Differential eosinophil and mast cell regulation: Mast cell viability and accumulation in inflammatory tissue are independent of proton-sensing receptor GPR65

Xiang Zhu, Eucabeth Mose, Simon P. Hogan, and Nives Zimmermann

Division of Allergy and Immunology, Department of Pediatrics, Cincinnati Children’s Hospital Medical Center, University of Cincinnati College of Medicine, Cincinnati, Ohio

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A few studies have examined the responses of specific immune cells to acidosis, with neutrophils being the most studied cell type. These studies have shown that neutrophil functions, such as chemokinesis and survival, are enhanced in acidic pH (5, 11, 24, 28, 32). Additionally, lymphocyte motility is activated by acidic pH (23). Dendritic cell activation, as well as antigen uptake, is also increased with mild acidosis (29). Recently, we demonstrated that eosinophil viability is enhanced in acidic conditions (10). However, the mechanism of how immune cells sense the acidification is incompletely understood.

Acidity can affect cells in many ways, including through activation of proton-sensing receptors. G protein-coupled receptor 65 (GPR65, also known as T cell death-associated gene 8) belongs to a group of acid-sensing receptors in the G2 accumulation receptor (G2A) family. This family includes three additional structurally related members, G2A, ovarian cancer G protein-coupled receptor 1 (OGR1), and G protein-coupled receptor 4 (GPR4). Members of this family, including GPR65, have been shown to sense extracellular acidity by proton transfer to the histidines in the first loop of GPR65, presumably causing a conformational change in GPR65 that activates Gα (13). This activity has been further demonstrated to induce cyclic adenosine 5′-monophosphate (cAMP) accumulation associated with adenyl cyclase activation (9, 10, 22, 30).

Increasing evidence demonstrates that extracellular acidification may affect cell functions through GPR65. For instance, GPR65 was suggested to be involved in extracellular acidification-induced inhibition of superoxide anion production in human neutrophils (20) and proinflammatory cytokine [tumor necrosis factor-α and interleukin (IL)-6] production in mouse macrophages (16). Moreover, GPR65 was shown to facilitate tumor development by promoting adaptation to the acidic environment and thereby enhancing cell survival and proliferation (8). More recently, we reported that eosinophil viability is increased by acidic pH in a cAMP- and GPR65-dependent manner (10). Furthermore, in an allergic asthma model, we found that GPR65 is required for optimal eosinophil accumulation in the pulmonary acidic environment and acts by affecting eosinophil survival (10). Collectively, these in vitro and in vivo studies suggest that GPR65 has different roles in different cell types.

Mast cells are critical for allergic responses, especially in the gastrointestinal tract. In this study, we aimed to examine 1) whether mast cells express GPR65; 2) whether GPR65 has an important role in regulating mast cell viability in vitro like it does for eosinophil viability; and 3) whether GPR65 is required...
for optimal mast cell and/or eosinophil accumulation by using an intestinal anaphylaxis model in which pronounced mastocytosis and eosinophilia develop in the jejunum. First, we identified GPR65 expression on mast cells. However, unlike for eosinophil survival, GPR65 was not required for mast cell survival in the acidic environment in vitro. Second, in the allergic gastrointestinal inflammation model, we found that GPR65 deficiency did not affect mast cell accumulation; however, it downregulated eosinophil accumulation in the inflammatory tissue, which is consistent with the phenotype in our prior asthma model (10). In conclusion, our present study demonstrated that mast cell viability and accumulation in inflammatory tissue are independent of proton-sensing receptor GPR65.

MATERIALS AND METHODS

Mice. Male and female 6- to 8-wk-old Gpr65+/− and Gpr65−/− mice (BALB/c background) were housed under specific pathogen-free conditions. Gpr65−/− mice contain a disrupted Gpr65 locus with an enhanced green fluorescent protein (EGFP) reporter knocked into the exon 2 to allow the analysis of GPR65 expression in living cells. All studies were reviewed and approved by the Cincinnati Children’s Hospital Medical Center Institutional Animal Care and Use Committee.

Culture of mast cells and eosinophils. To obtain mast cells for in vitro study, bone marrow (BM) cells were cultured in RPMI 1640 (Invitrogen) with 10% fetal bovine serum (FBS), 100 IU/ml penicillin, and 10 mM HEPES (Invitrogen). In addition, during the first 3 wk, the medium contained 10 ng/ml stem cell factor (SCF; PeproTech) and 20 ng/ml recombinant mouse IL-3 (PeproTech). During the 4th and 5th wk, the medium contained 20 ng/ml IL-3 only. The cell phenotype was subsequently identified by flow cytometry following FcεRIα and c-Kit double-positive mast cells (data not shown).

As a source of eosinophils, we primarily used BM-derived eosinophils, which were generated as described previously (4) with minor modifications. Briefly, BM cells were cultured in IMDM (Invitrogen) supplemented with 10% FBS (Cambrex), 100 IU/ml penicillin and 10 μg/ml streptomycin (Cellgro), 2 mM glutamine (Invitrogen), and 50 μM 2-ME (Sigma-Aldrich). From day 0 to 4, the medium contained 100 ng/ml SCF and 100 ng/ml FLT3 ligand (PeproTech). On day 5, the medium was replaced with fresh medium containing 10 ng/ml recombinant mouse IL-5 (PeproTech). On day 14, the cell phenotype was identified by flow cytometry following CCR3-FITC (R&D Systems) and Siglec-F-PE (BD Bioscience) staining and morphological examination following toluidine blue staining on the cytospan slides. Approximately 90–95% of harvested cells were consistently FcεRIα and c-Kit double-positive mast cells (data not shown). As a source of eosinophils, we primarily used BM-derived eosinophils, which were generated as described previously (4) with minor modifications. Briefly, BM cells were cultured in IMDM (Invitrogen) supplemented with 10% FBS (Cambrex), 100 IU/ml penicillin and 10 μg/ml streptomycin (Cellgro), 2 mM glutamine (Invitrogen), and 50 μM 2-ME (Sigma-Aldrich). From day 0 to 4, the medium contained 100 ng/ml SCF and 100 ng/ml FLT3 ligand (PeproTech). On day 5, the medium was replaced with fresh medium containing 10 ng/ml recombinant mouse IL-5 (PeproTech). On day 14, the cell phenotype was identified by flow cytometry following CCR3-FITC (R&D Systems) and Siglec-F-PE (BD Bioscience) staining and morphological examination following toluidine blue staining on the cytospan slides. Approximately 90–95% of harvested cells were consistently FcεRIα and c-Kit double-positive mast cells (data not shown). Because our original studies (10) were performed with intragastric feeding needles (01-290-2B; Fisher Scientific). Rectal temperatures were measured before and 30 min after OVA challenge. Diarrhea was assessed by visually monitoring mice for up to 60 min after intragastric challenge. Mice demonstrating profuse liquid stool were recorded as diarrhea-positive animals.

Preparation of single cell suspensions of the jejunum. For examination of EGFP expression in jejunal mast cells, fragments of the jejunum from OVA-challenged mice were dissected out and cut open longitudinally. Next, the tissue was first incubated in HBSS with EDTA (5 mM) on ice. After removal of EDTA, the tissue was minced into small pieces and incubated in serum-free RPMI 1640 containing collagenase A (2.4 mg/ml; Roche) and DNase I (0.1 mg/ml; Roche) with gentle shaking for 30 min at 37°C. Finally, the tissue was homogenized by pushing it through a 19-gauge needle and resuspended in PBS with 0.2% BSA for flow cytometry. FcεRIα/c-Kit−/7-AAD− cells were considered as live mast cells for examining EGFP expression under the control of GPR65 promoter activity.

ELISA. Total IgE in the serum was measured using the OpgEIA ELISA Kit (BD Bioscience) according to the manufacturer’s protocol. In brief, diluted serum samples were applied to anti-mouse IgE monoclonal antibody (mAb)-coated 96-well ELISA plates (Costar, Corning) after blocking with 10% FBS. After a 2-h incubation at room temperature, the plates were washed and added with the premixed Working Detector [biotinylated anti-mouse IgE mAb and streptavidin-horseradish peroxidase (HRP) conjugate] into each well. After a 1-h incubation, the colorimetric reaction was developed following the addition of tetramethylbenzidine substrate solution (BD Bioscience) and then stopped with 1 M H2SO4. Optical density at 450 nm was quantified with an ELISA plate reader (Synergy 2; BioTek). OVA-specific IgG1 in the serum was measured in a very similar way; OVA (100 μg/ml) was used to coat the ELISA plates, and diluted (1:1,000) HRP-conjugated anti-mouse IgG1 (X56; 0.5 mg/ml; BD Biosciences) was used to detect IgG1 in the serum. All diluted serum samples were performed in duplicate.

Total RNA extraction and real-time RT-PCR. Total RNA was extracted using TRizol Reagent (Invitrogen) as per the manufacturer’s instructions. RNA (1 μg) was treated by DNase I (Qiagen) for 15 min at room temperature before reverse transcription using the iScript cDNA Synthesis Kit (Bio-Rad). cDNA (2 μl) were subjected to real-time RT-PCR set up with iQ SYBR Green Supermix (Bio-Rad) and the following individual primer sets: Gpr65 (encoding GPR65), forward 5’-CGAGTTTGCACGCTCTCTT-
**A** Eosinophils  

**B** Mast cells  

**C** Viability (%)  

**D** Viability (%)  

**E** Viability (%)  

**F** Viability (%)  

**G** Viability (%)  

**H** Viability (%)  

**I** Viability (%)  

**J** Viability (%)  

**K** Viability (%)  

**L** Viability (%)  

**M** Viability (%)  

**N** Viability (%)  

**O** Viability (%)  

**P** Viability (%)  

**Q** Viability (%)  

**R** Viability (%)  

**S** Viability (%)  

**T** Viability (%)  

**U** Viability (%)  

**V** Viability (%)  

**W** Viability (%)  

**X** Viability (%)  

**Y** Viability (%)  

**Z** Viability (%)  

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**Fig. 1.** In vitro viability and intracellular cAMP assessment. **A:** flow cytometric identification of enhanced green fluorescent protein (EGFP) on mast cells and eosinophils from G protein-coupled receptor (Gpr) 65-positive (+/+) and Gpr65−/− (EGFP knock-in) mice. Representative out of 6 experiments is shown. **B:** flow cytometric assessment of eosinophil and mast cell viability after a 7-h incubation in media at the indicated pH (n = 5 experiments). The viable cells were defined as 7-AAD and Annexin V double negative. **C:** viable mast cell counting by trypan blue exclusion assay after incubation for the indicated time in media at pH 7.5-6.0 (solid lines) or absence (dashed lines) of interleukin (IL)-3 (10 ng/ml) (n = 3 experiments). **D:** comparison of Gpr65 transcript levels between wild-type (WT) mast cells and WT eosinophils by real-time RT-PCR following normalization by the housekeeping gene Actb. E: comparison of Gpr132 [encoding G2 accumulation receptor (G2A)], Gpr68 [encoding ovarian cancer G protein-coupled receptor 1 (OG1)], and Gpr4 [encoding G protein-coupled receptor 4 (GPR4)] transcript levels between WT and GPR65-deficient mast cells by real-time RT-PCR following normalization by Actb. F: eosinophils and mast cells were incubated at the indicated pH for 30 min in the presence of 3-isobutyl-1-methylxanthine (IBMXX), and accumulated intracellular cAMP was measured via a competitive enzyme-linked immunosorbent assay (ELISA) from cell lysates (n = 3 experiments). Data are expressed as means ± SD, and paired t-test was used for statistical analysis (B, C, and F). *P < 0.05; ns, no significant difference.

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CAGTC, reverse 5′-GCTCATGTCCTGCGCTTTTGA; Gpr132 (encoding G2A), forward 5′-TCACAGGAGGGTCCACAGACT, reverse 5′-ACGCCCAGTGACACCACCA; Gpr68 (encoding OR1), forward 5′-ACTGGCTGCTTTGCTGCTACCA, reverse 5′-GAGCAATTCTCCTGCTTGCA; Gpr4 (encoding GPR4), forward 5′-CTGTGACAGGAGCTGCTCG; reverse 5′-CATGACTTCAGATGCTCTTATTGC; Il13 (encoding IL-13), forward 5′-TGCCGCTTCTCTGCTGTTG; reverse 5′-CGGCGAGGTCCACTCTCATCAC; Cc11 (encoding eotaxin-1), forward 5′-GGCTACCCAGGCTCCATCC, reverse 5′-TTGGGTCCAGTGCTCTTGG; Cc24 (encoding eotaxin-2), forward 5′-CTCTTTCCTGTTGACACTCG, reverse 5′-GTGGATGGAGATTGACCGCTCCATT; Mcpt1, Mcpt2, and Mcpt4 (encoding mast cell proteinases 1, 2, and 4, respectively), forward 5′-GCTGAGCTGAGATTG, reverse 5′-CTCCGATGCTTGTATTTA; Mcpt2 reverse 5′-CCTCTCCGAGGAGATTG, Mcpt4 reverse 5′-TGCCAAATTGTTTTTTCAAGCCCTC; and the housekeeping gene Actb, forward 5′-TCAGGTGCTCCAGAATGG; and the housekeeping gene Actb, reverse 5′-TGGCCATGCTTCCAGATG.
to form a black precipitate, and counterstained with nuclear fast red. The sections were taken from the same position in the jejunum (3–5 cm distal to the stomach), and at least two random sections per mouse were analyzed. Mast cells were identified by staining for chloroacetate esterase (CAE) activity as previously described (6, 12). The eosinophils and mast cells within the jejunal lamina propria and crypt areas were counted and normalized from 15 to 25 fields of view (magnification ×100) individually by an observer blinded to treatment and genotype. Values were normalized by quantifying the total number of eosinophils or mast cells per square millimeter of tissue.

Statistical analysis. Student’s t-test (for experiments with 2 groups) and ANOVA (for experiments with >2 groups) were used to assess statistical significance. A P value of <0.05 was considered significant. All analyses were performed with Graphpad Prism 5.0 software.

RESULTS

GPR65 deficiency has no effect on mast cell viability in vitro. We and other groups have reported that a variety of leukocytes, including eosinophils, neutrophils, and T and B lymphocytes, express GPR65 (10, 20, 21). Another critical cell type that accumulates in allergic inflammation is the mast cell. First, we examined BM-derived mast cells to determine whether they express GPR65 by analyzing EGFP reporter that is knocked into the Gpr65 locus and is thus under the control of the endogenous Gpr65 promoter (21). We identified by flow cytometry that murine mast cells also express Gpr65 to a level that is comparable to eosinophils (P = 0.35, n = 6 experiments; Fig. 1A). This was further confirmed by comparing the Gpr65 transcript levels between wild-type (WT) mast cells and WT eosinophils as shown in Fig. 1D. No significant effect of EGFP expression was observed on the development of BM mast cells, in that the number of cells and expression levels of FceR1α/c-Kit were comparable after 35 days in culture (data not shown). Next, to investigate whether GPR65 regulates mast cell viability in the acidic environment, we incubated mast cells in media buffered to pH 6.0–7.5 in parallel with eosinophils. Consistent with our prior study in which eosinophils isolated from CD2-IL-5 transgenic mouse spleen were studied (10), WT eosinophils derived from BM also showed an increasing trend of viability from pH 7.5 to 6.0 after a 7-h incubation. Specifically, the viability at pH 6.0 was significantly higher than it was at pH 7.5 (Fig. 1B). However, the viability of GPR65-deficient eosinophils at pH 6.0 was significantly lower than that of WT eosinophils (Fig. 1B), showing that eosinophil survival in an acidic environment is dependent on GPR65. In contrast, WT mast cells did not show significant difference in viability between pH 7.5 and 6.0 after a 7-h incubation. Moreover, no significant difference was observed between WT and GPR65-deficient mast cells in their viability at pH 7.5 or 6.0 (Fig. 1B). We further investigated whether mast cell viability would be affected under acidic conditions with different incubation time (3–5 h or longer than overnight), with cytokine IL-3 withdrawal, or of even greater acidity (e.g., pH 5.5). Under no condition did we observe pH-dependent viability of WT mast cells or a significant difference in the viability between WT and GPR65-deficient mast cells (Fig. 1C and data not shown).

To explore the mechanism accounting for the lack of change in mast cell viability in response to acidity, we investigated several possibilities. First, we examined whether other proton-sensing receptors, including G2A, OGR1, and GPR4, are overexpressed in GPR65-deficient mast cells. No significant difference was observed in the expression of other proton-sensing receptors between WT and GPR65-deficient mast cells (Fig. 1E). Second, we tested whether mast cells signal in response to acidity. Because proton binding to GPR65 has been shown to induce intracellular cAMP accumulation and eosinophil viability under acidic conditions is dependent on cAMP (9, 10, 22, 30), we hypothesized that mast cells do not accumulate cAMP in response to acidic pH. Consistent with our prior report (10), WT eosinophils exhibited a significant, GPR65-dependent increase in cAMP at pH 6.0 compared with pH 7.5. In contrast, WT mast cells did not show cAMP induction at pH 6.0 compared with pH 7.5 (Fig. 1F). Collectively, these results demonstrate that GPR65 is expressed on mast cells but, unlike eosinophils, does not lead to cAMP induction or enhanced cell survival in response to an acidic environment.

Comparable sensitization and local inflammatory responses between Gpr65+/+ and Gpr65−/− mice. Our prior asthma study model together with in vitro data has suggested that GPR65 regulates eosinophil accumulation by affecting eosinophil survival (10). Because our present in vitro results suggested that GPR65 deficiency has no effect on mast cell survival, we speculated that GPR65 deficiency would not affect mast cell accumulation in vivo. To test this hypothesis and to directly compare the effect of GPR65 on eosinophils and mast cells within the same tissue, we used an oral antigen-induced intestinal anaphylaxis model that develops pronounced eosinophilia and mastocytosis in the jejunum. We first tested whether GPR65 deficiency would affect antigen sensitization, which in turn could affect the accumulation of mast cells and eosinophils in the GPR65-deficient mice following OVA challenges, by measuring the levels of total IgE and OVA-specific IgE in the serum of WT and GPR65-deficient mice by ELISA. We found that both of these Th2-type antibodies were significantly increased in OVA-challenged WT and GPR65-deficient mice compared with in saline-challenged controls (Fig. 2, A and B). However, there was no significant difference between WT and GPR65-deficient mice following OVA challenges (Fig. 2, A and B), suggesting that the sensitization to OVA antigen in WT and GPR65-deficient mice was comparable. Second, we examined the transcript levels of the major Th2 cytokines IL-4 and IL-13, as well as the eosinophil-selective chemokines eotaxin-1 and eotaxin-2, in the jejunum by real-time RT-PCR. The transcripts of all of these mediators were significantly increased in WT and GPR65-deficient mice following OVA challenges compared with in the saline-challenged control mice, but none of them was significantly different between OVA-challenged WT and GPR65-deficient mice (Fig. 2, C–F). Third, when performing OVA challenge, we recorded rectal temperature decrease and diarrhea occurrence, the two characteristic features in the intestinal anaphylaxis model. Neither the rectal temperature change nor the diarrhea occurrence showed a significant difference between WT and GPR65-deficient mice following OVA challenge (Fig. 2G). Collectively, these findings suggest that GPR65 deficiency does not have an effect on the antigen-induced systemic and local inflammatory responses.

Downregulated eosinophilia in the jejunum of OVA-challenged Gpr65−/− mice. We have observed that Gpr65−/− mice had attenuated airway eosinophilia in an allergic asthma model (10), suggesting that GPR65 was required for optimal eosino-
phil accumulation in allergic lung inflammation. Thus, given that extracellular acidification is commonly observed in inflammatory disorders, including allergic diseases (7, 13, 26), we hypothesized that GPR65 would also regulate eosinophil accumulation in the intestinal anaphylaxis model. Both WT and GPR65-deficient mice had significantly increased eosinophilia in the jejunum following multiple intragastric OVA challenges compared with the saline-challenged controls (Fig. 3). However, OVA-challenged GPR65-deficient mice had significantly reduced eosinophilia in their jejunum compared with the OVA-challenged WT mice (59.1 ± 9.2% decrease, n = 4 experiments). These data suggest that GPR65 regulates eosinophil accumulation in the jejunum during allergic gastrointestinal inflammation.

Mastocytosis in the jejunum of OVA-challenged Gpr65−/− mice. We further investigated whether GPR65 deficiency affects mast cell accumulation during the same allergic gastrointestinal inflammation. Following the confirmation of Gpr65

Fig. 2. Major immune responses and anaphylaxis outcomes in WT and GPR65-deficient mice. A and B: total IgE (1:20 dilution; A) and ovalbumin (OVA)-specific IgG1 (1:125,000 dilution; B) levels in the serum samples from saline- and OVA-challenged WT and GPR65-deficient mice were determined by ELISA. C–F: real-time RT-PCR analysis of the Th2 cytokines Il4 (C) and Il13 (D) and the eosinophil chemokines Ccl11 (E) and Ccl24 (F) in the jejunum of WT and GPR65-deficient mice after normalization by the housekeeping gene Actb. G: anaphylaxis was assessed by recording rectal temperature change and diarrhea occurrence following six challenges of either intragastric saline or OVA in WT and GPR65-deficient mice. The mice that evidenced diarrheal symptoms are in open symbols. Data shown are a representative of 4 experiments and expressed as means ± SD (n = 4–6 mice/saline group, n = 7–9 mice/OVA group). *P < 0.05; **P < 0.01; ns, no significant difference.

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expression on the jejunal mast cells (Fig. 4A), we evaluated the mastocytosis in WT and GPR65-deficient mice following OVA challenges. Both WT and GPR65-deficient mice developed significant mastocytosis in the jejunum following OVA challenges, and no significant difference was observed between these mice in terms of the overall mast cell numbers or their distribution within lamina propria and crypt areas (Fig. 4, B and C). Moreover, we compared the transcript levels of marker genes (reviewed in Ref. 14) for mucosal mast cells [mast cell protease (MCP)-1 and MCP-2] and connective mast cells (MCP-4) in the jejunum. The transcription of all of these genes was significantly increased in OVA-challenged WT and GPR65-deficient mice compared with saline-challenged control mice (Fig. 4, D–F). Notably, none of these markers had significantly different expression between WT and GPR65-deficient mice following OVA challenges, suggesting that GPR65 deficiency has no effect on these two mast cell subpopulations. Collectively, these findings suggest that GPR65 deficiency does not affect mast cell accumulation in allergic gastrointestinal inflammation.

**DISCUSSION**

In the present study, we demonstrated downregulated eosinophilia but unaffected mastocytosis in GPR65-deficient mice during allergic gastrointestinal inflammation. GPR65 deficiency did not affect systemic sensitization or local inflammatory responses, suggesting that a cell-intrinsic mechanism leads to decreased eosinophil accumulation. Mechanistic in vitro studies demonstrated that the viability of mast cells, in contrast to eosinophils, was not affected by acidification or GPR65 deficiency. This difference may explain our finding that GPR65 selectively affected eosinophil accumulation in allergic gastrointestinal inflammation.

Our previous study has shown that eosinophil development in the BM and eosinophil numbers in the blood and tissue at baseline are not affected by GPR65 deficiency (10). In the present study, we found no effect of GPR65 deficiency on allergic sensitization or local expression of major Th2 cytokines and eosinophil chemokines in the inflamed gastrointestinal tissue, all of which could affect eosinophil accumulation.
These results are consistent with previous studies showing that GPR65-deficient mice have normal major immune responses, including cytokine and antibody production in response to antigen (10, 21). On the basis of these data and our previous study (10) showing that GPR65 regulates eosinophil viability in response to acidic pH in vitro (also shown as control for mast cells in Fig. 1B), we postulate that the observed decreased intestinal eosinophilia in OVA-challenged GPR65-deficient mice is due to reduced eosinophil viability in the local microenvironment.

In contrast to the reduced eosinophilia in the intestine, comparable mastocytosis was observed in OVA-challenged WT and GPR65-deficient mice. Consistent with previous findings that disease outcomes, such as rectal temperature decrease (hypothermia) and diarrhea occurrence, are dependent on IgE and mast cells but not eosinophils in the allergic gastrointestinal inflammation model (2), we did not observe a significant difference in disease outcomes between allergen-challenged WT and GPR65-deficient mice (Fig. 2G). We were interested in the mechanism underlying the differential regulation of mast cells and eosinophils by GPR65. While mast cells express GPR65 in vitro and in vivo (Fig. 1A and data not shown), GPR65 deficiency did not have an effect on the viability of mast cells in physiological or acidic environment in vitro. There are several potential explanations for these findings. First, GPR65 function may be redundant on mast cells, since the other three structurally related G2A subfamily members (G2A, OGR1, and GPR4) were all shown to bind extracellular
protons as their primary ligands (13, 19, 30). Moreover, these receptors (at least G2A and GPR65) may have differential proton sensitivity in different cell types (22). Second, following extracellular proton-induced GPR65 activation, adenylyl cyclase activation leads to cAMP accumulation (9, 22, 30). However, cAMP may have different functions in different cell types, possibly stemming from cross talk with other pathways or differential levels of cAMP at baseline in individual cell types (1, 25). For instance, the level of cAMP in regulatory T cells is >10-fold higher than in CD4 effector T cells (1). Indeed, our study also demonstrated that eosinophils have a relatively higher baseline level of cAMP compared with mast cells (Fig. 1F). Third, cAMP accumulation may not necessarily be the only intracellular signaling pathway to be induced through GPR65. As a matter of fact, we observed no remarkable cAMP induction in WT mast cells in the acidic environment (pH 6.0 vs. 7.5) nor a significant cAMP reduction in GPR65-deficient mast cells (Fig. 1F). Thus, it remains possible that other signaling pathways, such as the inositol triphosphate signaling pathway associated with phospholipase C activation (13, 17) or the Rho signaling pathway (19), could be induced. However, it is unclear whether these possible early signaling events would have the potential to affect cell viability. Further studies are needed to address the cell-specific functions of GPR65 in inflammatory tissue and allergic disease.

In summary, our results have established these conclusions: 1) mast cells express GPR65; 2) GPR65 is not required for mast cell survival in an acidic environment; and 3) GPR65 regulates eosinophil but not mast cell accumulation in allergic gastrointestinal inflammation.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

Author contributions: X.Z. and E.M. performed experiments; X.Z. and E.M. analyzed data; X.Z. prepared figures; X.Z., E.M., S.P.H., and N.Z. interpreted results of experiments.

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