Marked Differences in the Signaling Requirements for Expression of CD203c and CD11b versus CD63 Expression and Histamine Release in Human Basophils

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Key Words

Activation markers · Histamine release · Desensitization · Actin polymerization

Abstract

Many techniques are being used to examine the status of circulating human basophils including the enhanced expression of a variety of cell surface proteins. There is accumulating evidence that there are at least two compartments containing these activation marker proteins but there are only some indications for the signaling requirements for each of the compartments. This study began with published reports by other investigators who potentially dissociated CD63 expression from anaphylactic degranulation with the p38 inhibitor, SB203580, a possible falsification of a previously proposed hypothesis regarding CD63 expression. To explore the signaling requirements for CD63, a variety of pharmacological agents were used to inhibit or enhance 4 endpoints of basophil activation. First, it was found that inhibition of both histamine release and CD63 expression with SB203580 was concordant. But it was also found that this agent had no effect on increased expression of CD203c and CD11b. Actin polymerization inhibitors caused marked enhancement of CD63 expression (concordant with their effects on degranulation) with no effect on expression of CD203c and CD11b.

The third generation syk inhibitor, NVP-QAB205, showed a 5-fold lower potency for inhibiting expression of CD203c and CD11b than for CD63. Finally, while desensitization of CD11b and CD203c expression occurs, it is slower than desensitization of the CD63 response. Taken together, these various observations demonstrate a marked difference in the early signaling requirements for the CD11b/CD203c compartment and CD63 degranulation and provide support for the hypothesis that CD11b and CD203c reside in a similar compartment.

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Introduction

Recent studies have begun to tease apart the conditions that lead to enhanced expression of various proteins that appear on the plasma membrane during different forms of stimulation of human basophils. Proteins such as CD63, CD203c and CD107a have been found to be useful replacement indicators of basophil activation and, with some caveats, markers of basophil histamine release [1–13]. However, various studies have noted that basophil responses measured by changes in surface proteins and mediator secretion are not always concordant [4, 5, 7, 14–18]. If these surface markers of activation are to be used

to understand the *in vivo* activation of basophils, then more information is needed about their signal transduction requirements to properly interpret what is occurring. For example, previous studies comparing changes in CD63 expression versus histamine release suggested that the two indicators of basophil activation could be dissociated under conditions where the type of degranulation was primarily of the so-called 'piece-meal' type [18–21]. Under these conditions, histamine release could occur without increased expression of CD63, but according to the model proposed, the reverse could not happen i.e. increased CD63 expression without histamine release. This is because additional CD63 only appears on the cell surface when histamine release occurs by anaphylactic degranulation.

This study developed from explorations into the signaling requirements for CD63 expression. There were two results that were discovered to further separate activation marker expression from histamine release. The first of these developed from three unrelated reports, that when taken together, suggested that CD63 expression could occur in the absence of histamine release, the precise condition that our proposed model predicted could not happen. A study by Gibbs et al. [16] demonstrated that the p38 inhibitor, SB203580, could inhibit histamine release. Inhibition in this study ranged from 60 to 70% for optimal levels of stimulation. In contrast, a study by Aerts et al. [5] indicated that SB203580 had little inhibitory effect on CD63 expression. In this study, optimal antigen-induced expression of CD63 was inhibited by only 11%. In a study by Ebo et al. [22], optimal stimulation with anti-IgE antibody was inhibited by only 14%. Taken together, the combined results might constitute a refutation of the proposed model of CD63 expression or at least strain the quality of the hypothesis. Therefore, this possibility needed to be examined in a side-by-side comparison of the inhibitor effects of this drug for histamine release and CD63 expression.

Since we were studying regulation of one activation marker, CD63, it was feasible to also study two additional markers from the same samples, CD203c and CD11b. There is developing information that CD203c expression is derived from a different compartment of the cell, although the precise nature of this compartment is not known. CD203c expression is clearly induced by substances that are not known to induce histamine release or CD63 expression [7, 17]. CD11b was included because there was suggestive evidence that the CD203c compartment might be a fast-release vesicle [4]. CD11b expression has been studied in some detail in neutrophils [23–27].

The protein is also expressed in basophils, being used by the basophil for the same purposes as the neutrophil [28]. Therefore, demonstrating similarity in the control of CD203c and CD11b would provide supportive evidence that CD203c was derived from the same compartment as CD11b and, therefore, immediately allow the base of knowledge about CD11b to be applied to CD203c.

The study expanded to several other pharmacological agents when unexpected results were found for regulation of CD203c/CD11b expression by SB203580. The actin polymerization inhibitors, latrunculin A or cytochalasins B or D have long been known to significantly upregulate mediator release [29–35]. One of the proposed mechanisms of this effect has been that actin polymerization modulates some of the early signal downregulatory events and, if so, it might be predicted that these drugs should act equally on events that resulted from early activation events. In addition, because CD203c expression appears to occur from a different compartment that might be a fast-release vesicle that is dependent on the actin network, it was useful to ask whether the effects of these actin polymerization inhibitors applied to this alternate compartment. The behavior of these agents was also unexpected and provided another example of the discordance of CD203c expression from mediator release.

Materials and Methods

Materials

The following were purchased: piperazine-N,N-bis-2-ethanesulfonic acid (PIPES), bovine serum albumin (BSA), EGTA, ethylenediamine N, N, N', N'-tetraacetic acid (EDTA), D(+)-glucose and fMLP (formyl-Met-Leu-Phe) – all from Sigma-Aldrich, St. Louis, Mo., USA, crystallized human serum albumin (HSA; Miles Laboratories, Elkhart, IN), SB203580 and latrunculin A (Calbiochem, EMD BioSciences, LaJolla, Calif., USA), cytochalasin D (Sigma), the 3rd-generation syk inhibitor, NVP-QAB205 (a gift from Glaxo-SmithKline), Percoll (Pharmacia, Piscataway, N.J., USA), anti-CD63 antibody (clone H5C6, BD BioSciences, San Jose, Calif., USA), anti-CD203c (clone NP4D6, BioLegend, San Diego, Calif., USA), anti-CD11b (clone Bear1, Beckman-Coulter, Sykesville, Md., USA) and mouse IgM anti-human IgE, HP6061P (Hybridoma Reagent Laboratory, Baltimore, Md., USA).

Buffers

PIPES-albumin-glucose (PAG) buffer consisted of 25 mM PIPES, 110 mM NaCl, 5 mM KCl, 0.1% glucose and 0.003% HSA. PAGCM was PAG-supplemented with 1 mM CaCl₂ and 1 mM MgCl₂. PAG-EDTA consisted of PAG supplemented with 4 mM EDTA, elutriation buffer and PAG containing 0.25% BSA. Phosphate-buffered saline (PBS) was 20 mM phosphate-buffered isotonic saline (pH 7.4) and was often supplemented with 4% BSA (Sigma).

Basophil Purification

While flow cytometry for CD63 or CD203 could have been performed with whole blood or mixed leukocytes, some of the experiments required purified basophils; to be consistent, all were performed with purified basophils. Residual cells of normal donors undergoing leukapheresis were enriched in basophils using a combination of Percoll density gradients and countercurrent-flow elutriation and negative selection, as previously described [36]. Basophil purities generally exceeded 99% purity. HIPPA (confidentiality and privacy) regulations do not allow identification or classification of the leukapheresis donors, so information regarding the atopic status of the subjects was not available.

Basophil Stimulation for Activation Markers and Histamine Release

When both activation markers and histamine release were assessed, purified basophils (600,000–800,000 per condition) were challenged in 60 μ l of PAGCM buffer for 20 min at 37°C, after which 10 μ l of cell suspension was transferred to cold EDTA-PAG and centrifuged to pellet the cells and recover the supernatant for the measurement of histamine content. Histamine was measured in supernatants by automated fluorometry [37]. To stop the reaction, 50 μ l of a 2% paraformaldehyde solution in PBS was added to the remaining cell suspension and incubated for 10 min at 37°C prior to adding 1.5 ml of a solution of 4% BSA in PBS for storage at 4°C until the day of flow cytometry. If only flow cytometry was performed, the removal of cells for histamine release was excluded from the protocol. For drug testing, the reaction normally included a 10-minute period of incubation with the drug at the specified concentrations prior to adding the stimulus. All drugs were dissolved in DMSO and the 'vehicle control' samples included DMSO at a concentration equal to that of the highest drug concentration being tested. Throughout the manuscript, the results are first calculated as a fraction of 'vehicle control response', meaning the response to stimulation in the presence of DMSO (see above). These fractional values are used for averaging.

For desensitization experiments, the reaction was carried out in 2 phases, a calcium-free phase ranging from 0 to 45 min followed by restoration of extracellular calcium to allow secretion for another 45 min. For the first phase, the cells were resuspended in PAG buffer containing 50 μ M EDTA $\pm$ anti-IgE antibody for different periods of time. The samples without anti-IgE were considered nondesensitized cells; they experienced the absence of extracellular calcium for the same periods as the stimulated cells. These conditions are included because depriving the cells of calcium for extended periods sometimes causes a small loss of responsiveness. After this 'desensitization' phase (calcium-free stimulation), the cell response was tested by the addition of extracellular calcium in the form of a PAG-CM buffer (containing appropriate concentrations of stimulus) and the secretion reaction allowed to proceed for an additional 45 min prior to the fixation of the cells for analysis of activation markers.

Flow Cytometry

Each sample of the stored suspension of fixed basophils was divided into 3, for labeling with either anti-CD63 (1/1,000 dilution from manufacturer's stock solution), anti-CD203c (1/500 dilution) or anti-CD11b (1/500 dilution). The primary metric of change in the flow cytometric studies was the difference in the

means of flow cytometric distributions between unstimulated and stimulated cells. For CD63, the alternative method of assessing the response that is common in the literature, net percent positivity, was also analyzed, but there were minimal quantitative differences from the mean measurement and no qualitative differences. Since these studies were done with purified basophils, the only gating criterion was a forward-scatter/side-scatter gate to remove nonintact cells from the analysis.

Statistics

In general, data is shown as the mean $\pm$ standard error of the mean, both in the text and figures. In some instances, the figure shows mean $\pm$ SEM but because the data includes stimulation $\pm$ drug, the appropriate statistical analysis uses a paired Student t test i.e. the reported p values are derived from paired analysis. For the dose-response curves shown in figure 1b, MANOVA statistics (2 conditions $\pm$ drug vs. 3 outcomes) were calculated using the area-under-the-curve metric for the results. An approximate statistical power to detect change in the dose-response curve calculation determined that a 27% change could be detected with 80% power. For the comparison of SB203580-mediated inhibition of CD63 expression and histamine release, the data points were fitted to a line; the slope and intercept and their respective errors of estimation were determined. The standard linear regression was chosen under the assumption that histamine release is the gold standard which is possibly predicting CD63 expression. However, the assay could also be viewed as 2 exchangeable outcomes; in which case, a Passing-Bablok test is more appropriate and was thus performed in addition to the standard linear regression.

Results

As noted in the introduction, a previous study by Gibbs et al. [16] demonstrated that SB203580 inhibits histamine release, while Aerts et al. [5] reported that this drug did not inhibit CD63 expression. We examined a short-series dose-response curve, from 0.3 to 10 μ M, and examined the expression of CD63, CD203c, CD11b (n = 4) and histamine release (in 3 of the 4 experiments) in response to anti-IgE antibody. Our primary end point in this series of experiments was to determine if there was discordance in the inhibition of CD63 expression and histamine release. Figure 1a plots all the data (from any drug concentration) from the 3 matched experiments and demonstrates a tight concordance. It shows the expectation for perfect concordance (dotted line) and the linear fit where the y-intercept (0.056 ± 0.112) and slope (0.846 ± 0.149) are not statistically different from 0 and 1.0, respectively. A Passing-Bablok statistical test on this result had the same conclusion: slope = 1.05, CI 0.75 to 1.35 and intercept = -0.0005 , CI -0.23 to 0.23 . These experiments also revealed an unexpected result, the absence of the inhibition of CD203c and CD11b (fig. 1b; for CD203c and

Fig. 1. Concordance of the inhibition of histamine release and upregulated CD63 expression by SB203580. **a** Using data from 3 experiments in which 4 concentrations of SB203580 were examined, a plot of the fractional response with histamine release versus CD63 expression as the outcome is shown. Dashed line: perfect concordance, solid line: the fit of the actual data. **b** In these experiments, purified basophils were stimulated with an optimal

concentration of anti-IgE antibody (0.5 μ g/ml) after a 10-minute preincubation with SB203580 at the various concentrations shown in **b**. The average stimulated response for these experiments was 82%. **b** Concentration dependence of the inhibition by SB203580 of the anti-IgE antibody-induced response. ■ = CD63; ○ = CD203c; ● = CD11b. Data is calculated relative to the vehicle control IgE-mediated response (n = 4).

CD11b, $p > 0.05$ and for CD63, $p = 0.0002$ for the difference from the control).

A variety of studies in both animal mast cell models and human basophils have shown that inhibition of actin polymerization by either latrunculin A or cytochalasin D markedly enhances histamine release [29–33]. Although one of the speculated mechanisms for this effect would predict that it should enhance CD63 and CD203c expression, there were other ways of viewing the effects of these drugs indicating a need to study these effects. Previous studies determined the concentration dependence of the drugs on the dissolution of polymerized actin in human basophils; a 2 μ M concentration of cytochalasin D or 200 nM latrunculin A optimally alters actin polymerization [34, 35]. So cells were tested with these concentrations. Figure 2a shows that latrunculin A had no statistically significant effect on CD203c expression while markedly enhancing CD63 expression in response to anti-IgE antibody. The enhanced expression of CD63 was consistent with previous studies of enhanced histamine release and in 1 of the experiments, also concordant with the enhancement of histamine release. Similar results were ob-

tained for cytochalasin D, with a 4.8-fold increase in IgE-mediated CD63 expression. Previous studies have noted that actin polymerization inhibitors also enhance non-IgE-dependent secretagogues. A similar pattern of behavior was confirmed for CD63 and CD203c when using fMLP (100 nM) as a stimulus. With latrunculin A, CD203c expression was 1.03 ± 0.19 -fold (n = 5, $H_0 = 1$; $p = \text{n.s.}$) of the response with the vehicle control and with cytochalasin D, 1.03 ± 0.28 -fold (n = 4; $p = \text{n.s.}$). In 3 experiments, the expression of CD63 was enhanced 1.5-, 5.1- and 5.6-fold with latrunculin A (4.5-fold average). In 2 experiments with cytochalasin D, CD63 expression was enhanced 1.8- and 4.5-fold (3.2-fold average). Figure 2b shows that there was no difference in the kinetics of CD203c expression following fMLP $\pm$ 200 nM latrunculin A.

It has recently been shown by other experimental maneuvers that IgE-mediated desensitization does not appear to be influenced by actin polymerization, despite the fact that overall mediator release is enhanced [35]; however, studies in other cell types have suggested that actin polymerization should have influenced the downregula-

Fig. 2. Effects of latrunculin A on CD63 or CD203c expression. **a** Purified basophils were incubated for 10 min $\pm$ 200 nM latrunculin A (or DMSO, vehicle control at a DMSO concentration equivalent to that in the latrunculin A condition) before stimulation with an optimal concentration of anti-IgE antibody (0.5 μ g/ml). **b** Kinetics of fMLP-induced CD203c expression $\pm$ 200 nM latrunculin A. $\bullet$ = Vehicle control; $\circ$ = 200 nM latrunculin A (n = 3). To average the data from 3 experiments, all data within a particular experiment was expressed as a fraction of the vehicle-control response at 5 min.

tion of the IgE-mediated response [38–40]. As noted above, it was found that latrunculin A, and by proxy, actin polymerization, had no effect on CD203c expression. If actin polymerization did, in fact, determine whether desensitization occurred, then the stimulated expression of CD203c might not show desensitization because expression occurred to the same extent regardless of the presence of actin inhibitors. A recent study by Lund et al. [41] demonstrated desensitization of CD203c expression using a sub-threshold method. The signaling requirements for this approach to demonstrating Fc ϵ RI-mediated desensitization have not been explored; so to link results for CD203c with our previous signaling studies, Fc ϵ RI-mediated desensitization was studied with the calcium-free method using anti-IgE antibody as the stimulus. The operational method of demonstrating the intrinsic self-termination mechanisms is to incubate cells in the absence of extracellular calcium with the stimulus for various periods of time, after which calcium is returned to the buffer (subsequent histamine release decays with time). This is a method with considerable history [see 42–46]. Figure 3 shows that desensitization of CD203c occurs. However, the rate is somewhat slower, and within this time frame the rate slows to the extent that it is difficult to detect at times greater than 30 min. Note that CD63 expression desensitized rapidly, at a rate consistent with desensitization measured by histamine release and consistent with many prior studies of this phenomenon [46, 47]. Once again, the behavior of CD11b during desen-

Fig. 3. Kinetics of IgE-mediated desensitization as measured by the expression of CD63 ($\circ$), CD203c ($\bullet$) or CD11b ($\blacksquare$) (n = 3). Basophils were incubated with an optimal concentration of anti-IgE Ab (0.5 μ g/ml) in the absence of extracellular calcium (50 μ M EDTA) for the time periods shown on the abscissa, after which extracellular calcium was returned to the buffer and the cells were incubated for an additional 20 min before fixation. Dashed line: results from 1 experiment to verify that the rate of desensitization measured by histamine release in this study is consistent with past experience.

Fig. 4. Inhibition of CD63, CD203c and CD11b and histamine release by inhibitors of early IgE-mediated signaling. **a** Syk inhibitor NVP-QAB205. Purified basophils were incubated for 10 min $\pm$ NVP-QAB205 (or DMSO, vehicle control at a DMSO concentration equivalent to that in the highest NVP-QAB205 concentration – drug responses are compared to this response) at the concentrations shown before stimulation with an optimal concentration of anti-IgE antibody (0.5 μ g/ml), CD63 ($\circ$), CD203c ($\bullet$), CD11b ($\square$) and histamine release ($\blacksquare$) (n = 3). **b** Src-family kinase inhibitor, PP1, reaction conditions and symbols are the same as in **a** (n = 3). The average stimulated histamine release in these experiments was 77%.

sitization looked very similar to CD203c. To determine if there was dependence of CD203c expression on the earliest elements of IgE-mediated signaling, basophils were stimulated in the presence of a syk inhibitor, NVP-QAB205 (a 3rd-generation syk inhibitor that has been extensively studied [48, 49]), effectively inhibiting CD203c, CD11b and CD63 expression (fig. 4a). But once again, there is a subtlety in the results for the syk inhibitor that raises questions about the nature of the signaling requirements for the expression of CD203c and CD11b, both of which are only inhibited at 4- to 5-fold higher concentrations of the drug. The earliest step in signaling is the activation of Src-family kinase(s), and the Src-family kinases inhibitor, PP1, inhibited all 4 outcomes at 10 μ M, with a slight enhancement of each outcome at 1–3 μ M, as demonstrated for histamine release in previous studies [50] (fig. 4b).

There have been mixed indications that expression of CD203c requires extracellular calcium [51]. To test this possibility, basophils were stimulated with fMLP, which generates a robust release of intracellular stores before a significant influx occurs [52, 53]. The addition of EDTA to the reaction buffer at the same time as the addition of fMLP (in order to prevent leaching of internal stores of calcium prior to the addition of the stimulus) resulted in a 40% inhibition of CD203c expression (data not shown). If the cells were first loaded with the internal calcium chelator BAPTA (15 μ M BAPTA-AM incubated with basophils for 30 min at 37°C, followed by washing prior to

stimulation [52, 53]), the simultaneous addition of EDTA (with the fMLP stimulus) resulted in a 100% inhibition of CD203c expression. Expression of CD63 was more sensitive to EDTA without the BAPTA step, showing 87% inhibition. It is also known that expression of CD203c is faster than histamine release or CD63 expression, especially during stimulation with phorbol myristate acetate [4, 18]. In a similar way, CD11b expression occurs rapidly during stimulation with phorbol myristate acetate (data not shown).

Discussion

This study demonstrated that there was no discordance between inhibition of CD63 expression and histamine release when inhibition was caused by SB203580. A serious challenge to the proposed mechanism of CD63 expression would have been complete inhibition of histamine release without any inhibition of CD63 expression, but even discordance with the partial inhibition of histamine release observed with SB203580 would have raised questions. The expectation is that the more sensitive indicator of inhibition would occur for CD63 expression because, in the proposed model, it is possible to have some histamine release without increased expression of CD63 if the histamine release shifts towards piece-meal degranulation. Indeed, throughout these studies of inhibition, CD63 tends to be inhibited somewhat more ef-

fectively than histamine release, suggesting that as stimulation pathways are weakened, piecemeal degranulation may be favored. A similar pattern of behavior was seen in our previously published studies [18] with the unique exception of supraoptimal stimulation which has been shown to generate a stronger early cytosolic calcium response [52]. In other words, scanning through the anti-IgE dose-response curve is somewhat unique in that the sub-optimal side of the polyclonal anti-IgE antibody dose-response curve favors piecemeal degranulation, so that there is a qualitative shift in the nature of degranulation as one moves through the dose-response curve to its supraoptimal side.

These experiments also demonstrated a marked discordance in the regulation of CD63 and CD203c expression. It is not entirely clear how SB203580 inhibits secretion because this drug has equal potency for the inhibition of at least 5 kinases: GAK (cyclin-G-associated kinase), RIPK2 (receptor-interacting serine-threonine kinase 2), NLK (nemo-like kinase), CSNK1D (casein kinase 1 delta) and JNK (c-Jun N-terminal kinase), the first 3 of which are likely expressed in basophils based on the presence of a strong mRNA signature (unpublished results), including p38 [54]. But current evidence suggests that all of the known kinases that are inhibited by SB203580, including p38, are considered late-signal transduction elements whose activation occurs downstream of the earliest signaling steps in IgE-mediated activation. This later signaling nevertheless alters both histamine release and CD63 expression but appears to have no influence on CD11b and CD203c expression.

We also found that the actin polymerization inhibitors, latrunculin A and cytochalasin D, resulted in concordant enhancement of CD63 expression and histamine release, but once again found discordance in the changes in expression of CD203c and CD11b. Surprisingly, despite the marked effects of these agents on secretion induced by physiological secretagogues and some nonphysiological agents [34, 35], there was no effect on CD203c expression; indeed, there was a modest trend towards inhibition. The role of actin polymerization in the maintenance of activation pathways is controversial. There are indications in studies of rodent mast cells that actin polymerization mediates down-regulation of the earliest steps of FcεRI-dependent activation [38, 55, 56]; if this were the case, it is reasonable to speculate that all downstream functions would be enhanced. In this context, one would expect that actin-polymerization inhibitors would enhance all indicators of basophil activation, including ac-

tivation markers. But actin polymerization inhibitors did not influence CD11b and CD203c expression. This is a surprising result.

To carry this issue one step further: if actin polymerization did have a role in termination mechanisms and we do not observe an effect of actin-polymerization inhibitors, then one might be led to conclude that desensitization might be different for CD11b and CD203c. Desensitization is a general term for the sum of the various processes that serve to terminate an active signal. Some of these processes operate on very early signal transduction steps and would therefore be expected to influence all functions downstream of these early steps. We therefore examined desensitization. Although it appears to occur for all the measured endpoints, some caution is reserved since for CD11b and CD203c, its rate was somewhat altered (especially at later time points), raising the possibility that the mechanisms for down-regulating these 2 responses may still differ, in part, from the mechanisms used to turn off histamine release and, by extension, CD63 expression. Recent studies have also demonstrated the desensitization of CD203c expression, but as noted above, the methodology used a low-dose escalation to establish some degree of desensitization [41]. Taken together, it seems clear that some aspects of desensitization are operating on the expression of CD203c and CD11b. Finally, the observation that actin-polymerization inhibitors did not enhance CD203c or CD11b expression, but that desensitization still occurs, provides further evidence that actin polymerization is not regulating termination mechanisms in human basophils, which supports previous studies that came to this conclusion [35]. These issues aside, the basic result further demonstrates that the signaling pathways for CD11b/CD203c expression differ substantially from the pathways for CD63 expression and histamine release.

The potency of the syk inhibitor, NVP-QAB205, was shifted 4–5 rightward for both CD11b and CD203c expression. This inhibitor is considered a reasonably selective inhibitor of human syk with little inhibition of Src-family kinases [48, 57], so, while the potency shift could reflect inhibition of other kinases, there is no strong evidence for this explanation. If it were the explanation, it would imply a lack of dependence on syk which would be a surprising conclusion. Alternatively, the strength of the signaling needed for CD203c expression may be smaller than for histamine release, so that more effective inhibition of syk is needed. However, our previous studies on cellular sensitivity do not strictly support this interpreta-

tion [18]. Without a better model of expression or secretion, it may be difficult to translate the cellular sensitivity studies to this situation. Therefore, it is unclear what the shift in potency implies about the dependence on syk for signaling to CD203c expression, although it does highlight another difference in the signaling requirements for CD203c when compared to CD63. The results with PP1 do suggest that a Src-family kinase is needed for the response, which is not surprising, since, unlike the requirement for syk, no study has dissociated any basophil function from inhibition by PP1.

We have found that CD203c and CD11b appear to follow the same rules of expression. First, they are both rapidly expressed during stimulation with phorbol myristate acetate. Second, they are not inhibited by SB203580. Third, they are not enhanced with actin polymerization inhibitors. In addition, their sensitivity to the syk inhibitor, NVP-QAB205, was shifted 4- to 5-fold. In a previous publication, we also found a 3- to 4-fold shift in their sensitivity to a Btk inhibitor [58]. There have been many studies of CD11b expression in neutrophils [23–27, 59] identifying a vesicular granule that contains CD11b that fuses rapidly with the plasma membrane during stimulation. The concordant characteristics and signaling requirements between the expression of CD11b and CD203c suggest that these 2 proteins may be located

in the same compartment in human basophils, and the rich literature on the expression of this molecule in neutrophils may also aid the understanding of the signaling requirements for CD203c expression. This study also demonstrates that the signaling requirements for this alternative intracellular vesicular fusion event are unique from those of normal degranulation. Nevertheless, these are indirect measures of similarity; electron microscopy would be necessary to colocalize the 2 proteins in the same compartment.

In summary, several agents known to modify signal transduction in human basophils appear to differentially alter the expression of CD63/histamine release versus CD203c/CD11b expression. This study supports previous indications for different signaling pathways for these 2 classes of outcomes and advocates further caution when interpreting the effects of stimuli on the expression of the various activation markers being used to study human basophils.

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