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CD200R surface expression as a marker of murine basophil activation

M. N. Torrero, D. Larson, M. P. Hübner, and E. Mitre

Department of Microbiology and Immunology, Uniformed Services University of the Health Sciences, Bethesda, MD, USA

Summary

Background—Basophils are increasingly recognized as playing important roles in the immune responses of allergic diseases and helminth infections. One of the main obstacles to studying basophils has been the lack of a simple and rapid assay to measure basophil activation in mice.

Objective—The purpose of this study was to develop an assay to measure murine basophil activation.

Methods—Mouse blood cells were stained with various combinations of positive and negative markers for basophils – sorted and then assessed for basophil purity by May-Grünwald staining of cytopins. Once a flow cytometric strategy for staining basophils was determined, basophil surface expression of CD200R was assessed by multi-colour flow cytometry after stimulation of whole blood with anti-IgE, ionomycin or N-formyl MetLeuPhe (fMLP). Confirmation of basophil activation was assessed by concomitant staining of cells for intracellular IL-4. To test the ability of flow cytometric basophil CD200R measurements to assess for antigen-specific IgE-mediated activation of basophils, surface CD200R expression in response to *in vitro* stimulation with media alone, helminth antigen or ovalbumin was measured on basophils obtained from control mice, mice infected with helminths and mice sensitized to ovalbumin.

Results—Using anti-IgE-FITC as a positive marker and a combination of anti-CD4-PERCP and anti-B220-PERCP as negative markers resulted in a well-separated basophil population. Additional staining with anti-CD200R-PE demonstrated that (1) basophil CD200R expression increases in response to anti-IgE, ionomycin and fMLP, (2) most CD200R-positive basophils also stain positively for IL-4 and (3) CD200R expression increases after antigen-specific activation of basophils in murine models of helminth disease and allergy.

Conclusion—We developed a multi-colour flow cytometry assay that measures murine basophil activation by utilizing CD200R as an activation marker. This assay is straightforward and rapid, taking approximately half a day for obtaining blood, *in vitro* stimulation and flow cytometric analysis.

Keywords

allergy; basophil; CD200R; cellular activation; flow cytometry; helminth; IgE; murine

Introduction

Basophils are the least common white blood cells, typically constituting less than 0.5% of circulating leucocytes and nucleated bone marrow cells. Because of their rarity, basophils have

not been as extensively studied as other leucocytes. In recent years, however, there has been increasing recognition that basophils play important roles in the immune responses induced by allergic diseases and helminth infections [1,2].

To date, the study of basophil function in murine models of allergy and helminth infection has been greatly limited by the lack of a simple assay to measure murine basophil activation. Unlike human basophils, on which CD203c and CD63 expression can be measured to rapidly assess cellular activation by flow cytometry [3,4], no activation markers have yet been identified on murine basophils.

CD200R (aka CD200R1 [5]) is an inhibitory receptor that belongs to the immunoglobulin superfamily [6,7]. While initially described as being primarily expressed on cells of the myeloid lineage, recent studies demonstrate that CD200R is also expressed on granulocytes, T cells, and mast cells in both mice and humans [8–11]. Although surface basophil expression of CD200R has not yet been evaluated in mice, studies of human cells demonstrate that all basophils express CD200R [11]. Further, in comparison with other circulating leucocytes in humans, basophils have the greatest amount of surface CD200R expression by relative brightness on flow cytometry [12]. Murine basophil surface CD200R expression and changes in response to cellular activation, however, have not been previously assessed.

In this study, we sought to develop a flow cytometric assay to detect murine basophil activation. This was done by initially testing the ability of various antibody combinations to correctly identify murine basophil populations by flow cytometry. Once this was accomplished, changes in surface expression of CD200R were assessed in response to IgE-mediated activation in normal mice and in murine models of helminth infection and allergic disease. Our results demonstrate that we have developed a rapid flow cytometric assay for the detection of murine basophil activation utilizing CD200R as an activation marker.

Materials and methods

Animals

Female BALB/C mice (NCI Mouse Repository, Frederick, MD, USA) were maintained at the Uniformed Services University (USUHS) animal facility. Experiments were performed with mice between 4 and 8 weeks of age under a protocol approved by the USUHS Institutional Animal Care and Use Committee. At study endpoints, mice were euthanized with carbon dioxide and whole blood obtained by cardiac puncture. For all experiments, whole blood was collected in heparinized microfuge tubes (Sarstedt, Numbrecht, Germany).

Antibodies

Purified rat anti-mouse IgE (R35-92) was used for basophil stimulation. The following anti-mouse antibodies were used for flow cytometry: anti-Fc ϵ RI α FITC (MAR-1), anti-IgE FITC (R35-72), anti-CD123 FITC (5B11), anti-CD123 PE (5B11), anti-B220 PERCP (RA3-6B2), anti-CD49b APC (HMA2), anti-CD200R-PE (OX-110), anti-CD200R-Alexa Fluor 647 (OX-110), anti-CD4 PERCP (RM4-5), anti-CD117 c-Kit APC (2B8) and anti-IL-4 APC (11B11). Antibodies MAR-1 and 5B11 were purchased from eBioscience (San Diego, CA, USA), OX-110 from AbD Serotec (Kidlington, Oxford, UK), 2B8 from Biolegend (San Diego, CA, USA) and the rest from BD Pharmingen (San Diego, CA, USA).

Identification of murine basophils by flow cytometry

Whole blood (100 μ L) was diluted with 100 μ L of RPMI 1640 (Cellgro; Mediatech, Herndon, VA, USA). Cells were washed twice with 2 mL of PBS and centrifuged at 500 $\times$ g for 5 min. Supernatants were aspirated and the cells were lysed with a whole blood lysing reagent kit

(Beckman Coulter, Fullerton, CA, USA). Immuno-Lyse was diluted 1: 25 in PBS and 1 mL of working solution was added to each tube and incubated 1 min at room temperature. Cells were immediately fixed with 250 μ L of fixative solution and washed twice with 2 mL of PBS and centrifuged at 500 $\times$ g for 5 min. Supernatants were aspirated and non-specific binding sites on cells blocked by re-suspending in 100 μ L of 1% BSA/PBS and incubating at 4 °C for 1 h. Cells were stained for 30 min with various two-, three- and four-colour combinations of positive and negative markers for murine basophils. Positive markers included anti-FcERI α FITC, anti-IgE FITC, anti-CD123 FITC, anti-CD123 PE, anti-CD200R PE, anti-CD49b APC and anti-CD200R-AlexaFluor 647. Negative markers included anti-CD4 PERCP, anti-B220 PERCP and anti-CD117 c-Kit APC. Cells were then washed twice with 2 mL of PBS and centrifuged at 500 $\times$ g for 5 min. Cells were re-suspended in 200 μ L PBS and analysed using a BD LSR II Optical Bench flow cytometer (Beckman Coulter) and Diva software (Beckman Coulter).

Staining strategies that resulted in well-separated putative basophil populations were then repeated using 300 μ L aliquots of murine blood. Cells falling within the putative basophil gate were sorted using a BD FACSAria high-speed cell sorter. May-Grünwald stains were then made of cytopins of sorted cells and evaluated for basophil purity.

Basophil activation assay

Whole blood (100 μ L) was diluted with 100 μ L of RPMI 1640 (Cellgro; Mediatech). Tubes with blood were incubated with media, 25 μ g/mL ionomycin (EMD Biosciences, LaJolla, CA, USA), anti-mouse IgE (at 0.031, 0.125 μ g/mL, or at various concentrations as described in 'Results'), *Litomosoides sigmodontis* antigen at 20 μ g/mL (LsAg, prepared from a homogenate of lyophilised adult *L. sigmodontis* worms), ovalbumin at 20 μ g/mL or N-formyl MetLeuPhe (fMLP, at 0.5 and 1 μ M) for 2 h at 37 °C in 5% CO₂. When intracellular IL-4 was measured along with CD200R, Monensin (BD GolgiStop protein transport inhibitor; BD Biosciences, San Diego, CA, USA) was added after 1 h of incubation at 2 μ M final concentration and the tubes were incubated for 2 more hours at 37 °C in 5% CO₂. Cells were washed twice with 2 mL of PBS and centrifuged at 500 $\times$ g for 5 min. Supernatants were aspirated and the cells were lysed and fixed using a whole blood lysing reagent kit (Beckman Coulter). Immuno-Lyse was diluted 1: 25 in PBS and 1 mL of working solution was added to each tube and incubated 1 min at room temperature. Cells were immediately fixed with 250 μ L of fixative solution and washed twice with 2 mL of PBS and centrifuged at 500 $\times$ g for 5 min. Supernatants were aspirated and non-specific binding sites blocked by re-suspending in 100 μ L of 1% BSA/PBS and incubating at 4 °C for 1 h or overnight. Cells were then stained with anti-IgE FITC, anti-CD4 PERCP, anti-B220 PERCP and anti-CD200R PE for 30 min at 4 °C, washed twice with 2 mL of PBS and centrifuged at 500 $\times$ g for 5 min. In studies in which intracellular IL-4 was also evaluated, cells were stained in a two-step manner. After surface staining and two washes, cells were permeabilised with BD Perm/Wash buffer, resuspended in 1% BSA/PBS, stained with anti-IL-4 APC for 30 min at 4 °C, and washed twice. Cells were resuspended in 200 μ L PBS and analysed using a BD LSR II Optical Bench flow cytometer and Diva software.

For all flow cytometry experiments, antibodies were individually titrated before use and compensations assessed using BD compBeads bound to the antibodies being utilized for that experiment. For basophil activation assays, cut-off gates for CD200R-PE, and IL-4-APC positivity were established using the fluorescence-minus-one (FMO) technique [13].

Infection of mice with *Litomosoides sigmodontis*

Jirds that were exactly 4 days post-exposure to mites infected with *L. sigmodontis* were obtained from TRS Labs in Athens, Georgia. Upon arrival to our laboratory, jirds were euthanized and pleural lavage immediately performed for the recovery of infective-stage L3 larvae. Recovered larvae were then washed by allowing the larvae to sediment to the bottom

of a petri dish at 1×g aspirating the supernatants, and adding 10 mL of fresh RPMI media. Female BALB/c mice were then inoculated subcutaneously between the shoulder blades with 40 L3 larvae in 150 µL RPMI.

Sensitization of mice with ovalbumin

BALB/c mice were sensitized to ovalbumin by administration of 100 µL of a 0.5 mg/mL solution of ovalbumin (Sigma, St. Louis, MO, USA) adsorbed to inject alum (Pierce) by intraperitoneal injection every 2 weeks for a total of two doses. Control mice were vaccinated with PBS alone.

Specific immunoglobulin E and interleukin-4 enzyme-linked immunosorbent serologic assays

Blood was collected from *L. sigmodontis* infected female BALB/c mice or ovalbumin-sensitized female BALB/c mice at various time-points by cardiac puncture and analysed for LsAg-specific IgE measured by colorimetric sandwich ELISA. Flat-bottom Immulon 4 plates (Thomas Scientific, Swedesboro, NJ, USA) were coated overnight at 4 °C with 20 µg/mL LsAg. Blocking was performed with 5% bovine serum albumin (BSA) PBS. IgG was adsorbed by incubating serum samples with GammaBind G Sepharose (Amersham Biosciences, Uppsala, Sweden) overnight at 4 °C. Then, samples were centrifuged and supernatants used for the assay. Plates were washed six times and incubated with 2 µg/mL biotinylated rat anti-mouse IgE (clone R35-118) in PBS. Plates were washed again and incubated with 1 mg/mL of alkaline phosphatase-conjugated streptavidin (BD Pharmingen). Substrate *p*-nitro-phenyl phosphate disodium (Sigma) was added at 1 µg/mL in sodium carbonate buffer after six washes. Colorimetric development was detected at 405 nM using a Perkin Elmer Victor V microplate reader. IL-4 was quantified using a commercial IL-4 kit according to manufacturer's instructions (eBioscience).

Statistical analysis

Comparisons between groups of paired data were performed with the nonparametric Wilcoxon signed rank test and strength of correlation derived using the Spearman Rank test (Prism 4 Statistics software; GraphPad Software, San Diego, CA, USA).

Results

Identification of murine basophils by flow cytometry

To identify murine basophils by flow cytometry, whole blood samples from BALB/c mice were stained with various two-, three- and four-colour combinations of positive and negative markers for basophils. Positive markers included anti-FcERIα FITC, anti-IgE FITC, anti-CD123 FITC, anti-CD123 PE, anti-CD200R PE, anti-CD49b APC and anti-CD200R-AlexaFluor 647. Negative markers included anti-CD4 PERCP, anti-B220 PERCP and anti-CD117 c-Kit APC. Several antibody combinations worked well to identify basophil populations in murine blood. These included anti-FcERIα FITC/anti-CD49b APC/anti-CD4 PERCP/anti-B220 PERCP, anti-IgE FITC/anti-CD49b APC/anti-CD4 PERCP/anti-B220 PERCP, anti-IgE FITC/anti-CD117 c-Kit APC/anti-CD4 PERCP/anti-B220 PERCP and anti-IgE FITC/anti-CD4 PERCP/anti-B220 PERCP. In general, approaches that used anti-IgE antibody resulted in greater separation of the basophil population than approaches using anti-FcERIα. Figures 1a and b demonstrate the simplest effective strategy for identifying basophils by flow cytometry. Initially, a large gate is drawn that encompasses the typical lymphocyte region and the lower half of the typical granulocyte region on the forward and side scatter dot plot (Fig. 1a). Gated cells that stain positively for IgE and negatively for CD4 and B220 are identified as basophils (Fig. 1b). Confirmation that this strategy results in identification of

basophils comes from the findings that this gated population stains very brightly for CD49b (Fig. 1c), a cell surface marker known to be highly expressed on basophils, and that sorting of this population routinely results in recovery of cells that are >98% basophils (Figs 1d and e). Addition of anti-CD49b-APC to the anti-IgE FITC/anti-CD4 PERCP/anti-B220 PERCP strategy resulted in no substantial increase in basophil purity after sorting, whereas purity can be increased to >99% by addition of anti-cKit-APC as an additional negative marker (data not shown).

Basophil CD200R surface expression increases upon cellular activation

To assess whether CD200R is a marker of IgE-mediated basophil activation, whole blood samples from five uninfected and 14 *L. sigmodontis* infected BALB/c mice were incubated with media or 0.125 µg/mL anti-IgE for 2 h and then stained for flow cytometry with anti-IgE FITC, anti-CD4 PERCP, anti-B220 PERCP and anti-CD200R PE. Basophil populations were identified as IgE+/CD4-/B220- cells as depicted in Fig. 1b, and the cut-off for anti-CD200R PE positivity determined using the FMO approach (Fig. 2a). In all mice evaluated, anti-IgE stimulation resulted in increases in the percentages of basophils expressing CD200R compared with media stimulation (Fig. 2b, geometric mean (GM) = 23.1% CD200R+basophils after anti-IgE stimulation vs. 6.3% after incubation in media alone, $P = 0.0001$). Similarly, the mean fluorescence intensity (MFI) of CD200R PE staining of the basophil population increased in all mice evaluated (basophil CD200R PE MFI = 355 after anti-IgE stimulation vs. 250 after incubation in media alone, $P < 0.0001$, data not shown).

To determine whether CD200R expression is up-regulated after IgE-independent activation of basophils, whole blood samples from six uninfected and four *L. sigmodontis* infected BALB/c mice were evaluated for CD200R expression on basophils by flow cytometry after stimulation with media or 25 µg/mL ionomycin. As with anti-IgE stimulation, incubation of whole blood with ionomycin resulted in increases in the percentages of CD200R+basophils in all mice studied (Fig. 2c, GM = 12.6% CD200R+basophils after ionomycin stimulation vs. 3.8% after incubation in media alone, $P = 0.002$). Similarly, as seen in Fig. 2d, MFI of CD200R PE staining on basophils increased in response to activation with all tested basophil activating agents as compared to media. This suggests that both percentage CD200R positivity and changes in basophil CD200R MFI can be used to detect activation. Further, increases in CD200R expression occur in response to both IgE-independent (ionomycin, fMLP) and IgE-mediated pathways of activation.

Up-regulation of CD200R expression on basophils correlates with basophil interleukin-4 positivity

As previous studies have demonstrated that intracellular basophil IL-4 positivity as detected by flow cytometry increases upon basophil activation [14,15], we sought to confirm that CD200R is a marker of murine basophil activation by correlating surface expression of CD200R with basophil IL-4 positivity. For these experiments, whole blood samples from uninfected and 1-week *L. sigmodontis* infected BALB/c mice were incubated for 3 h with either media alone or anti-IgE, with addition of monensin after the first hour of incubation. Cells were initially stained for surface markers (IgE, CD4, B220 and CD200R) and then permeabilized and stained for intracellular IL-4.

As expected, anti-IgE stimulation of basophils resulted in increased percentages of IL-4+, CD200R+ and IL-4+CD200R+basophils (Figs 3a and b) compared with media. As seen in Fig. 3b, there was a correlation between basophil surface CD200R expression and intracellular IL-4 expression (Spearman's rank correlation coefficient $r = 0.838$, $P < 0.0001$). Correlation of IL-4 expression and CD200R staining was not complete, as on average 60% of IL-4+basophils stained positively for CD200R (median 60, range 10–97%) whereas only 36% of CD200R

+basophils stained positively for IL-4 (median 31, range 10–62%). While it is possible that CD200R may be a more sensitive marker of basophil activation than IL-4 expression, it is likely that some of the differential staining of IL-4 and CD200R may have been due to differences in the time-points at which these molecules are optimally expressed.

Basophil CD200R expression is dose- and time-dependent

To determine dose- and time-dependent activation parameters of basophil CD200R expression, whole blood samples from BALB/c mice were incubated either with increasing anti-IgE concentrations or with 0.125 µg of anti-IgE for 30 min, 1, 2, 3 or 4 h and then stained for flow cytometric analysis of CD200R expression. As shown in Fig. 4a, CD200R expression demonstrates dose-dependent activation in response to increasing concentrations of activating anti-IgE antibody.

CD200R expression was also found to be time-dependent, with time-course studies showing that the percentages of basophils staining positively for CD200R were significantly increased after 30 min of incubation with anti-IgE as compared with incubation with isotype control antibody (52% CD200R+vs. 1%), peaked after 1–2 h of incubation with anti-IgE (72–80% CD200R+vs. 1%), and then decreased to undetectable expression after 4 h (Fig. 4b).

Basophil CD200R expression increases in response to antigen-specific immunoglobulin E mediated stimulation

To assess whether CD200R expression can be used to detect antigen-driven IgE-mediated basophil activation, we measured CD200R positivity on basophils obtained from mice infected with *L. sigmodontis*, an invasive filarial nematode of rodents, after *in vitro* challenge with parasite antigen (LsAg). Whole blood samples from BALB/c mice infected for 1 ($n = 4$) or 8 weeks ($n = 5$) with *L. sigmodontis* were incubated with LsAg at 20 µg/mL for 2 h and then assessed for basophil surface CD200R expression by flow cytometry. BALB/c mice infected for only 1 week, at which time there was no detectable circulating LsAg-specific IgE (Fig. 5a), demonstrated no increase in percentages of CD200R positive basophils in response to LsAg (Fig. 5b, GM = 2.9% CD200R+basophils after media incubation vs. 3.4% after LsAg incubation, $P = 0.84$). In contrast, basophils of BALB/c mice infected for 8 weeks, when there were substantial amounts of serum LsAg-specific IgE (Fig. 5a), exhibited significant increases in CD200R positivity when incubated with LsAg as compared with media (GM = 4.8% CD200R+basophils after media incubation vs. 25.6% after incubation with LsAg, $P = 0.03$; Fig. 5c).

To confirm that LsAg and anti-IgE actually activate basophils of mice infected with *L. sigmodontis* for 8 weeks, we measured supernatant IL-4 concentrations of whole blood after 2 h of incubation with media, LsAg or anti-IgE. As seen in Fig. 5d, both LsAg and anti-IgE stimulation of blood from mice infected with *L. sigmodontis* for 8 weeks substantially increase supernatant IL-4 concentrations. As basophils are the only cells within whole blood known to have the ability to release IL-4 within 2 h of activation [16], these results confirm that both anti-IgE stimulation and LsAg activate basophils of 8-week infected mice.

To evaluate whether CD200R expression can be used to detect Ag-specific IgE activation in a murine model of allergy, mice were sensitized against ovalbumin by repeated injection of ovalbumin adsorbed to alum (OVA-sensitized, $n = 5$) or given injections of PBS–alum alone (control group, $n = 5$) and then assessed for basophil surface expression of CD200R by flow cytometry after stimulation with either media alone or ovalbumin. Serum OVA-specific IgE was detectable by ELISA in all sensitized mice and in none of the control mice (data not shown). Whereas control mice demonstrated no increase in percentages of CD200R+basophils after

incubation of whole blood with ovalbumin, sensitized mice developed significantly greater percentages of CD200R+basophils after incubation with ovalbumin (Fig. 6, $P = 0.032$).

Discussion

Recent studies indicate that basophils play significant roles in the immune responses induced by helminth infections and allergic diseases [1,2]. To date, however, study of their roles in murine models of disease has been hampered by the lack of a direct assay to detect their activation in mice. This study demonstrates that CD200R can be utilized as a marker of murine basophil activation.

Murine basophil surface expression of CD200R, as detected by flow cytometry, increased in response to *in vitro* activation of whole blood with anti-IgE, ionomycin or fMLP. These results demonstrate that CD200R up-regulation occurs in response to both IgE-dependent and independent pathways of basophil activation. Additionally, incubation of whole blood obtained from mice infected with *L. sigmodontis* or sensitized against ovalbumin resulted in up-regulation of basophil CD200R surface expression in response to LsAg or ovalbumin once circulating antigen-specific IgE had developed. Further evidence that CD200R is a murine basophil activation marker came from the finding that its expression on basophils increased in proportion to increases in intracellular basophil IL-4 positivity after cellular activation. Finally, basophil CD200R surface expression was found to increase in a dose-response fashion to increasing concentrations of anti-IgE.

One of the advantages of this whole blood assay is that it does not require any basophil priming cytokines or enrichment steps, which in human basophils have been shown in some cases to affect expression of surface activation markers [17]. If one wanted to conduct such manipulations before assessing basophil activation by surface CD200R expression, however, it would be important to first assess whether such manipulations were altering baseline surface CD200R staining.

CD200R surface up-regulation was found to be rapid and transient, as time course studies demonstrated CD200R surface up-regulation occurs as early as 30 min after IgE-mediated activation, peaks at 1–2 h, and then returns to baseline by 4 h after activation. This time-course is similar to the kinetics of CD63 up-regulation on human basophils, which becomes maximal 60 min after activation, but is slower than that observed for human basophil CD203c expression, which peaks within 15–20 min [18]. While the reason for different kinetics of human basophil activation markers is not known, it is presumed due to differences in the pathways by which these markers translocate from cytoplasmic compartments to the extracellular space [18].

While CD200R has been observed to be present on human basophils in prior studies [11,12], to our knowledge this study is the first to demonstrate that CD200R is also present on murine basophils and that its expression on these cells increases in response to cellular activation. As ligation of CD200R inhibits FcERI-mediated activation and degranulation of human basophils and mast cells [8,12,19], it is possible that the transient up-regulation of CD200R we observed on the surface of activated murine basophils represents a negative-feedback mechanism that enables inhibition of further FcERI-mediated activation in the hours immediately following initial cellular activation.

Recently, a number of research groups have discovered that the mouse genome contains a family of CD200R-like proteins with close homology to CD200R (aka CD200R1). These include CD200R2 (CD200RLc), CD200R3 (CD200RLb), CD200R4 (CD200RLa) and CD200R5 [5,11,20]. The antibody we utilized in our studies (anti-CD200R, OX-110) binds to CD200R but not to CD200R3 and CD200R4 [11]. CD200R, CD200R2, CD200R3 and

CD200R4 have all been shown capable of binding to CD200 [5], a membrane protein expressed by thymocytes, activated T cells, B cells, neurons and endothelial cells [6]. However, while ligation of CD200R typically results in inhibitory signals, other CD200R family members contain the positively charged amino acid lysine in their trans-membrane regions and thus can associate with ITAM- or YxxM motif-bearing adaptor molecules such as DAP12 that mediate stimulatory signals [5,11,20]. As such, it has been speculated that CD200R and CD200R-like receptors may serve as balancing inhibitory and activating receptors [5]. With respect to basophils, ligation of CD200R3 molecules has recently been demonstrated to result in activation of murine basophils and mast cells [21]. As ligation of CD200R inhibits FcERI-mediated activation of these cells [8,12,19], it is possible that releasability of murine basophils is controlled by the relative surface expression of CD200R and other CD200R-like molecules.

In conclusion, we have shown that the inhibitory receptor CD200R is rapidly up-regulated after murine basophil activation. By utilizing this marker and a combination of positive and negative basophil markers, we have developed a flow cytometric assay for detection of basophil activation in whole blood. This assay is straightforward and rapid, taking approximately 6 h for obtainment of blood, *in vitro* stimulation and flow cytometric analysis. Additionally, given the known inhibitory properties of CD200R ligation on basophils, the finding of CD200R up-regulation in response to FcERI-mediated basophil activation suggests CD200R may function as a negative feedback mechanism in the hours immediately after basophil activation.

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Fig. 1.

Gating strategy to identify basophils by flow cytometry. (a) Initial gating by forward and side scatter. (b) Basophils identified as CD4-B220-IgE+ cells (0.76% of all circulating leucocytes). (c) Gated basophils stain brightly for CD49b. May-Grünwald staining of cytopsin from basophils obtained after sorting on CD4-B220-IgE+CD49b+ cells shown at 200× (d) and 1000× (e).

Fig. 2.

Basophil CD200R expression increases in response to both IgE dependent and IgE independent pathways of activation. (a) Representative dot plots of basophil CD200R expression. Basophils were gated as shown in Figs 1a and b. Left panel demonstrates setting of PE cut-off for PE positivity by fluorescence-minus-one staining (cells stained with all antibodies except anti-CD200R PE). Middle and right panels demonstrate CD200R staining of basophils after incubation of whole blood with media and anti-IgE. (b) Percentage of basophils that are CD200R⁺ after whole blood incubation with media or anti-IgE. (c) Percentage of basophils that are CD200R⁺ after whole blood incubation with media or ionomycin. (open circles: uninfected mice, closed circles: infected mice). (d) Fold increase over media in mean fluorescence intensity of CD200R on basophils after stimulation with various concentrations of ionomycin, fMLP and anti-IgE ($n = 7$ per condition). Means $\pm$ SEMs are shown.

Fig. 3. Basophil surface CD200R expression correlates with basophil IL-4 positivity. (a) Representative dot plots of basophil surface CD200R expression and intracellular IL-4 positivity after incubation with media and anti-IgE. (b) Correlation between CD200R+ basophils and IL-4+ basophils. Solid squares represent whole blood incubated with anti-IgE and open squares represent whole blood without stimulation.

Fig. 4. Basophil CD200R expression as detected by flow cytometry is dose- and time-dependent. (a) Percentages of basophils expressing CD200R as detected by flow cytometry after 2 h of incubation with increasing quantities of anti-IgE. (b) Percentages of basophils expressing CD200R in response to 125 ng/mL anti-IgE after 0.5, 1, 2, 3 and 4 h of incubation.

Fig. 5.

In vitro parasite antigen exposure increases CD200R expression in basophils of parasite-infected mice once they develop parasite antigen-specific IgE. (a) Plasma LsAg-specific IgE levels 1 and 8 weeks post-infection. Percentages of basophils that are CD200R+ as determined by flow cytometry after incubation of whole blood in media or LsAg at 1 week (b) and 8 weeks (c) post-infection. (d) Supernatant IL-4 concentrations after 2h incubation of whole blood obtained from 8 week *Litomosoides sigmodontis* infected mice (results representative of three independent experiments).

Fig. 6.

OVA increases CD200R expression in basophils of BALB/c mice in OVA-alum vaccinated mice that have developed OVA-specific IgE. Mice were injected i.p. with OVA-alum or PBS and whole blood was incubated with ovalbumin or media alone. Percentages of basophils that were CD200R positive were measured by flow cytometry. (open bars: PBS/alum vaccinated mice, closed bars: OVA/alum vaccinated mice). Means $\pm$ SEMs are shown.