Brief report

Increased expression of formyl peptide receptor-1 by basophils from patients with mastocytosis

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Background: Symptoms in patients with systemic mastocytosis (SM) are associated with an increase in mast cell burden and release of mast cell–derived mediators. The most frequent presentation of SM is indolent SM (ISM), with moderate symptoms and prognosis. Basophil numbers in these patients are generally normal. However, when examining basophil activation in patients with ISM, we noted an abnormal response to N-formylmethione-leucyl-phenylalanine (fMLP).

Objective: Our aim was to compare basophil responsiveness to fMLP and anti-IgE in healthy volunteers and patients with ISM and relate the findings to fMLP receptor (FPR) expression.

Methods: Basophils isolated from peripheral blood of 15 patients with ISM and 14 healthy volunteers were stimulated with fMLP or anti-IgE. CD63 expression to assess basophil activation and expression of FPRs were assessed by flow cytometry.

Results: Baseline expression of CD63 on basophils was similar between the healthy volunteers and patients with ISM. fMLP induced higher expression of CD63 on basophils from patients with ISM, whereas responses to anti-IgE were similar between groups. Basophils from patients with ISM also had higher fMLP1 receptor (FPR1) expression, wheresas FPR2 and FPR3 were not detected. fMLP blocked the binding of anti-FPR1 antibody to FPR1, consistent with the conclusion that fMLP signals through FPR1.

Conclusions: Level of fMLP-induced basophil activation is higher in patients with ISM, which is associated with an increase in FPR1 expression. Further investigation is needed to determine why FPR1 expression is elevated, whether such expression might serve as an additional surrogate marker in the diagnosis of ISM, and whether enhanced responses of basophils to fMPL might have some relationship to unexplained episodes of mediator release. (J Allergy Clin Immunol Global 2024;3:100296.)

Key words: *Basophils, mast cells, mastocytosis, basophil activation test, fMLP, FPR1*

INTRODUCTION

Mastocytosis is a disease that is characterized by the accumulation of clonal mast cells (MCs) in tissues often associated with enhanced release of mediators such as histamine, cytokines and proteases.[1,2] Indolent systemic mastocytosis (ISM) refers to cases with a relatively low burden of MCs and a more indolent clinical course with better prognosis than that of more aggressive forms of the disease.[3] Basophils, which share many functional characteristics with MCs and are involved in both innate and adaptive immune responses against parasites and initiation of allergic reactions, remain generally normal in number in mastocytosis.[4] Basophils may be activated and contribute to the increase in inflammatory mediators after engagement of FcεRI and other receptors such as the formyl peptide receptors (FPRs), the latter of which are G protein–coupled receptors (GPCRs) that bind a chemotactic factor derived from bacteria, namely, N-formylmethionyl-leucyl-phenylalanine (fMLP).[5,6] Despite the recognized contributions of basophils to allergic reactions, it is unknown whether basophil responses might be enhanced in patients with ISM due to an increase in inflammatory mediators in these patients.[6,7]

The basophil activation test (BAT) using flow cytometry is a widely used diagnostic method to evaluate allergic hypersensitivity.[8] The BAT measures activation markers such as CD63 on basophils in response to allergens, with responses to fMLP serving as a control.[9] In the process of examining the responsiveness of basophils from patients with ISM to FcεRI cross-linking, we unexpectedly found that they were abnormally responsive to fMLP. This observation led us to further compare the basophil reactivity to fMLP in patients with ISM with that in healthy volunteers and to also explore fMLP receptor expression.

RESULTS AND DISCUSSION

It is unknown whether the increased presence of vasoactive and proinflammatory mediators in patients with ISM alters basophil responsiveness and, in turn, might contribute to symptoms in these patients. To measure the reactivity of basophils in ISM, we used the flow cytometry–based BAT, which is commonly used for

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Abbreviations used
BAT: Basophil activation test
fMLP: N-formylmethione-leucyl-phenylalanine
FPR1-3: Formyl peptide receptors 1 through 3
GPRC: G protein–coupled receptor
ISM: Indolent systemic mastocytosis
MC: Mast cell
MFI: Mean fluorescence intensity

evaluation of agents provoking anaphylaxis[10] and for diagnosing food allergies.[11,12] In human peripheral blood, basophils are characterized as $CD193^{+}CD123^{+}HLA\text{-}DR^{-}$ cells. For this study, we gated on this population and analyzed cell surface CD63 expression as an activation marker by using flow cytometry (see Fig E1 in the Online Repository at www.jaci-global.org). The patients with ISM evaluated for basophil responsiveness were aged 32 to 76 years and carried the *KIT* D816V mutation, as identified either in bone marrow or in peripheral blood. The patients' serum tryptase levels varied from 14 to 225 ng/mL (see Table E1 in the Online Repository at www.jaci-global.org).

At baseline, the basophils from healthy volunteers and those from patients with ISM showed similar CD63 expression levels (geometric mean fluorescence intensity [MFI]) and proportions of cells expressing CD63 (Fig 1, *A* and *B*). Similarly, in response to anti-IgE, the percentage of basophils expressing CD63 in patients with ISM did not differ from that in healthy volunteers, although there was a trend toward a higher MFI. (Fig 1, *C* and *D*). Unexpectedly, we noted that when basophils were stimulated with fMLP (Fig 1, *E* and *F*), an activation control frequently used in BAT assays, both the percentage of basophils expressing CD63 (Fig 1, *F* [*left*]) and CD63 expression levels (Fig 1, *F* [*right*]) were significantly increased on basophils from patients with ISM.

To explore a basis for the difference in responses to fMLP, we next examined the expression of fMLP receptors (FPR1-3) on basophils from healthy volunteers and from patients with ISM. Increases in levels of FPRs have been reported in neutrophils of patients with Crohn disease[13] and in tumor-infiltrating neutrophils in human colorectal cancer samples.[14] Further, treatment of neutrophils, monocytes, and macrophages with LPS or inflammatory cytokines can upregulate FPR1 mRNA levels by transcriptional mechanisms and by stabilization of the transcript. Stimulation of neutrophils can also promote the rapid recycling of preformed FPR1 present in secretory vesicles to the cell surface. Depending on the cell type and context, these factors can thus prime cells for enhanced responses to fMLP.[15,16] However, to our knowledge,

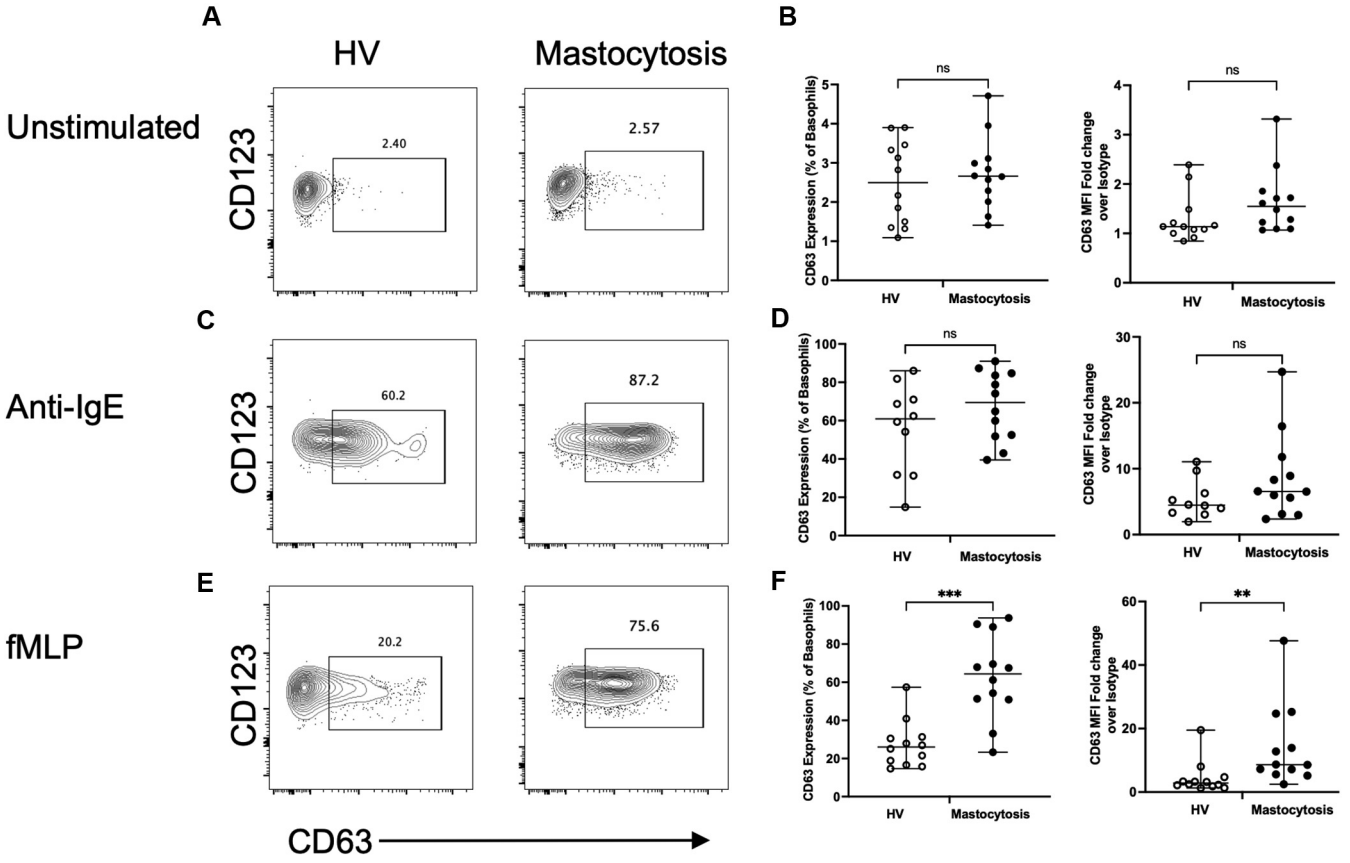

FIG 1. Basophil activation in patients with ISM and healthy volunteers (HVs). **A, C,** and **E,** Representative fluorescence-activated cell sorting dot plots showing CD63 expression (percentage and MFI) on unstimulated (**A**), anti-IgE–stimulated (**C**), and fMLP-stimulated (**E**) basophils (n = 3-10). **B, D,** and **F,** Percentage of $CD63^{+}$ cells (*left*) and relative MFI of CD63 expression level (*right*) (n = 10-12). $^{**}P < .01$; $^{***}P < .001$. *ns,* No statistically significant difference.

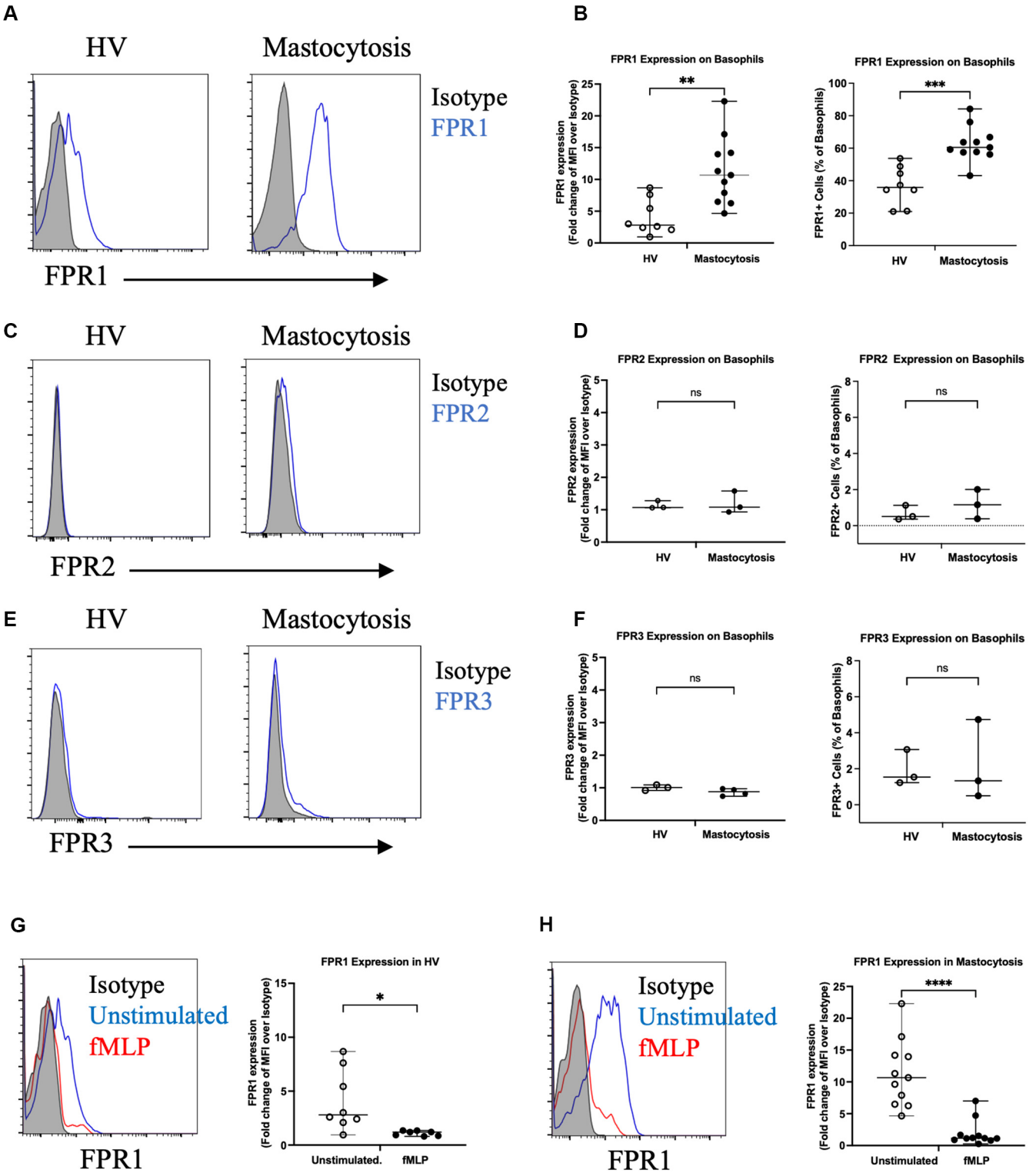

FIG 2. FPR1 expression on basophils in patients with ISM and healthy volunteers (HVs). **A, C,** and **E,** Representative fluorescence-activated cell sorting histograms showing FPR1 (**A**), FPR2 (**C**), and FPR3 (**E**) expression on basophils (n = 3-10). **B, D,** and **F,** Relative MFI of FPR1 expression and percentage of FPR1^{+} cells (**B**), FPR2 expression (**D**), and FPR3 expression (**F**) on basophils from patients and from controls. **G** and **H,** Representative fluorescence-activated cell sorting histograms of FPR1 expression (*left*) and graphs showing relative MFI (*right*) of FPR1 expression on basophils after fMLP stimulation (2 μM) for 20 minutes in HVs (**G**) and patients (**H**) (n = 8-11). *P < .05; **P < .01; ***P < .001. *ns*, No statistically significant difference.

regulation of FPR1 expression has not been studied in basophils. In our study, we observed a higher level of expression of FPR1 (Fig 2, *A* and *B* [*left*]) on the surface of basophils and a higher percentage of $FPR1^+$ cells (Fig 2, *B* [*right*]) in samples from patients with ISM than in samples from healthy volunteers (Fig 2, *A* and *B*). In contrast, we did not detect FPR2 and FPR3 expression on basophils from either group (Fig 2, *C-F*), although these receptors were expressed on monocytes from healthy controls (see Fig E2 in the Online Repository at www.jaci-global.org). In addition, to evaluate whether signals in the serum of the patients could induce such upregulation, we coincubated blood basophils from 3 healthy volunteers with plasma from patients with ISM and found that FPR1 expression level was not affected (see Fig E3 in the Online Repository at www.jaci-global.org). However, because of the short-term viability of basophils, longer incubations were not possible, and thus we cannot exclude the possibility of long-term effects of patient sera involving processes of mRNA synthesis, mRNA, or protein stability.

Activation of immune cells can alter the expression of FPRs and lead to changes in subcellular localization of the receptors.[17] Although fMLP-induced activation of FPR1 has, in many instances, been shown to increase long-term expression of FPR1 in some cell types, other studies have shown an immediate decrease in FPR1 expression after fMLP treatment because of endocytosis of activated receptors, which commonly occurs after activation of many GPCRs.[18] In agreement with this concept and with an involvement of FPR1 in fMLP-induced activation, we found that fMLP treatment for 30 minutes resulted in a significant reduction in FPR1 cell surface expression levels on basophils from both healthy volunteers (Fig 2, *G*) and patients with ISM (Fig 2, *H*). In contrast, basophil activation with anti-IgE did not reduce FPR1 cell surface expression but instead seemed to enhance its expression, indicating specificity to fMLP-FPR1 interaction (see Fig E4, *A* and *B* in the Online Repository at www.jaci-global.org). These data are consistent with the conclusion that fMLP engages FPR1 and induces FPR1 internalization, a process thought to represent a negative feedback mechanism to prevent excessive activation of immune cells and maintain immune homeostasis.[19]

By identifying enhanced responsiveness of basophils from patients with ISM to fMLP, this study opens new avenues for investigating the underlying mechanisms of unexplained mediator release in ISM and perhaps for ultimately identifying novel therapeutic targets. Increased gut permeability, which is common in patients with mastocytosis, could increase the availability of fMLP and other bacterial products,[20] contributing to periodic basophil activation and release of vasoactive and proinflammatory mediators. Furthermore, this study highlights the importance of investigating the functional responses of basophils in addition to that of MCs in patients with ISM.

This preliminary study has limitations. First, the sample size was small, and the study was cross-sectional. Second, we measured only CD63 expression as a marker of basophil activation and did not assess other markers of basophil activation, such as CD203c or histamine release. Third, in our experiments examining FPR1 internalization, we could not exclude the possibility that fMLP binding with FPR1 results in the blockage of antibody binding to the receptor, although fMLP incubation with fixed anti-IgE–activated basophils ($FPR1^+$ cells) did not affect FPR1 level (Fig E4, *C*), suggesting no blockage of the binding of anti-FPR1 in fMLP- occupied FPR1. Finally, our study did not investigate the relationship between fMLP-induced basophil activation and clinical symptoms in mastocytosis.

In conclusion, this study provides evidence of abnormal basophil activation in patients with ISM and will, it is hoped, encourage further investigation into the functional responses of basophils in this disease and possible involvement in unexplained episodes of mediator release.

DISCLOSURE STATEMENT

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