD200R mRNA, it was clear that CD200R shared a similar cellular and tissue distribution in both species. The highest levels of expression were observed in bone marrow-derived macrophages from the mouse, and monocyte-derived dendritic cells in humans. Significant gene expression, however, was also seen in polarized Th2 T cells, mast cells, and dendritic cells of both mice and humans. Interestingly, although mouse bone marrow-derived macrophages expressed very high levels of CD200R mRNA, freshly isolated human peripheral blood monocytes expressed only low levels of CD200R mRNA. The differentiation status of these particular cell types is, however, not comparable, and it is thus...
possible that CD200R expression is up-regulated on tissue macrophages. B cells, fibroblasts, and endothelial cells from both mice and humans expressed little, if any, detectable CD200R mRNA. In whole tissues, the highest expression of CD200R mRNA was observed in bone marrow, lymph nodes, spleen, and lung, while the lowest expression was seen in liver, spinal cord, and kidney (Figs. 7 and 8). Tissue macrophages of the CNS (microglia) are known to express low levels of CD200R in the rat (3); however, in whole spinal cord tissues, the level of mRNA contributed by these cells was negligible. The thymus was the only tissue that showed differential expression between mice and humans. Mouse thymus showed significant expression of CD200R mRNA, whereas human thymus had essentially undetectable levels.

The cellular distribution of mCD200RLa and mCD200RLb was also quantified by PCR on the same panel of mouse cDNA libraries (Fig. 7). The highest level of mCD200RLa was observed in unstimulated mast cells. This expression was, however, significantly decreased in mast cells triggered via FcγRI. Strong mCD200RLa expression was also seen in bone marrow-derived (2.0 μM) at 100 μl/min over immobilized hCD200RCD4d3+4-biotin at high (1815 RU) and medium (895 RU) levels and also a negative control CD4d3+4-biotin (2185 RU), and data were collected at 10 Hz. The data were then normalized (100% at the start of the dissociation phase), and first order exponential decay curves were fitted to the dissociation data (hCD200R medium) and yielded a $k_{off}$ value of 0.09 s⁻¹. For clarity, only every tenth data point is shown.
FIGURE 6. Organization of CD200 and receptor genes in the human and mouse genomes. The approximate positions of the CD200 and CD200R and related genes are shown as oblongs in their approximate positions on the genome. The numbering of the bases is from the current NCBI human and mouse databases. Arrows indicate direction of transcription.

FIGURE 7. Expression of mCD200R, mCD200RLa, and mCD200RLb by mRNA analysis in various cell lines (A) and tissues from C57BL/6 mice (B). Results are presented as fold expression relative to normalized ubiquitin levels.
macrophages and dendritic cells, and to a lesser extent in polarized Th2 T cells. mCD200RLb, however, was primarily expressed in mast cells and polarized Th2 T cells and to a lesser degree in bone marrow-derived dendritic cells. Unlike mCD200R and mCD200RLa, mCD200RLb was undetectable in bone marrow-derived macrophages and was expressed at relatively low levels in bone marrow-derived dendritic cells. cDNA for hCD200RLa was isolated from peripheral blood, but was not found in significant levels in cDNA libraries. Analysis of the amino acid sequence revealed that it lacked two cysteines when compared with the mouse homologue and other genes in this group (Fig. 1). This may explain why hCD200RLa was not expressed even in the presence of DAP12, DAP10, FceRIγ, or CD3γ. Indeed, reintroducing the cysteine residues restored expression in the presence of DAP12 (G. Brooke, unpublished data). It is likely therefore that hCD200RLa is nonfunctional in humans.

**Distribution of mouse and human CD200R by mAb staining**

mAb specific for mCD200R and hCD200R were generated to investigate the cellular distribution of the CD200R protein. In mouse peripheral blood, mCD200R (DX109) was strongly expressed on all granulocytes (CD11b⁺, Gr-1⁺) and monocytes (CD11b⁺⁺, Gr-1⁻) with weaker labeling of most T cells (CD3⁺) (Fig. 9). T cell staining for CD200R was primarily restricted to CD4⁺ T cells (data not shown). Weak mCD200R expression was observed on a subset of NK cells (DX5⁺, CD3⁻), NKT cells (CD3⁺, DX5⁺), and B cells (CD19⁺). In the spleen, mCD200R showed an identical cellular expression pattern to that observed in the peripheral blood, although with somewhat higher levels of expression of CD200R on splenic T cells (data not shown). Consistent results were obtained with another rat anti-mouse CD200R mAb OX110 (data not shown).

In human peripheral blood, hCD200R (DX136) displayed a similar cellular distribution to that observed in mouse peripheral blood (Fig. 10). The majority of human peripheral blood T cells (both CD8⁺ and CD4⁺), all neutrophils (CD14⁺⁺, CD16⁺⁺), and all basophils (CD9⁺⁺, FceRI⁺⁺; data not shown) expressed high levels of hCD200R. Similar to mouse peripheral blood, the majority of human monocytes expressed moderate levels of hCD200R; this expression was significantly up-regulated when monocytes were induced to differentiate into dendritic cells in vitro (CD14⁺⁻, HLA-DR⁺⁺) in the presence of GM-CSF and IL-4 (data not shown). The majority of NK cells (CD56⁺, CD3⁻) did not express hCD200R. Low levels of hCD200R expression, however, were
observed on a subset of the CD56 bright NK cells (CD56^+^, CD3^-^) as well as NKT cells (CD56^+, CD3^+^). A small subset of B cells (CD19^+) demonstrated hCD200R expression in most donors analyzed. hCD200R was not expressed on freshly isolated peripheral blood platelets or RBC (data not shown). Similar results were obtained with an anti-human mCD200R mAb OX108 (data not shown).

**Distribution of mouse CD200RLa and CD200RLb by mAb staining**

Rat mAb specific for mCD200RLa (DX87) and mCD200RLb (DX116) were generated to investigate the expression of these proteins on mouse leukocytes. Unlike mCD200R, mCD200RLa was not expressed on T cells (Fig. 11). Strong expression of mCD200RLa was observed on NK cells, monocytes, and a subset of NKT cells, while low expression was seen on B cells and granulocytes. Although data for peripheral blood leukocytes are shown, similar expression patterns were also observed on splenic populations of leukocytes (data not shown). mCD200RLb, although strongly expressed on bone marrow-derived mast cells (data not shown), was not significantly expressed on peripheral blood leukocytes (Fig. 11).

**Binding of mCD200 to mCD200RLa and mCD200RLb**

To analyze the ability of mCD200 to bind mCD200RLa and mCD200RLb, we used soluble fusion proteins consisting of the extracellular domains of mCD200 fused to the Fc binding domain of hIg and Ba/F3 cell transfectants expressing either mCD200RLa or mCD200RLb. Although mCD200-hIg readily bound to transfectants expressing the mCD200R protein (Fig. 3), we were unable to demonstrate any significant binding of mCD200 to either the mCD200RLa or mCD200RLb proteins (data not shown). Attempts to demonstrate mCD200 binding to these receptors by varying mCD200 concentrations, binding times, and binding temperatures have also failed to show specific receptor binding. Presumably, mCD200RLa and mCD200RLb bind alternative ligands despite the relatively high sequence similarity to the mCD200R.

**Discussion**

We report on the characterization of the CD200 receptor family in both humans and mice. Analysis of the human and mouse genomes together with cDNA sequence analysis indicates that there has been extensive gene duplication in the CD200R genes with up to five related genes in mice on chromosome 16 and two genes in humans on chromosome 3. The human homologue of mCD200R,
designated hCD200R, shows a high degree of sequence and structural similarity to its mouse counterpart, and indeed interacts with hCD200 protein in an analogous manner to that reported in rodents (3). Of particular interest are the highly conserved tyrosines and associated amino acids in the cytoplasmic domain of both mouse and human CD200R. The high degree of identity in the tyrosine-rich regions of the cytoplasmic tails of mCD200R and hCD200R suggests that these proteins recruit similar signal transduction machinery. The molecular identity of the cytoplasmic receptor signaling proteins is presently unknown; however, both mouse and human CD200R have a conserved phosphotyrosine binding site for Shc (NPXY) in the cytoplasmic domains (21, 22). Studies are presently underway to delineate the mechanisms and the structural requirements for signal transduction via the CD200R.

Analysis of mRNA expression in mouse and human cDNA libraries reveals that CD200R is expressed in a variety of cell types of the myeloid/monocytic lineage, in particular, macrophages, dendritic cells, and mast cells. Interestingly, CD200R mRNA is also highly expressed in polarized Th2 T cells in both mice and humans, indicating that this receptor may have a more extensive immunological role than previously anticipated because it has a broader distribution than observed with the original anti-rat CD200R mAb (3). mAbs specific for mCD200R and hCD200R were generated to investigate the expression of CD200R protein on freshly isolated leukocyte populations. In both mice and humans, CD200R is prominently expressed on peripheral blood neutrophils. Because neutrophils play a major role in most inflammatory responses (23), the high expression levels of CD200R suggest that...
this receptor may play an important regulatory role in neutrophil biology; this concept has been reviewed (5). Experiments are presently underway to investigate the functional role of CD200R in neutrophil phagocytosis, superoxide production, and chemokine responses.

As expected from the mRNA distribution analysis, CD200R is also expressed on freshly isolated CD4/H11001 T cells from mouse and human peripheral blood. The mRNA analysis showed a restricted expression of CD200R to polarized Th2 cells; however, cellular expression of the protein indicates that the majority of CD4/H11001 T cells in the mouse and human peripheral blood expressed CD200R. It is possible that CD200R is down-regulated by the cytokines required to polarize Th1 cells, while remaining stably expressed on committed Th2 cells. The expression of CD200R on Th2 cells has important implications for immune regulation. Th2 cells have been strongly implicated in variety of pathological conditions, including allergy, asthma, and hypersensitivity (24–27). Proinflammatory cytokines, such as IL-3, IL-4, IL-5, IL-13, and GM-CSF, are produced by Th2 cells and are believed to be the major factors in allergic pathologies, such as inflammation, mucous hypersecretion, and airway constriction. The strong expression of CD200R, an inhibitory receptor, on Th2 cells may indicate that this receptor plays an important regulatory role in Th2-mediated responses. The expression of CD200R on Th2 cells also suggests that therapeutic strategies directed toward triggering CD200R on Th2 cells may function to inhibit allergic inflammation and associated pathologies. The distribution of CD200R and related proteins is summarized in Table II.

Our previous studies in the mouse have implicated CD200R in the regulation of normal macrophage/myeloid function (3, 4). Consistent with these earlier studies, mCD200R was strongly expressed on peripheral blood monocytes and splenic macrophages. Indeed, preliminary immunocytochemistry analysis demonstrates the expression of CD200R on subsets of macrophages from normal mouse skin, lung, and intestine. mAb staining in the mouse clearly shows that, although CD200R is associated with the macrophage/ml

Table II. Summary of tissue distribution of human and mouse CD200R and related proteins

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*Compilation of data from expression studies by mRNA analysis and mAb staining on normal tissues and cell lines (see Figs. 7–11). Human macrophage data are from skin (data not shown).
myeloid lineage, it is not ubiquitously expressed on all tissue-bound myeloid cells. In the human, however, CD200R is expressed on the majority of monocyte/myeloid lineage cells in the peripheral blood. In addition to neutrophils and monocytes, CD200R was strongly expressed on basophils and dendritic cells derived from monocytes. Likewise, preliminary immunocytochemical analysis demonstrates a strong expression of CD200R on myeloid cells of normal human skin and lung (data not shown).

Mast cells, which are derived from hemopoietic progenitors, play a critical effector role in allergic diseases and IgE-dependent immune responses. Engagement of the high affinity FcR for IgE on mast cells and basophils triggers a series of biochemical events resulting in the secretion of a variety of inflammatory mediators, including histamine, sulfated proteoglycans, proteinases, and cytokines (28). The prominent expression of CD200R on both mouse and human mast cells suggests that this receptor may function as a constitutive regulator of mast cell biological responses. Although we have not yet investigated the expression of CD200R on freshly isolated mast cells, mast cells derived from hemopoietic progenitors clearly express both high levels of specific mRNA for CD200R as well as cell surface protein (data not shown). Previous in vivo studies have implicated the CD200R in the regulation of experimental autoimmune encephalomyelitis (EAE) and collagen-induced arthritis (3, 4, 7). The conclusion from these studies was that CD200R played a major role in the regulation of these autoimmune models by modulating macrophage function. Macrophages are known to contribute to the onset and severity of brain inflammation in the EAE model (29). Several recent studies using mast cell-deficient mice, however, have shown that EAE and collagen-induced arthritis are dependent upon functional mast cells (30, 31).

It is possible, therefore, that some of the effects of CD200R in these models are manifested through the regulation of mast cell functions. The molecular and biological functions of CD200R expression on mast cells are presently a major focus of ongoing studies.

Two of the mouse genes of the CD200 receptor family (mCD200RLa and mCD200RLb) associate with DAP12 and require DAP12 for stable cell surface expression. DAP12 is a small disulfide-bonded homodimer that is structurally similar to FcεRIγ chain and the TRCγ chain (32, 33). The transmembrane domain of DAP12 contains a negatively charged aspartic acid residue that allows pairing with a variety of cell surface receptors with positively charged amino acid residues in their transmembrane regions. The cytoplasmic domain of DAP12 contains a conserved immunoreceptor tyrosine-based activation motif, which when phosphorylated recruits protein tyrosine kinases (32–35). Pairing of mCD200RLa and mCD200RLb with DAP12 clearly defines these proteins as potentially activating receptors. mCD200RLa and mCD200RLb do not bind mCD200, despite extensive sequence similarity in the extracellular regions at least for CD200RLa (84% identity for CD200RLa and CD200RLb with CD200R, respectively). The ligands of CD200RLa and CD200RLb and the functional and biological significance of these receptors are unknown; however, it is possible that these receptors have evolved to interact with viral proteins in a manner similar to that recently described for a DAP12 pairing member of the Ly-49 family and mouse CMV (36, 37). The human homologue of mCD200RLa, hCD200RLa, is structurally very similar to the mouse gene, including the positively charged residue in the transmembrane domain. It seems likely that hCD200RLa is a nonfunctional gene, as no expression could be detected despite many attempts with a variety of adapter molecules.

CD200R is a member of a group of proteins expressed on myeloid cells, but we now report expression on many T cells in mouse and human in contrast to earlier data in the rat (3). In the mouse, there is clearly a form that can give an activating signal through DAP12, and in this regard it resembles other gene families such as signal regulatory proteins and killer Ig-related receptors that have both activating and inhibitory forms (38–41). Manipulating the CD200R has been shown to affect immune responses, but these data indicate that the cells being affected may include both myeloid cells and T cells. Importantly, the definition of a family of closely related CD200-like proteins (at least in the mouse) suggests that different effects in vivo and in vitro may be expected using CD200-Fc fusion proteins, shown in this study to be specific for CD200R, and Abs against the various related members that may show different cross-reactivities.

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References


