Modulation by balancing activating and inhibitory receptors constitutes an important mechanism for regulating lymphocyte and myeloid cell effector responses. Using a microarray screen during parasitic helminth infection, we identified CD200 receptor-like 3 as a transcript highly expressed in basophils. Novel splice variants were present that generated proteins that differed in surface expression. The second immunoglobulin-like domain, encoded by exon 4, was required for cell surface expression and recruitment of DAP12 to the cell surface. Splice variants also generated unique cytoplasmic domains, which contributed to efficient pairing with DAP12. Despite expression on basophils and mast cells, which are integral components of allergic immunity, the absence of DAP12 did not alter effector cell recruitment or the host response elicited by helminth infection with *Nippostrongylus brasiliensis*.

The immune system protects multicellular organisms against uncontrolled infections with various pathogens. However, prolonged activation of effector cells can be harmful, and regulatory mechanisms have developed to down-modulate potentially damaging responses to normal tissues. Frequently, the effector functions of immune cells are regulated by inhibitory and activating receptors expressed at the cell surface. For example, the activity of NK cells is inhibited by recognition of self-major histocompatibility complex I molecules by Ly49, CD94/NKG2A, or killer cell inhibitory receptors (1). Also, IgG molecules induce an inhibitory signal on macrophages and B cells by binding to FcγRIib, and mice deficient in this receptor develop autoimmune disease (2, 3). SIRPα, an inhibitory receptor expressed on myeloid cells, inhibits phagocytosis upon engaging its ligand, CD47, which is expressed on many cells, including erythrocytes (4). Most of these inhibitory receptor families also contain family members with activating functions, such as Ly49D, NKG2C, killer cell inhibitor receptor 2DS, FcγRIII and SIRPβ.

In general, these activating receptors contain a positively charged amino acid in their transmembrane domains and short cytoplasmic tails that lack signaling capacity. To transduce signals, the receptors must interact with a family of adaptor proteins, including DAP12, DAP10, CD3ζ, and FcγRIγ, which provide tyrosine-based activation motifs as docking sites for downstream signaling components via Src homology 2 domain interactions. These receptor-adaptor pairs have been studied mainly in lymphoid cells. However, DAP12-associated activating receptors, including the orphan receptors TREM-1, -2, and -3 (where TREM is triggering receptor expressed on myeloid cells), myeloid DAP12-associated lectin 1, and SIRPβ are also expressed on myeloid cells (5–7).

The OX-2, or CD200 receptor (CD200R), 1 belongs to the immunoglobulin superfamily, contains two Ig-like extracellular domains, and mediates inhibitory signals in myeloid cells (8). Mice deficient for the ligand CD200 develop enhanced experimental allergic encephalomyelitis and collagen-induced arthritis (8, 9). The augmented tissue reactivity in these mice is due mainly to unrestrained activation of monocytes/macrophages. Additional CD200R-like proteins have recently been identified in mouse and man (10). Unlike CD200R, these receptors may function as activating receptors because they contain short cytoplasmic tails and a lysine residue in the transmembrane region and they are likely to signal via adaptor proteins such as DAP12, DAP10, FcγRIγ, or CD3ζ. Indeed, pairing of two of these CD200R-like receptors with DAP12 was recently shown (10).

We describe the identification of CD200R family members in basophils and mast cells, including new splice variants for CD200R3 that show selective surface expression and pairing with signaling adaptor proteins. To assess the role for signaling via these receptors in basophils and mast cells, we analyzed the requirements for DAP12-associated signaling during a type 2 immune response induced by infection with the parasitic helminth, *Nippostrongylus brasiliensis*.

**EXPERIMENTAL PROCEDURES**

**Mice**—C57BL/6 mice were purchased from Jackson Laboratories (Bar Harbor, ME). DAP12-deficient mice (11) were back-crossed nine generations with C57BL/6 mice. Interleukin-4 reporter mice (4get mice) (12) were back-crossed 10 generations with BALB/c mice. 

**Cloning of CD200R Genes**—CD200R-like receptors CD200R3 and

1 The abbreviations used are: CD200R, CD200 receptor; CD200RL, CD200R-like proteins; RT, reverse transcription; GFP, green fluorescent protein; IL, interleukin; mAb, monoclonal antibody; FACS, fluorescence-activated cell sorter; FcγR, Fcγ receptor; NFAT, nuclear factor of activated T cells; NKG, natural killer gene; SIRP, signal regulatory protein.
CD200R3 Expression in Basophils and Mast Cells

CD200R4 were cloned from lung cDNA prepared from mice infected 10 days previously with the parasitic helmint, N. brasiliensis. CD200R2 was cloned from bone marrow-derived basophil/mast cell cultures. Primers flanking the coding sequences were designed based on the following template sequences at the NCBI data base: NM_020635 for CD200R2, XM_148097 for CD200R3, and AK089262 for CD200R4. The primer sets were: CD200R2, forward: 5'-agcatctttaatgatgatatg-3' and reverse: 5'-catggagagagctggcag-3'; CD200R3, forward: 5'-gacctcctaactctggtgcagc-3' and reverse: 5'-tcatggatgtctggggtg-3'; CD200R4, forward: 5'-gacctcctaactctggtgcagc-3' and reverse: 5'-atggatgacgatatcgct-3'. RT-PCR products were cloned into the pCR2.1-TOPO sequencing vector, sequenced, and subcloned into the BamHI/Xhol double-digested pMX-pie bicistronic retroviral internal ribosomal entry site eGFP expression vector (13). The CD200R3-GFP fusion proteins were generated by PCR cloning the entire CD200R3 lacking the stop codon in-frame with pcDNA3.1C/T-GFP-TOPO vector (Invitrogen). The generation of expression plasmids for FLAG-tagged protein has been described (14). FLAG-tagged CD200R3 protein was generated by cloning CD200R3C without signal peptide sequence into a pcDNA3.1-based vector containing the N-terminal CD8 signal peptide sequence followed by the FLAG-tag. The different splice versions of CD200R3 were cloned into pCR2.1-TOPO after isolation of individual bands from the ethidium bromide-stained gel. Splice variants B and D were generated by exchanging exons 1-2 and 3-4 between the variants A and E. The sequences for all splice variants have been submitted to the NCBI database (GenBank accession numbers AY703837–AY703842).

Multiple Tissue RT-PCR—RNA from indicated tissues and cell lines was prepared using the total RNA isolation kit (Fluka) and transcribed into cDNA with the Superscript II reverse transcriptase kit (Invitrogen). cDNAs were amplified with primer pairs for CD200R2, CD200R3, and CD200R4, respectively, and reverse primers as indicated above. In addition, the following primers were used: CD200R, forward (5'-gacctcctaactctggtgcagc-3') and reverse (5'-gtctctttctgtagctagc-3'); α-actin, forward (5'-tgtgagagtctgctgct-3') and reverse (5'-ttggtctgctgctgc-3'). PCR conditions were 35 cycles with 30 s at 94 °C, 30 s at 58 °C, and 60 s at 72 °C followed by a final elongation for 10 min at 72 °C. For amplification of DAP10 and DAP12 (5'-catggatgtctggggtg-3'), PCR conditions were 40 cycles with 30 s at 94 °C, 30 s at 58 °C, and 60 s at 72 °C followed by a final elongation for 10 min at 72 °C.

Cell Culture and Transfections—Mast cell/basophil cultures were generated from bone marrow cells by in vitro culture for 6 days in RPMI 1640 supplemented with 10% fetal calf serum, 2% L-glutamine, 100 mg/liter chloramphenicol, and 5 ng/ml recombinant mouse IL-3 (R&D Systems, Minneapolis, MN). Mouse mastocytoma P815 cells and human embryonic kidney 293T cells were cultured in Dulbecco’s modified Eagle’s medium 1640 supplemented with 10% fetal calf serum, penicillin/streptomycin, and 1640 glutamine (Sigma-Aldrich). Cell lysates were precleared with 60 μg/ml proteinase K before transfection, 293T cells were dispersed in 24-well tissue culture plates and transduced with plasmid DNA with lipofectamine 2000 (Invitrogen). Immunoprecipitations and Western Blots—1 × 10^7 human embryonic kidney 293T cells that were transiently transfected with human MycDAP12 and CD200R3C-GFP or CD200R3F-GFP were solubilized in 1 ml of Brij-Nonidet P-40 lysis buffer (0.875% Brij 97, 0.125% Nonidet P-40, 10 mM Tris base, 150 mM NaCl, and protease inhibitors, Sigma–Gmaid). Cell lysates were preheated with 60 μl of protein G-Sepharose beads (Amersham Biosciences) for 1 h at 4 °C. Anti-human DAP12 mAb DX37 (14) or isotype-matched control mAb-coated beads were used for immunoprecipitation of preheated lysates for 3 h at 4 °C. Samples were separated on a 12% SDS-PAGE, transferred to Immobilon P membrane (Millipore, Billerica, MA), blocked with 5% milk, incubated with rabbit anti-GFP antiserum (a kind gift from Mathias Wabl, University of California, San Francisco) followed by horseradish peroxidase-conjugated donkey anti-rabbit IgG (Amersham Biosciences), and visualized with chemiluminescent substrate (Pierce).

Reporter Cell Assay—An NFAT-GFP reporter construct (generously provided by Hisashi Arase, Osaka University, Japan) was stably transfected into BaF3 cells, and a clone was selected that expressed GFP upon treatment with 10 μg/ml phorbol 12-myristate 13-acetate and 1 μM ionomycin (Sigma) (15). Plasmids encoding DAP12 (14) and FLAG-tagged-NKp44 or FLAG tagged-CD200R3C were transfected into IL-3+ BaF3 NFAT-GFP reporter cells using the Amaxa Nucleofection Technology (Amaxa, Koen, Germany). Briefly, 5 × 10^5 cells were resuspended in 100 μl of nucleofector solution V, mixed with 2.5 μg of each plasmid, and transfected using program T-20 following the Amaxa guidelines for cell line transfection. Tissue culture plates were coated with 1 mg/ml 1,2-dioleoyl-3-trimethylammonium-propane (Sigma–Aldrich) for 10 min at room temperature before being buffered with saline. Plasmids were incubated with mAbs diluted in 0.1 M bicarbonate buffer (pH 9.0) for 16 h at 4 °C, and then washed once with phosphate-buffered saline and once with RPMI 1640. 24 h after transfection, cells were incubated for 20 h on antibody-coated plates and then analyzed for GFP expression by flow cytometry.

Isolation of Basophils from the Lung—Lungs of IL-4 reporter mice (4get mice) were isolated 10 days after N. brasiliensis infection and digested with collagenase/DNase for 30 min at 37 °C. Cell suspensions were blocked with Fc-Block and stained with allophycocyanin-conjugated anti-CD49b mAb DX5 (BD Biosciences). Basophils were sorted on a MoFlo cell sorter (Dako-Cytomation, Fort Collins, CO) based on their expression of eGFP and DX5 in 4get mice as described (17) and processed for RNA isolation as described above.

N. brasiliensis Infection—Mice were infected subcutaneously with 1.3 larval stages (500 organisms) in 200 μl of saline at the base of the tail and placed on antibiotic-containing water (2 g/liter neomycin sulfate, 100 mg/liter chloramphenicol) for 5 days, as described (18). Mice were analyzed on day 10 after infection.

Flow Cytometry—Lungs were perfused with 10 ml of cold phosphate-buffered saline via the right cardiac ventricle, excised, transferred into 10 ml of cold medium (RPMI 1640 supplemented with 10% fetal calf serum and 100 mg/liter penicillin) and minced with a nylon mesh (Corning Costar, Acton, MA). The cell suspension was washed once in medium, and erythrocytes were lysed with hypotonic lysis buffer. To determine the frequency of Thy2 cells in the lung, 5 × 10^6 total lung cells were stimulated for 2 h in vitro with 40 ng/ml phorbol 12-myristate 13-acetate and 1 μg/ml ionomycin and then stained for CD4 and IL-4 secretion using the IL-4 capture kit (Milltenyi Biotec) according to the manufacturer’s instructions. Blood samples were collected from the chest cavity in heparin-containing FACS buffer (phosphate-buffered saline, 2% fetal calf serum, 1 mg/liter NaN₃). To determine the frequency of eosinophils, cell suspensions were washed once with FACS buffer and the resuspended cell pellets were incubated for 5 min with anti-CD16/CD32 mAb (2.4G2) before staining with phycoerythrin-conjugated anti-CD11b (BD Biosciences). Basophils were identified by staining with biotinylated anti-IgE (BD Biosciences) followed by streptavidin-allophycocyanin (Molecular Probes, Leiden, Netherlands). For flow cytometric analysis of transfected 293T cells, cells were trypsinized 2 days after transfection and washed once in FACS buffer. Cell pellets were stained with M2, a biotinylated mouse anti-FLAG mAb (Sigma–Aldrich), followed by allophyocyanin-conjugated streptavidin.

IgE Enzyme-linked Immunosorbent Assay—Serum IgE levels were determined by a standard enzyme-linked immunosorbent assay protocol using the anti-mouse IgE monoclonal antibody B1E3 for coating and the biotinylated anti-mouse IgE monoclonal antibody EM95 for detection.

Microarray—The microarray experiment comparing gene expression of basophils and eosinophils from the lung of N. brasiliensis-infected mice has been described in detail elsewhere (17).

RESULTS

To identify genes that are differentially expressed in mouse basophils and eosinophils, we compared the gene expression profiles of these innate effector cells during an acute infection with the helmint parasite, N. brasiliensis. The most differentially expressed gene in basophils was the RIKEN clone 483340F19Rik (Fig. 1A). BLAST search analysis revealed that this clone encodes a member of the OX2/CD200 receptor (CD200R) family (10). In addition to the originally described inhibitory receptor CD200R, there are at least four potentially activating receptors in the mouse, which have been named CD200R-like proteins, a–d (CD200Rla–d). However, this alphabetic nomenclature has been revised with the understanding that mouse CD200RLa is not the ortholog of human CD200R and to accommodate potential splice variants (Fig. 1D) (19). To determine the tissue distribution of CD200R-like receptors, we performed RT-PCR analysis on selected tissues. The CD200R4 (CD200RLa) and CD200R3 (CD200Rlb) receptors were expressed in the lungs of N. brasiliensis-infected mice but not in the lungs of noninfected mice (Fig. 1C). The inhibitory receptor CD200R (now also named CD200R1 (19)) was
expressed at relatively high levels in the spleen and at lower levels in other tissues, including thymus, lung, liver, and small intestine. Increased expression of CD200R was also observed in the lungs of *N. brasiliensis*-infected mice. As compared with infected wild-type mice, lungs from infected rag-deficient mice showed lower expression levels of CD200R4, undetectable levels of CD200R3, and comparable levels of CD200R. In *vitro* generated mast cell/basophil cultures, the mastectomy cell line P815, and *ex vivo* isolated basophils expressed all four CD200R-like receptors. A BLAST search revealed a fifth member of this family, which we named CD200R5. This receptor has highly homologous. Each of these receptors, except for the inhibitory CD200R, contains a charged amino acid residue in the transmembrane domain and has a short cytoplasmic tail (Fig. 2A). CD200R3 is more distantly related to the other family members and in addition can be expressed in variant splice forms (Figs. 1B and 2).

To study whether the different splice versions of CD200R3 result in a change in the amino acid sequence and whether this determines their pairing with adaptor proteins, we cloned and sequenced the different bands indicated in Fig. 1B. First, we identified a different translation start upstream from the previously published start codon (10), which generates a signal peptide with higher homology to the other CD200R-like receptors (Fig. 2A). Full-length CD200R3 consists of seven exons. The first four exons encode two Ig-like domains that form the extracellular part of the molecule. Exon 5 encodes the transmembrane domain and can be alternatively spliced to either exon 6A (50 bp) or exon 6B (95 bp), which are separated by a 600bp intron. Alternatively, exons 6A and -B can be spliced out, allowing direct alignment with exon 7 (Fig. 3A). Each of these three splice variants generates a protein with a different cytoplasmic tail. In addition, exon 4 can be present or absent. Therefore, a total of six possible CD200R3 splice forms can be formed.

Different splice forms of mouse NKG2D have been shown to pair selectively with either DAP12 or DAP10, a process controlled by a short cytoplasmic sequence in mouse NKG2D (20). Because CD200R3 can have three different cytoplasmic tails, we analyzed the capacity of each of these splice forms to bring DAP12 to the cell surface. 293T cells were transiently transfected with FLAG-tagged DAP12 together with splice forms of CD200R3, which were expressed as bicistronic constructs linked to eGFP via an IRES, as described (14). Surface expression of DAP12 was determined by staining with anti-FLAG monoclonal antibody and flow cytometric analysis for eGFP and FLAG expression. Splice variant A, which contains exons 4 and 6A, could bring DAP12 to the cell surface comparably to CD200R4 and CD200R2, whereas splice variant E, which lacks exon 4 and contains the alternative exon 6B, could not (Fig. 3B). Thus, either the cytoplasmic tail (exon 6) or the extracellular part of the CD200R3 receptor (exon 4) plays an important role in DAP12 recruitment or surface expression of the receptor. To address these possibilities, we tested the remaining four splice variants. Using this system, we observed that exon 4 was required to detect DAP12 on the cell surface, whereas the cytoplasmic tail played a less critical role (Fig. 3C).

Basophils express at least three different signaling adaptor molecules: the FcRγ chain as part of the high affinity IgE receptor and both DAP10 and DAP12, as revealed by RT-PCR of *ex vivo* isolated basophils (Fig. 3D). To analyze whether the different splice variants of CD200R3 can pair with adaptor molecules other than DAP12 and whether other adaptors would have the capacity to bring exon 4-deficient splice variants to the cell surface, we cotransfected 293T cells with all six splice versions of CD200R3 together with FLAG-tagged DAP12, DAP10, FcRγ, or CD3ζ. As indicated in Fig. 4, exon 4-containing splice variants paired preferentially with DAP12 and, to a lesser extent, with DAP10 in this transfection assay. Splice variant CD200R3B showed reduced specific pairing with both DAP12 and DAP10, indicating that the cytoplasmic tail might influence the fine-tuning of receptor-adaptor pairing. Exon 4-deficient splice variants showed only a weak capacity to bring FcRγ or CD3ζ to the cell surface, and essentially no pairing was observed with either DAP12 or DAP10.

To exclude the possibility that the splice variants lacking exon 4 cannot generate a stable protein, we constructed C-terminal GFP fusion proteins with exon 4-positive and -negative splice variants. The expression levels of both fusion proteins in transiently transfected 293T cells were comparable based on the fluorescence intensity for GFP and Western blot analysis of total cell lysates (Fig. 5A and data not shown), indicating that both constructs generated a stable protein. However, as seen before, the exon 4-deficient splice variant (CD200R3F-GFP) could not bring DAP12 to the cell surface. In addition, communoprecipitation experiments showed that DAP12 pairs more efficiently with CD200R3C-GFP than with CD200R3F-GFP (Fig. 5B). To analyze further the signaling capacity of CD200R3C, we transfected BaF/3 reporter cells expressing GFP under control of
NFAT promoter elements with FLAG-tagged CD200R3C and DAP12. Surface cross-linking of FLAG-CD200R3C with anti-FLAG antibody induced the expression of GFP from the reporter cell line, indicating that an intact signal transduction pathway via DAP12 can be induced from this receptor (Fig. 5C).

DAP12 appears to be the preferential binding partner for the basophil/mast cell-specific activating receptor CD200R3 and other activating receptors; however, the relative importance of this adaptor molecule during an allergic type 2 immune response has not been described. Therefore, DAP12-deficient mice were infected with the helminth parasite *N. brasiliensis* and analyzed 10 days later for recruitment of eosinophils, ba-
sophils, and Th2 cells to the lung, worm expulsion from the intestine, and serum IgE levels. No major differences compared with wild-type mice were observed (Fig. 6). Thus, despite its role in transducing signals from this basophil/mast cell-specific receptor, DAP12 plays no obvious requisite role during N. brasiliensis infection, a well characterized model for type 2 immune responses.

DISCUSSION

Lymphoid and myeloid cells provide important effector functions during acute and chronic immune responses, when their activity is regulated by balanced expression of inhibitory and activating receptors. This provides protection against pathogens and prevents overreactive immune responses against host tissues. The major myeloid effector cells during type 2 immune responses associated with parasitic worms and allergic reactions are eosinophils, basophils, and mast cells. Understanding the regulation of effector function in these cell types has the potential to guide novel therapeutic interventions.

Results from our microarray and RT-PCR analysis indicate that basophils and eosinophils express relatively high levels of the signaling adaptor proteins DAP12 and DAP10, although the associated activating receptors are largely unknown (Fig. 3D and Ref. 17). Recently, a new DAP12-associated immunoglobulin-like activating receptor, myeloid-associated immunoglobulin-like receptor, or leucocyte mono-Ig-like receptor, was described in mouse mast cells (21, 22). However, this receptor is expressed in a broad range of other cells. The role of DAP12 has thus far been studied in type 1 inflammatory immune responses, where it plays an important role in regulating NK cell and macrophage function (11, 23–26). Here, we have investigated the relative importance of this adaptor molecule in mediating a type 2 immune response in vivo. Infection of mice with the helminth N. brasiliensis is a well established mouse...
model for type 2 immunity that shows the main features of an allergic response, including high serum IgE and markedly increased numbers of Th2 cells, basophils, and eosinophils in blood and the lungs. The analysis of DAP12-deficient mice, however, revealed no major differences compared with wild-type mice, suggesting that either DAP12-associated activating receptors are not critically involved in this immune response or that other signaling adaptors, such as DAP10 or FcR/H9253, provide redundant mechanisms (Fig. 6).

We found activating receptors of the CD200R family to be expressed by mouse basophils and mast cells. Expression of CD200R2 was detected only in in vitro generated bone marrow-derived mast cell/basophil cultures, the mouse mastocytoma cell line P815, and ex vivo isolated basophils, whereas CD200R3 could be detected in these cells as well as in N. brasiliensis-infected lung tissue from wild-type but not rag-deficient mice. Thus, these receptors are expressed preferentially in basophils and mast cells. Consistent with our previous observation, the reduced expression of these receptors in the infected rag-deficient mice reflects that T cells are required in the recruitment of basophils into the lung (17). CD200R3 appears to be an unusual member of this receptor family because it can be expressed with different splice versions, and it is only distantly related to the other family members. Transient transfection of this receptor into 293T cells revealed that exon 4, which encodes the second Ig-like domain, was required for stable pairing with DAP12 and cell surface expression of the receptor complex. This suggests that the extracellular part of the receptor may interact with a molecular chaperone in the endoplasmic reticulum and that exon 4 may be required for this interaction or that exon 4 could directly interact with the short extracellular part of DAP12 to form a stable complex. Exon 4-deficient splice versions did show a weak capacity to bring FcR/H9253 or CD3/H9256 to the cell surface, which indicates that these receptors might be surface-expressed with these other adaptors, although these interactions might reflect only weak associations under non-physiological conditions of overexpression by transfection (Fig. 4). For the exon 4-containing splice versions, DAP12 appeared to be the primary adaptor as assessed using this transient transfection assay. Interestingly, the cytoplasmic tail of CD200R3 seems to influence the pairing with adaptor proteins because CD200R3A and CD200R3C had better pairing ability with DAP12 and DAP10 as compared with CD200R3B in 293T cells (Fig. 4). Previous work demonstrated that the cytoplasmic tails of DAP10 or DAP12 themselves were not required for pairing and that the transmembrane regions were sufficient to confer specific interactions with NKG2D and CD94/NKG2C, respectively, although the extracellular part of DAP10 might increase the affinity for association with NKG2D (14). Different splice versions of mouse NKG2D have recently been shown to regulate pairing with DAP12. The short splice version lacks a 13-amino acid cytoplasmic extension and can pair with DAP12, whereas the long splice version, which con-

![Fig. 5. Expression of CD200R3 splice variants. A, plasmids encoding GFP fusion proteins of exon 4-containing and exon 4-lacking CD200R3 variants were transiently transfected together with FLAG-tagged DAP12 into 293T cells. Expression of the fusion proteins (GFP) and surface expression of DAP12 (FLAG) was determined by flow cytometry. B, communoprecipitation of DAP12 and CD200R3. 293T cells were transiently transfected with DAP12 and CD200R3-GFP or CD200R3F-GFP. Cell lysates were precipitated with anti-DAP12 antibody, separated on a SDS-gel, and then blotted and stained with anti-GFP antibody. IP: immunoprecipitation. C, signal transduction through CD200R3C. BaF/3 cells expressing GFP under control of NFAT promoter elements were transfected with Myc-tagged DAP12 and FLAG-tagged CD200R3C or, as a positive control, FLAG-tagged Nkp44. The receptors were cross-linked with plate-bound anti-FLAG antibody, and the expression of GFP was analyzed 20 h later. All dot plots show cells gated on total live cells.](http://www.jbc.org/)

![Fig. 6. Analysis of DAP12-deficient mice after N. brasiliensis infection. DAP12-deficient and wild-type (WT) mice were infected with 500 L3 larvae of N. brasiliensis and analyzed 10 days later. The frequency of lung and blood eosinophils (A) and basophils (B) and the frequency of Th2 cells in the lung (C) was determined by flow cytometry as described under “Experimental Procedures.” D, serum IgE levels were analyzed by enzyme-linked immunosorbert assay.](http://www.jbc.org/)
thereby forced the evolution of activating receptors in the host. Conceivably, these activating receptors have evolved to counteract the engagement of SIRP-associated activating receptor on myeloid cells, might have tomegalovirus infection (30, 31). Similarly, the ubiquitously ex-...cell activation. However, resistant mice have evolved the related
ceptors remains to be analyzed.

It was recently proposed that OX-2, the ligand for the inhibitory receptor CD200R, is also a ligand for CD200R2, CD200R3, and CD200R4 (19); however, another group has previously reported that OX-2 does not bind these receptors (10). Further studies are required to clarify these conflicting results. The activating CD200 receptors could, however, play an important role in IgE-independent mast cell and basophil activation. Interestingly, herpesviruses and poxviruses encode OX-2-like molecules, suggesting that viruses capture this gene from the host to down-modulate the immune response against infected cells via the inhibitory receptor CD200R (27). In fact, it has been shown that the OX-2-like protein of the human Kaposi sarcoma virus inhibits myeloid cell activation and binds CD200 with an affinity similar to the natural ligand CD200 (28). However, another group reported that the human Kaposi sarcoma virus-encoded CD200-like protein activates rather than inhibits myeloid cells (29). Whether mouse herpesviruses encode CD200-like proteins that can bind to CD200R-like receptors remains to be analyzed.

Mast cells and basophils are generally not associated with anti-viral immune responses. However, mucosal mast cells would be positioned to mediate an early immune response against viral infections inoculated at that site. Mouse cytomegalovirus encodes the major histocompatibility complex-like molecule m157, likely captured from the host genome, which binds to the inhibitory receptor Ly49H and down-modulates NK cell activation. However, resistant mice have evolved the related activating receptor Ly49H, which protects mice from mouse cytomegalovirus infection (30, 31). Similarly, the ubiquitously expressed ligand CD47 binds the inhibitory receptor SIRPα, and *Poxviridae* encode CD47-like proteins, although it is not known whether they can bind to SIRPα. SIRPβ3, an orphan DAP12-associated activating receptor on myeloid cells, might have evolved to counteract the engagement of SIRPα by Poxviruses (16, 27). Conceivably, these activating receptors have evolved because of the selective pressure imposed by viruses or other pathogens, which initially targeted inhibitory receptors and thereby forced the evolution of activating receptors in the host.

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