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I, Sribava Sharma, hereby submit this original work as part of the requirements for the degree of Master of Science in Immunology.

It is entitled:
Deletion of ?dblGata motif leads to increased predisposition and severity of IgE-mediated food-induced anaphylaxis response.

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Deletion of ΔdblGata motif leads to increased predisposition and severity of IgE-mediated food-induced anaphylaxis response.

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Abstract

Background: Previous studies have revealed an important role for the transcription factor GATA-1 in mast cell maturation and degranulation. However, there have been conflicting reports with respect to the requirement of GATA-1 function in mast cell dependent inflammatory processes. Herein, we examine the requirement of GATA-1 signaling in mast cell effector function and IgE-mast cell-dependent anaphylaxis.

Objective: To study the requirement of GATA-1 dependent signaling in the development and severity of IgE-mast cell-dependent anaphylaxis in mice.

Methods: Wildtype (Balb/c) and mutant ΔdblGata (Balb/c) mice were employed to study the role of GATA-1 signaling in in vitro IgE-mediated activation of bone marrow derived mast cells (BMMCs). Murine models of passive IgE-mediated and oral antigen-induced IgE-mediated anaphylaxis were employed. Frequency of steady state mast cells in various tissues (duodenum, ear, and tongue), peritoneal cavity, clinical symptoms (diarrhea, shock, and mast cell activation), and intestinal Type 2 cellular analysis including CD4+ TH2 cells, type 2 innate lymphoid cells (ILC2), and IL-9 secreting mucosal mast cells (MMC9) were assessed.

Results: In vitro analysis revealed that ΔdblGata BMMCs exhibit a reduced maturation rate, decreased expression of FcεRIα, and degranulation capacity when compared to their wildtype (WT) counterparts. These in vitro differences did not impact tissue resident mast cell numbers, total IgE, and susceptibility to or severity of IgE-mediated passive anaphylaxis. Surprisingly, ΔdblGata mice were more susceptible to IgE-mast cell-mediated oral antigen induced anaphylaxis. The increased allergic response correlated with increased Type 2 immunity (antigen-specific IgE, and CD4+ TH2 cells), MMC9 cells and subsequent increased small intestine (SI) mast cell load.

Conclusion: GATA-1 is necessary for steady state mast cell FcεRIα expression, proliferation, and degranulation activity. However, GATA-1 signaling is not required for IgE-mediated anaphylaxis due to heightened Type 2 immune responses which compensate for the ΔdblGata deletion impact on steady state mast cell frequency.
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**List of Abbreviations**

MC – Mast Cell

BMMC – Bone Marrow derived Mast Cell

pMC – Peritoneal Mast Cell

CTMC – Connective tissue mast cells

DC – Dendritic Cell

HSC – Hematopoietic Stem Cell

EoE – Eosinophilic Eosiphagitis

IL - Interleukin

APC – Antigen Presenting Cell

PAF – Platelet Activating Factor

ILC – Innate Lymphoid Cell

MMC9 – IL-9 secreting mucosal mast cell

MCPt-1 – Mouse mast cells protease-1

SI – Small intestine
Chapter 1: Introduction

1. Allergy

Allergic diseases are characterized by hypersensitive immune reactions in response to environmental factors (1-3). These diseases exhibit in various forms such as atopic dermatitis, food allergies, asthma, allergic rhinitis, and eosinophilic esophagitis among others (1, 2, 4-8). The World Allergy Organization estimates that, globally, approximately 300 million people suffer from asthma causing 250,000 deaths annually and a further 250 million people have food allergies (2). These numbers have been on the rise dramatically over the past years affecting individuals across age, gender, socio-economic, and national boundaries worldwide (9-11). In addition, allergic diseases are accompanied by financial burdens, social and economic impacts, and loss in quality of life (12). Without a cure incidences, prevalence, and severity of the diseases are expected to continue rising (2, 6). It is currently the subject of a great public health concern.

Allergic diseases are characterized as Type I Hypersensitivity reactions mediated by IgE immunoglobulins (3, 5, 13). The reactions can present with local or systemic symptoms such as itchy eyes, runny nose, rashes, hives, trouble breathing, diarrhea or anaphylactic shock (1, 14). Digestive, circulatory, respiratory, and nervous systems are among the systems that can be affected by allergic reactions leading to possible life-threatening conditions (15, 16). While some are rapid-onset symptoms, others manifest over time (3). Treatments include antihistamines, glucocorticoids, and antileukotrienes to reduce and manage inflammatory responses, and lifestyle changes are required to avoid triggers as a prevention method (14, 17). In severe cases of anaphylactic shock, epinephrine injections are administered to patients (18).

The causes of allergic diseases are currently not known. Evidence indicates that both environmental and genetic factors contributing to the development of allergic diseases in individuals (19). Genetic factors include sex and race while environmental risk factors including pollution, diet, and exposure to allergen and infectious diseases are also thought to contribute (19-21). In addition, stress has been shown to exacerbate
allergic conditions and stress management can reduce symptoms in individuals (22). Since allergic reactions are abnormal immune reactions with undesirable consequences, improper immune development is the subject of the hygiene hypothesis (23). It states that a lack of or reduced exposure to microbes, both infectious and symbiotic, as well as helminths can lead to immune tolerance deficiencies (19, 24). Longitudinal studies have shown that the prevalence of asthma and allergic diseases have increased as nations became more modern and developed (25, 26). Reduced microbial exposure to children in urban areas led to higher prevalence compared to children in rural areas with increased microbial exposure (25, 27). Experimentally, investigators have shown that early mice neonatal exposure to microbiota led to protection against developing asthma, while germ-free mice developed asthma (28). Although the hygiene hypothesis provides a plausible explanation, it further highlights the complicated nature of the development of allergies and the need for continued studies for prevention and treatment options.

1.1 Food Allergy

Food allergies are a type of allergic reactions where individuals experience adverse immune reactions upon ingestion of certain substances (4). An estimated 15 million Americans suffer from food allergy of which 6 million are children (6). 39% of food allergic children experience severe reactions with nuts being the major culprit of these adverse effects (6). In addition to the health complications, the financial burden, currently estimated at $25 billion per year, poses a further problem for the patients and families (12).

Food-induced anaphylaxis can have gastrointestinal and systemic involvement including cutaneous, cardiovascular, respiratory, and neurological manifestations (15, 16). Due to the lack of a cure, complete avoidance of the food allergens is the primary prevention method (14). Treatment options such as antihistamines, glucocorticoid steroids, and epinephrine exist based on the severity of the reaction (14, 17). It is estimated that 1/5th – 1/3rd of anaphylaxis reactions treated in hospitals are due to food allergic reactions with 150 – 200 deaths each year (16, 18).
As a Type I Hypersensitivity reaction, food allergies are mediated by IgE antibodies produced against the allergens (29). Non-IgE mediated responses can also occur many hours after ingesting the food (30, 31). These reactions are cell-mediated and are poorly defined (30, 31). In IgE-mediated responses, individuals first go through a sensitization phase where they are exposed to the allergen and develop antigen-specific IgE (5, 32, 33). Individuals do not experience any adverse effects during this primary exposure. The antigens from the ingested food are taken up by antigen presenting cells (APCs), such as dendritic cells (DCs), and presented to naïve CD4+ T cells which then become activated. Activation leads to differentiation into CD4+ TH2 cells followed by secretion of cytokines such as interleukin (IL)-4 and IL-13 (34). These cytokines promote class switching in B cells resulting in the production of antigen-specific IgE antibodies (35). These antibodies bind to the high-affinity FcεRIα receptor on mast cells and basophils and prime them for degranulation upon secondary exposure (29). Allergens ingested during secondary exposure bind and crosslink on the epitopes of IgE-FcεRIα and thereby trigger the degranulation of the cells through a signaling cascade (29). Upon degranulation, chemical mediators such as histamines, tryptase, leukotriene, proteases and platelet activating factor (PAF) are released into the system whose effects are presented as local and systemic symptoms in the patient (36-38). Direct effects of these chemicals include vasodilation, increase in vascular permeability, and bronchoconstriction among others. Treatments against food allergic reactions attempt to reduce the symptoms experienced by patients as a result of the chemicals released (14). For example, antihistamine medications can suppress histamine induced responses by binding to H1 histamine receptors as antagonists, blocking histamine binding, or as inverse agonists, reducing receptor activity levels (39). Since these treatments are designed to alleviate symptoms alone, and not prevent future reactions, the best method of prevention is identification and avoidance of allergens (14).
2. Immunology

2.1 Mast Cells (MCs)

Mast cells (MCs) are granular leukocytes derived from bone marrow hematopoietic stem cells (HSCs) and fall under the myeloid cell lineage (40). They are categorized as granulocytes due to presence of granules within the cell containing mast cell mediators and are identified as expressing high levels of receptors c-kit and FcεRI on their cell surface (41, 42). Immature MCs exit the bone marrow into circulation and migrate to various tissues (43) where they mature following interactions with c-kit ligand stem cell factor (SCF) and IL-3 (41, 44-46). Tissue resident MCs are found in the skin, tongue, digestive tract, lungs, and mucosal membranes (47). Importantly, these locations are points of entry for foreign antigens into the host (47). MCs function to induce inflammation through the release of their mediators in response to parasitic and bacterial infections (48). They can be activated via pattern recognition receptors (PRRs) binding to pathogen associated molecular patterns (PAMPs) on pathogens (48, 49). In addition, MCs also serve in physiological processes such as angiogenesis and vasodilation (48). However, undesired activation of mast cells can lead to MC activation disorders including allergic reactions, anaphylactic responses, and autoimmune diseases (48).

The role of MCs in allergic reactions has been well established through clinical and experimental studies (36-38, 50, 51). Clinically, elevated levels of antigen specific IgE and primary MC mediators, such as tryptase, histamine, and PAF are found in patients experiencing allergic reactions (36-38). Consistent with this, studies involving FcεRI knockout mice and MC deficient mice have demonstrated a requirement for MCs in the onset of symptoms of IgE-mediated responses (50, 51). Furthermore, a subset of MCs known as IL-9 secreting mucosal mast cells (MMC9) have been identified as the main source of IL-9 in the gut, and function to promote MC maturation, intestinal mastocytosis, and heighten IgE-mast cell-dependent responses (52). Research currently focuses on the interactions of MMC9 cells with other immune cells, such as CD4+ T_{H}2 cells, and understanding genetic factors that can predispose individuals to develop over active MCs.
2.2 CD4\(^+\) T\(_2\) cells

CD4\(^+\) T cells, also known as T helper cells, are a type of T cell involved in the adaptive immune system. They are part of the lymphoid lineage and are involved in immune response regulation and mediation (53). Structurally, T helper cells express CD4 protein on their surface and recognize major histocompatibility (MHC) class II molecules on APCs (53, 54). CD4\(^+\) cells are capable of differentiating into various effector T cell subsets depending on the immune response required within the host. These include T\(_2\), T\(_1\), T\(_9\), T\(_17\), T\(_22\), Regulatory T cells (T\(_{reg}\)), and T Follicular cells (T\(_{FH}\)) (53, 54). The subset determines the cytokines that will be produced and the effector function of the CD4\(^+\) T cells (53, 54).

CD4\(^+\) T\(_2\) cells in particular play a role in IgE-mediated responses (13, 55). CD4\(^+\) T\(_2\) responses, also referred to as Type 2 immune responses, are characterized by the production of T\(_2\) cytokines such as IL-4, IL-5, IL-9, and IL-13 (13, 55). The effector cells responsible for these include B cells, MCs, eosinophils, and basophils (55). In B cells, IL-4 and IL-13 induce class switching of immunoglobulins to IgE (35) and promote proliferation (34). However, Type 2 immune responses can also be stimulated by non-pathogenic substances such as allergens, culminating in IgE-mediated responses described as allergic reactions (13). Experimentally, IL-4 has been shown to enhance antigen uptake within the intestine by upregulating CD23, an IgE receptor that binds to antigen specific IgE (56), and elevated levels of CD4\(^+\) T\(_2\) cells are observed in allergy induced mice (52). Clinically, elevated levels of antigen specific IgE are observed in patients with food allergy (57, 58). Furthermore, studies involving IL-4 and IL-13 knockout mice (IL-4\(^{-/-}\), IL-13\(^{-/-}\), and IL-4/13\(^{-/-}\)) showed that these mice had IgE production deficiency and, when induced, did not develop IgE-mediated food allergic symptoms (34, 59, 60). Indeed, when IL-4\(^{-/-}\) mice were given serum from sensitized WT mice and then exposed to the allergen, they displayed symptoms of IgE-mediated responses and food aversion was restored (59). Therefore, it is evident that T\(_2\) responses are a crucial part of IgE-MC-dependent responses.
2.3 Type 2 Innate Lymphoid Cells (ILC2)

Innate lymphoid cells (ILC) are innate immune cells originating from a common lymphoid progenitor (61). These cells are lineage marker negative (CD4, CD11b, CD11c, CD8, B220, Gr-1, TCR-β, TCR-γδ), lack receptors for antigens, and are identified by their cytokine expression profile (61). Their cytokine repertoire is similar to that of the various CD4+ T cell subsets. Type 1 innate lymphoid cells (ILC1) express transcription factor T-bet (T-box transcription factor) and produce cytokines such as IFNγ and TNF, TH1 cytokines (62). Type 2 innate lymphoid cells (ILC2) express transcription factors GATA-3 and RORα while producing high levels of IL-5 and IL-13, TH2 cytokines (63-65). Type 3 innate lymphoid cells (ILC3) express transcription factor RORγt and produce IL-17 and IL-22, TH17 cytokines. These cells function to form a bridge between innate and adaptive immune responses and defend the host against the various types of pathogens (62).

ILC2s, similar to CD4+ TH2 cells, work to combat parasitic and extracellular infections (66, 67). They have also been shown to be important mediators of allergic diseases (68-70). ILC2s are capable of inducing CD4+ T cell responses (71), CD4+ TH2 polarization and TH1 suppression (72), thereby promoting Type 2 immune responses. It has been observed that CD4+ TH2 and ILC2 interactions enhance airway inflammations (72, 73) and that Ig-E mediated responses can be enhanced via IL-13 production by ILC2s (74). Intestinal IL-25 and antigen-induced CD4+ TH2 cells are capable of promoting IL-13 production in ILC2s as well (75). In addition, ILC2-deficient mice could not produce a sufficient CD4+ TH2 response when exposed to allergens (68, 76, 77). It was also observed that IL-13 secreted by ILC2 cells was necessary for CD4+ TH2 differentiation in mouse lymph nodes and induction of Type 2 immune response against local antigen exposure (68, 76, 77). Therefore, ILC2s have a role to play in IgE-MC-dependent responses.

2.4 Basophils

Basophils are granulocytes originating from bone marrow HSCs. The granules contain chemical mediators similar to those present in MCs, and are involved in
inflammatory responses (78, 79). They are the least common granulocyte population, accounting for less than 1% of leukocyte cells (80). Similar to MCs, basophils express FcεRIα on their surface, however, unlike MCs, they do not regress from the bone marrow until fully mature (81, 82). Basophil development is regulated by cytokines IL-3 and thymic stromal lymphopoietin (TSLP) (83-86) and transcription factors such as GATA-2, C/EBPα, and STAT5 (83, 87, 88).

Physiologically, basophils are involved in Type 2 immune defense against extracellular pathogens. They can be activated via antibody (IgE) crosslinking with FcεRIα (89-91), cytokines such as IL-3, IL-18, and IL-33 (85, 92-94), and protease activity (95, 96). Pathologically, basophils have been associated with various diseases including allergic reactions (79, 97, 98), asthma (99, 100), Crohn’s disease (101), and myeloid leukemia (102, 103). In the context of food allergy, it has been shown that basophils promote T_H2 sensitization to food allergens and the consequent development of IgE-mediated reactions in epicutaneously sensitized mice (104). Basophils were shown to aggregate at the site of sensitization and were capable of secreting IL-4 leading to the polarization of naïve CD4^+ T cells (104). This is a key finding as basophil derived IL-4 drives T_H2 polarization and, thereby, promotes IgE-mediated food allergic responses. Further, basophils were shown to be a major contributor to IL-4 secretions during secondary antigen exposure (105). IgE-FcεRIα crosslinking induces rapid IL-4 secretion which aides Type 2 immune responses (105, 106). Basophil derived IL-4 secretion was observed within 2-4 hours of antigen exposure while CD4^+ T derived IL-4 secretion took longer, indicating that basophils are important in driving Type 2 immune responses and anaphylaxis response (105), (107).

3. Mast Cell Development

The development and maturation of mast cells from HSCs is regulated by various transcription factors including PU.1, MTIF, STAT5, C/EBPα, and GATA-2 (81, 108-111). GATA-1, a zinc-finger protein, is expressed in a number of hematopoietic lineages such as mast cells, eosinophils, megakaryocytes, and erythroid cells (112, 113). Sertoli cells are the only non-hematopoietic cells that express GATA-1 (114). Loss of function studies
have revealed an important role for GATA-1 in the development of megakaryocytes and erythroids (115-117). Indeed, genetic deletion of GATA-1 results in the pre-term death of mice due to anemia and improper megakaryocyte development (115-117). To study GATA-1 biology, investigators have generated a number of mice lines with reduced GATA-1 expression (118-121). Notably, studies utilizing these mice have revealed somewhat conflicting roles for GATA-1 in mast cell development and function (118-121). Studies utilizing GATA-1<sup>low</sup> mice revealed that decrease of GATA-1 expression results in reduced MC FcεRIα expression and altered immature MC numbers (118). In contrast, a study employing conditional GATA-1 knockout mice (Gata1<sup>−/−</sup>) showed that deletion of GATA-1 in adult mice had little to no effect on tissue resident MCs or <i>in vitro</i> bone marrow mast cell cultures (BMMCs) (119). Finally, using the ∆dblGata mice, where the palindromic double GATA site upstream of the GATA-1 promoter has been genetically deleted (120, 122, 123), investigators revealed normal MC levels in the context of airway inflammation (121).

4. Gap in knowledge

The contribution of GATA-1 to MC development and function has not been fully delineated. Furthermore, the requirement of GATA-1 signaling for IgE-MC dependent responses has not been investigated. In this thesis, we employ ∆dblGata mice to investigate the role of GATA-1 signaling in MC development and IgE-mast cell dependent anaphylactic reactions.

5. Hypothesis

1. Deletion of ∆dblGata region in GATA-1 genome increases susceptibility IgE-mast cell responses <i>in vitro</i>.
2. Reduced GATA-1 signaling has no impact on mast cell development and IgE-activation <i>in vivo</i>.
3. GATA-1 signaling has no impact on CD4<sup>+</sup> T<sub>TH2</sub> function in the context of food allergic reactions.
Chapter 2: Material and Methods

**Animals.** ΔdblGata (BALB/C) mice were generously provided by Dr. Stuart H. Orkin (Harvard, MA, USA) (120) and wild-type (WT) BALB/C mice originally provided by Charles River Laboratories, (Wilmington, MA, USA) were bred in-house at Cincinnati Children's Hospital Medical Center (CCHMC) (Cincinnati, OH, USA). Age-, sex-, and weight-matched littermates were used in all experiments. The mice were maintained and bred in a clean barrier facility and were handled under an approved Institutional Animal Care and Use Committee protocol at CCHMC animal facility. All experimental procedures were approved by the Institutional Animal Care and Use Committee of Cincinnati Children’s Medical Center.

**IgE-mediated experimental food allergy.** For the skin sensitization food allergy model, mice were first sensitized by applying (painted on skin surface) 20 μl of MC903 (0.1 μM Calcipotriol, TOCRIS Bioscience) and 5 μl of OVA (200mg/ml) to the right ear for 14 days consecutively. After the sensitization phase, the mice were fasted for 4 hours and then orally gavaged with OVA (50 mg in 250 μl saline) eight times every other day. The mice were observed for evidence of allergic symptoms 60 minutes following challenges as described previously (124).

**Clinical measurement parameters of food allergy.** Mice were scored using a scoring system after the eighth challenge (125). 0 for no clinical symptoms, 1 for repetitive nose and ear scratching, 2 for lethargy, pilar erecti and puffy nose, ears, and mouth. 3 for periods of motionless for >1 min and lying prone. 4 for no response to whisker stimuli or prodding. 5 for tremor, convulsions, and death. Occurrence of diarrhea, in the form of liquid excretion post challenge, was tracked within 1 hour after every challenge.
**ELISA and histological measurements.** Serum samples from blood drawn after cardiac puncture were analyzed using ELISA kits for OVA-specific IgE (MD Bioproducts, Oakdale, MN, USA), MCPt-1 (Invitrogen, Carlsbad, CA, USA), and OVA-specific IgG1 (Alpha Diagnostic International, San Antonio, TX, USA). Total IgE (Bioscience, San Diego, CA, USA) was performed for steady state analysis. For intestinal histological analyses, duodenal tissue was fixed in 10% formalin and processed by standard histological procedures as previously described (126). 5µm tissue sections were stained with Leder stain for Chloroacetate esterase (CAE) and counter-stained with hematoxylin and eosin. Sections were imaged at 20x and 40x magnifications. Stained cells were quantified as previously described (126). At least eight random images were taken from at least three random sections per mouse. Quantification of stained cells per square millimeter was performed by morphometric analysis using Image Processing Software (ImagePro, Media Cybernetics, MD, USA).

**Lamina propria mononuclear isolation.** Mice were euthanized in CO₂ and small intestine (SI) were removed surgically from mice, cut longitudinally, and cleaned in HBSS as previously described (127). In brief, samples were incubated in HBSS with 5mM EDTA (HBSS-EDTA) at 4°C for 5 minutes followed by vortexing in fresh HBSS-EDTA for four cycles to remove epithelial cells. The remaining tissues were minced in 8 mL RMPI 1640 (Gibco, Grand Island, NY, USA) with 2.4mg/mL collagenase A (Roche Basel, Switzerland) and 0.2mg/mL DNase I (Roche, Basel, Switzerland) at 37°C for 30 minutes. Single cell suspensions were obtained by passing the digested tissue 4 times through a 10mL syringe using a 19G needle and filtering homogenate. Filtered cells were washed once with RPMI 1640, suspended in 5mL of 44% Percoll and underlayered with 3mL of 67% Percoll before centrifugation for 20 minutes at room temperature (24°C) at reduced acceleration and deceleration. Lamina propria cells were collected from the interface between 44% and 67% Percoll, washed in media, resuspended in RPMI with 10% FBS, counted, and stained for flow cytometric analysis (52).
**Flow cytometric analysis.** Lamina propria cells from SI of ΔdblGata or WT mice were first stained with phycoerythrin (PE)- conjugated anti-c-kit, fluorescein isothiocyanate (FITC)-conjugated anti-β7 integrin, Horizon V500-conjugated CD4, APC-Cy7-conjugated anti-CD3e (BD Pharmingen, Mountain View, CA, USA), allophycocyanin (APC)-conjugated anti-IL-17RB, PE-Cy7-conjugated anti-FcεRIα (Biolegend, San Diego, CA, USA) and biotinylated anti-T1/ST2. Subsequently, cells were counterstained with PerCP-Cy5.5-conjugated monoclonal antibodies against lineage (Lin) markers (CD11b, CD11c, CD45R (BD Pharmingen, Mountain View, CA, USA) CD8α, Ly6G, and Pacific Blue labelled Streptavidin (Biolegend, San Diego, CA, USA)) before analysis with a FACS Canton II (BD Bioscience, Mountain View, CA, USA). CD4+ T H2 cells were identified as Lin-, CD3+, CD4+, and IL17RB+ populations. ILC2 cells were identified as Lin-, CD3-, CD4-, 1L17RB+, and cKit+ populations. MMC9 cells were identified as Lin-, CD3-, CD4-, 1L17RB-, cKit+, and FcεRIα+ populations as previously described (52). All cytometric data was acquired using BD FACSCanto II and data analysis was performed using Flowjo software (FlowJo, Ashland, OR, USA).

**Bone marrow derived mast cell culture.** Femur and tibia were harvested from age and gender matched WT and ΔdblGata mice. Bone marrows were flushed into complete RPMI 1640 containing penicillin-streptomycin (Gibco, Grand Island, NY, USA) and 10% fetal bovine serum (Gibco, Grand Island, NY, USA). Cells were centrifuged, resuspended in media, and filtered to remove debris. mIL-3 and mSCF (20ng/mL each) were added to the media and cells plated at 1 x 10⁶/mL. Media was changed frequently, and cells were re-plated at a concentration of 1 x 10⁶/mL. Mast cell maturation and purity was tracked weekly via FACS analysis for seven weeks. Cells were stained with phycoerythrin (PE)-conjugated anti-c-kit (BD Pharmingen, Mountain View, CA, USA), PE-cy7-conjugated anti-FcεRIα (Biolegend, San Diego, CA, USA) and biotinylated anti-T1/ST2. They were subsequently counterstained with Pacific Blue labelled streptavidin (Biolegend, San Diego, CA, USA). Analysis was performed on FACS Canton II (BD Bioscience, Mountain View, CA, USA).
**Bone marrow mast cell degranulation assay.** β-hexosaminidase assays were performed on the bone-marrow derived mast cells once they had reached maturation (≥85% ckit⁺ FcεRIα⁺ cells). As previously described, cells were incubated overnight with 0.5μg/mL mouse anti-IgE (α-TNP-BSA) (128). The following day, unbound IgE was washed with Tyrode’s buffer and 150,000 cells were loaded with 0.1, 1, or 10 ng/mL TNP-BSA and incubated for 45 minutes. Lysates were prepared by two freeze/thaw cycles by placing cells in -70°C for 15 minutes followed by 37°C heat bath for 5 minutes. Supernatant was extracted from both incubated and lysed cells and plated with P-nitrophenyl-N-acetyl-beta-D-glucosaminidase (PNAG), the hexosaminidase substrate. Plate was incubated for 90 minutes and the reaction stopped with 2M NaOH. Readings were obtained at 405nm (128). Percent lysates were calculated by dividing treated WT and ΔdblGata BMMCs by their respective lysates and then multiplying by 100.

**Passive anaphylaxis.** Mice were injected intravenously with 20μg/200μL of anti-IgE (IgG₂a mAb to mouse IgE; EM-95; obtained from Fred Finkelman, CCHMC) (129). The severity of shock was assessed by means of rectal temperature change with a rectal probe and a digital thermocouple thermometer (Model BAT-12; Physitemp Instruments, Clifton, NJ, USA). Blood was drawn intro heparinized capillary tube and centrifuged for 5 min at 10,000 rpm. Hematocrit (percentage of packed RBC volume) was calculated as the length of packed RBCs divided by the total length of serum and red cells in the capillary tube, multiplied by 100 (130, 131).

**Peritoneal wash.** Naïve Balb/c and ΔdblGata mice were euthanized and their peritoneal cavity was flushed by injecting 10mL of PBS in 10% FBS into peritoneal cavity, massaging the abdomen, and then drawing out the fluid. Cells were kept on ice, centrifuged at 1200 rpm for 5 mins, and supernatant removed. Pellets were lysed with 5mL Red Blood Cell Lysis Buffer (Sigma-Aldrich, St. Louis, MO, USA) for 5 minutes at room temperature, 5mL of RPMI 1640 media added to neutralize lysis, and centrifuged for 5 mins. Pellet was re-suspended in media and counted. Cells were stained with phycoerythrin (PE)- conjugated anti-c-kit (BD Pharmpingen, Mountain View, CA, USA), PE-cy7-conjugated anti-FcεRIα
(Biolegend, San Diego, CA, USA) and biotinylated anti-T1/ST2. They were subsequently counterstained with Pacific Blue labelled streptavidin. Analysis was performed on FACS Canton II (BD Bioscience, Mountain View, CA, USA) and analyzed using Flowjo (Flowjo Software, Ahsland, OR, USA).

**Statistical analysis.** All data is represented with mean unless otherwise stated. In experiments comparing multiple experimental groups, statistical differences between groups were analyzed using the one/two-way ANOVA parametric test. In experiments comparing two experimental groups, statistical differences between groups were determined using a Student’s t-test. Results are considered significant at $P \leq 0.05$. All data was analyzed using Prism (GraphPad Software, San Diego, CA, USA).
Chapter 3: Results

Reduced GATA-1 function alter in vitro bone marrow mast cell (BMMC) phenotype

Bone marrow cells from WT and ∆dblGata mice were cultured in mast cell growth conditions (IL-3 and SCF) for seven weeks and mast cell frequency and maturity were characterized by ckit⁺ FcεRIα⁺ analysis (Fig 1A – B). At seven weeks, MCs were the predominant cell population (≥ 85%) of the culture (Fig 1A – B). Notably, MC maturation was associated with high expression of FcεRIα and degranulation capacity (Fig 1A – D). In contrast, ∆dblGata cultures had a lower percentage of mature cells at seven weeks and the mast cells had reduced levels of FcεRIα expression and degranulation capacity (Fig 1B – D). Based upon these studies, we concluded that GATA-1 signaling contributes to MC differentiation in vitro.

Role of GATA-1 signaling in passive IgE-mediated anaphylaxis

Given the reduced mast cell differentiation efficiency in ∆dblGata mice, we examined the requirement for GATA-1 signaling in IgE-mediated anaphylaxis. Administration of anti-IgE to WT mice induced hypovolemic shock as evidenced by ≤ 4°C loss in body temperature and increased hemoconcentration (Fig 2A – C). Surprisingly, in ∆dblGata mice, we observed a similar response to that of WT mice (Fig 2A – C). Moreover, ∆dblGata mice experienced loss in body temperature and increased hemoconcentration (Fig 2B – C). We concluded that reduced GATA-1 signaling does not impact passive IgE-mediated responses in mice at steady state.

Requirement of GATA-1 signaling for steady state tissue MCs

Given these paradoxical observations of decreased FcεRIα expression and degranulation capacity of ∆dblGata BMMCs and normal IgE-mediated responses in vivo, we examined steady state tissue-resident mature mast cell levels in WT and ∆dblGata mice. Histological analyses of SI, ear, and tongue of WT and ∆dblGata mice revealed no
significant differences in MC numbers (Fig 3A – F). Analysis of peritoneal cavity revealed a 2-fold increase in peritoneal mast cell (pMC) levels in ∆dblGata mice compared to WT (Supplementary Figure S1). Notably, level of FcεRIα expression on pMCs was reduced in ∆dblGata mice when compared to WT mice (Supplementary Figure S1). Based upon these studies we concluded that a reduction of GATA-1 signaling does not impact SI, skin, and tongue MC levels, and increased pMC frequency.

**Requirement for GATA-1 signaling for IgE-MC-dependent responses *in vivo***

To determine the contribution of GATA-1 signaling in MC-dependent active inflammatory responses, we employed an active food allergy model (132). Antigen sensitization and repetitive oral challenges of WT mice induced anaphylaxis as evidenced by diarrhea and clinical symptoms (Fig 4A – C). Notably, development of symptoms of food-induced anaphylaxis was associated with MC activation (Fig 4D). Sensitization and repeated antigen challenge of ∆dblGata mice also induced food-induced anaphylactic response; surprisingly the ∆dblGata mice were more susceptible to IgE-MC-dependent food allergy. Moreover, only 50% of WT mice had diarrhea on the eighth challenge compared to 100% of ∆dblGata mice (Fig 4B). The ∆dblGata mice were also more susceptible to severe reactions (Fig 4C). Notably, the ∆dblGata mice developed more severe symptoms following the eighth challenge including repetitive nose and ear scratching as well as lethargy, pilar erecti (Fig 4C).

The heightened disease severity was associated with significantly increased mast cell activation (MCPt-1) and SI mast cell numbers as compared with WT mice (Fig 4D and Supplementary Figure S2). These data are consistent with previous reports demonstrating a positive correlation between SI mast cell levels and activation and severity of oral antigen-induced anaphylaxis (124). Together, these results show that ∆dblGata mice are more susceptible to IgE-mediated food allergy and reduced GATA-1 signaling increased mucosal mast cells in murine model of food allergy.
IgE-mediated responses in ΔdblGata mice driven by Th2 immunity

Type 2 immunity has been shown to be critical for the IgE-mast cell response in murine models of food allergy (13, 34, 133). To determine whether increased food allergy susceptibility in ΔdblGata is associated with increase Type 2 immunity, we assessed antigen specific IgE and IgG1 levels (Fig 5A – B). Notably, while both WT and ΔdblGata mice generated antigen-specific IgE and IgG1 responses, ΔdblGata mice had significantly higher levels of both OVA-specific IgG1 and IgE compared to WT mice (Fig 5A). Given the relationship between antigen specific IgE and Type 2 immune response, we examined for presence of CD4+ Th2 cells (CD4+ IL17RB+) (Fig 5F and I) and ILC2 (Lin-, cKit+ IL17RB+) cells (Fig 5E and Fig H) within the SI of OVA-sensitized and challenged WT and ΔdblGata mice (Fig 5C). We identified a significant increase of CD4+ Th2 cells in the SI of ΔdblGata mice as compared to WT mice (Fig 5F and I). Although there was a significant decrease in population percentage of ILC2 cells, the difference was not significant in total cell numbers between WT and ΔdblGata mice (Fig 5E and H). The significant increase of CD4+ Th2 cells confirms the involvement of Type 2 immunity for heightened disease severity in ΔdblGata mice.

MMC9 cells have been recently shown to be the main source of IL-9 in the SI (52) which promotes mast cell maturation and intestinal mastocytosis (52, 132). Analysis of MMC9 levels in WT and ΔdblGata mice revealed that OVA sensitization and repeated challenge induced SI MMC9 levels in both WT and ΔdblGata mice (Fig 5D and G). Consistent with elevated mature MC levels in ΔdblGata mice, levels of SI MMC9 cells were also increased in ΔdblGata mice compared with challenged WT mice. To determine whether the observed differences in Type-2 immunity in ΔdblGata mice was a steady state phenotype, we examined SI immune profile of WT and ΔdblGata mice at steady state. Notably, levels of total serum IgE and CD4+ Th2, ILC2, and MMC9 cells levels in the SI were comparable between WT and ΔdblGata mice at steady state (Supplementary Figure S3). These studies suggest that antigen sensitization and challenge of ΔdblGata mice leads to elevated Type 2 immune response which results in increased IgE-MC-dependent responses.
Figure 1. Bone marrow derived MC phenotype in ΔdblGata mice. (A) Representative flow cytometry plot of BM MC phenotype (FcεRIα⁺ c-kit⁺ cells) in the culture at seven weeks of age. (B) The percentage of FcεRIα⁺ c-kit⁺ cells in WT and ΔdblGata mice BM culture over seven weeks. (C) The mean fluorescence intensity (MFI) of FcεRIα on WT and ΔdblGata BMMCs. (D) The level of β-hexosaminidase activity following IgE-mediated degranulation of WT and ΔdblGata BMMCs. BM cells were isolated from 6-8 week old mice were cultured in the presence of IL-3 (20 ng/ml) and SCF (20 ng/ml) for 7 weeks, and BMMCs were examined for FcεRIα, and c-Kit expressions by flow cytometry analyses. 7 week cultured WT and ΔdblGata BMMCs (5 × 10⁶/ml) were sensitized with IgE-TNP (0.1 - 100 ng/ml) and challenged with BSA-TNP and supernatant was assayed for β-hexosaminidase activity as described in materials and methods. Data represents mean ± SEM and is representative of three separate experiments. Statistical significance is * p ≤ .05, *** p < 0.001 compared with negative control (IgE and BSA-TNP negative). n.s. not significant.
Figure 2. IgE-mediated passive anaphylaxis in WT and ΔdblGata mice. (A) Rectal temperature 0-60 minutes, (B) maximal temperature change at 30 minutes, and (C) hemocrit concentration at 60 minutes in anti-IgE-treated (20 μg/200 μl saline) WT and ΔdblGata mice. Data represent mean ± SEM. Data were analyzed using a Student’s T-test. (A) represented as mean ± SEM; n = 4 to 8 mice per group from triplicate experiments. (B) and (C) line indicates mean and symbols represent individual mice. Dashed line indicates steady state hemacconcentration at baseline. n.s. not significant.
Figure 3. Systemic mast cells in WT and ΔdblGata mice at steady state. Representative photomicrographs and quantification of Mast cells/Hpf in: a) jejunum, (b) ear skin and (c) tongue of WT and ΔdblGata mice. Images were taken at 40x magnification. Data represent mean ± SEM (n = 4-6) and is representative of two separate experiments. Data were analyzed using a Student's T-test. n.s. not significant.
Figure 4. Active IgE-MC-dependent anaphylaxis in WT and ΔdblGata mice. (A) Oral antigen-triggered anaphylaxis experimental regime. (B) Diarrhea occurrence, (C) clinical score and (D) serum mast cell protease-1 (MCPT-1) levels in OVA-sensitized, i.g. Veh- or OVA-challenged BALB/c WT and ΔdblGata mice. The percentage in panel B represent the number of mice that experienced diarrhea over the number of total mice challenged as a percentage. Panels C and D were analyzed following eighth challenge of OVA-sensitized, i.g. Veh- or OVA-challenged mice. (C) and (D) line indicates mean and symbols represent individual mice. Values represent mean ± SEM; n = 6 to 10 mice per group. Statistical significance is *** p < 0.01. ND non-detected.
Figure 5. OVA-specific Ig, CD4+ Th2 cell, MMC9 and ILC2 frequency in SI of WT and ΔdblGata mice. (A and B) Antigen-specific IgE and IgG1 in serum, (C) detection and frequency of SI Lin−ST2+FcεRlα+c-Kit+ β7integrinlow MMC9 (D and G), Lin−IL-17RB+c-KIT−ILC2s (E and H) and Lin−CD3+CD4+IL-17RB+ Th2 cells (F and I) from OVA-sensitized, i.g. OVA-challenged BALB/c WT and ΔdblGata mice. All analyses were performed following eighth challenge of OVA-sensitized, i.g. Veh- or OVA-challenged mice. A, B and D-I, line indicates mean and symbols represent individual mice. Statistical significance is * p ≤ .05, ** p ≤ .01, and *** p ≤ .001.
Supplemental Figure 1. Peritoneal wash mast cells. (A) Peritoneal cells were obtained from 4-6 week old ∆dblGata mice and BALB/c WT as described in materials and methods. Mast cells were identified as FcεRIα⁺ c-kit⁺ cells, (B) frequency detected, and (C) MFI of FcεRIα calculated. (B) and (C) line indicates mean and symbols represent individual mice and is representative of three separate experiments. Data were analyzed using a Student’s T-test. Statistical significance is ** p ≤ .01.
Supplemental Figure 2. Increased intestinal mastocytosis in allergy induced ΔdblGata mice. (A – C) Representative photomicrographs and quantification of Mast cells/mm². (C) line indicates mean and symbols represent individual mice. Images were taken at 20x and 40x magnification. Data representative of four separate experiments. Statistical significance is *** p ≤ .001.
Supplemental Figure 3. Total IgE, CD4⁺ T\(\text{H}2\) cell, MMC9 and ILC2 frequency in SI of WT and \(\Delta\text{dblGata}\) mice at steady state. (A) Total IgE in serum. (B) detection and frequency of SI Lin⁻ST2⁺Fc\(\varepsilon\)R\(\alpha\)⁺c-Kit⁺β\(7\)integrin\(\text{low}\) MMC9, (C) Lin⁻IL-17RB⁺c-KIT⁻ILC2s, and (D) Lin⁻CD3⁺CD4⁺IL-17RB⁺ T\(\text{H}2\) cells from BALB/c WT and \(\Delta\text{dblGata}\) mice at steady state. (A-D) line indicates mean and symbols represent individual mice. Data representative of three separate experiments. n.s. not significant.
Chapter 4: Discussion and Future Direction

In the present study, we have investigated the requirement of GATA-1 signaling in the development and maturation of MCs and IgE-induced MC function in vitro and in vivo. We show that in vitro that diminished GATA-1 function reduces MC maturation rate and degranulation capacity. However, in vivo analyses revealed that diminished GATA-1 activity impacts neither tissue MC frequency nor MC functionality at steady state. Surprisingly, employing models of passive and active IgE-mediated anaphylaxis, we show that diminished GATA-1 functionality has no impact on IgE-MC-dependent responses in the naïve state, however, antigen sensitization models revealed that low GATA-1 activity amplifies IgE-MC-dependent responses. The increased response appeared to be associated with a stronger pro-Type 2 response associated with increased antigen-specific IgE and CD4+ T\(_H\)2 response. Despite, GATA-1 intrinsic requirement for MC maturation and IgE-functionality in vitro, in vivo analyses reveals that a compensatory involvement for GATA-1 in the regulation of CD4+ T\(_H\)2 responses leads to heightened MC functionality and IgE-MC-dependent immune responses in vivo.

There have been a number of studies employing various transgenic and gene-deficient mice defining the involvement of GATA-1 in MC biology (42, 118-120) and these studies have identified a complex role for GATA-1 in the differentiation, maturation and functionality of MCs. Some studies have reported that GATA-1 is not required for MC differentiation and maturation, however other studies report an important role for MC maturation and functionality (42, 118, 119). There are many potential explanations for these discrepancies related to the level of GATA-1 promoter inactivation in the various genetically modified mice. Moreover, MC-intrinsic differences in GATA-1 activity (GATA\(_{\text{low}}\) versus ΔdblGata mice) contribute to the differences in surface expression of FcεRI on BMMCs (118, 120). These studies are supported by the observations of GATA-1 binding to the promoter region of FcεRI, ckit and cpa3 genes in BMMC’s (134, 135) and the identification of GATA-1 binding motifs in the 5’-end of group A tryptase (119). Collectively these studies indicate that GATA-1 is dispensable for MC differentiation (119) however, GATA-1 plays a conserved role in later stage MC maturation through its involvement in transcriptional regulation of expression of several MC specific genes including FcεRIα,
FCεRIβ and Cpa3 (136-138). MC differentiation and maturation involves a complex interplay by several transcription factors including PU.1, MTIF, STAT5, C/EPBα, and GATA-2 (81, 108-111). Unlike following GATA-1 deletion, GATA-2 deletion leads to loss of MC lineage specification (139) suggesting that GATA-2 plays a more dominant role in mast cell differentiation. Elegant studies by Ohneda and colleagues revealed that GATA-2 is required for cell lineage specification and the maintenance of MC in the differentiated state whereas GATA-1 regulates expression of MC specific group A tryptase gene expression (119).

The observed in vitro effects on diminished GATA-1 signaling on FcεRI expression and MC cell degranulation capacity prompted us to examine the impact of diminished GATA-1 signaling on IgE-MC dependent responses. To our surprise we observed no difference in IgE-mediated MC-dependent shock responses between WT and ΔdblGata mice. We speculated that the observed discrepancy between the in vitro and in vivo observations could be explained by heightened MC activation following IgE crosslinking or altered steady state tissue MC levels. However, examination of the surrogate MC activation marker (Mcpt-1) revealed comparable level of MC activation between groups and peripheral tissue MC levels at steady state in ΔdblGata mice were not significantly different to WT mice. The observed similarities in distribution or frequency of MC populations in peripheral tissues are consistent with previous studies employing ΔdblGata and Gata1−/− mice (119). To our knowledge this is the first in vivo examination of MC function in ΔdblGata mice. Interestingly, IgE-FcεRI-MC-dependent anaphylaxis in mice is dependent on connective tissue mast cells (CTMC) and histamine (140). Examination of frequency and phenotype of MCs in the peritoneal lavage of ΔdblGata mice revealed higher MC numbers and reduced expression of FcεRI. The increased level of MCs in peritoneal lavage is consistent with the observed higher incidence of immature MCs (c-kithigh FcεRIlow) observed in peritoneal lavage of GATA-1low mice (118). Previous studies have reported a central role for GATA-2 and MITF in CTMC differentiation and IgE-MC-mediated anaphylaxis (140). MITF and GATA-2 critically regulate HDC gene expression and histamine synthesis (140) whereas GATA-1’s effects on MC is likely restricted to regulation of expression of specific MC genes related to group A Tryptase gene
expression (e.g. Mcpt-6) which are not necessary for IgE-FcεRI-MC-dependent anaphylaxis.

Employing our active models of IgE-MC-dependent anaphylaxis (124), we revealed heightened IgE-MC-dependent shock response during diminished GATA-1 activity. Diminished GATA-1 activity had an impact on intestinal mastocytosis and MC-dependent activity. The enhanced IgE-MC response in the ΔdblGata mice was associated with heightened Type-2 immune responses as evidenced by increased CD4⁺ T_H2 cells and antigen-specific IgE. We have previously reported an important role for IgE and CD4⁺ T_H2 cells in food-induced anaphylaxis in mice (34, 52, 124, 141).

We demonstrate that ΔdblGata mice exhibit a more severe disease phenotype in our active model. This is in contrast to a similar study that was recently published observing that there was no difference in IgE-mediated anaphylactic response in between ΔdblGata and WT mice when food allergy was induced (104). However, this study employed an active model consisting of fewer oral gavage challenges (two) and, upon inspection, showed slightly elevated levels of mast cells in the SI and intestinal mastocytosis, albeit not significantly different (104). We hypothesize that this observation is heightened in our active model containing a higher number of oral challenges (eight) and, thereby, eliciting a more robust response leading to elevated symptoms.

GATA-3 is necessary for CD4⁺ T_H2 development (142) and previous work has shown that GATA-3 is capable of auto-activation in a STAT-6-independent manner leading to T cell commitment to CD4⁺ T_H2 lineage and Th1 inhibition (143, 144). GATA-3 expression can also be activated by heterologous GATA family members such as GATA-1, GATA-2, and GATA-4 inducing CD4⁺ T_H2 development in STAT6-deficient T cells (145). This led to the speculation that GATA-3 genome could consist of GATA binding sites to which GATA proteins can bind with varying degrees of affinities activating GATA-3 (145), similar to the GATA regulatory elements found in GATA-1 promoter region (122, 146). Therefore, mechanistically, in the absence of the high affinity GATA binding site in GATA-1 DNA, increased numbers of GATA proteins may be capable of binding to GATA sites on GATA-3 inducing elevated CD4⁺ T_H2 development and Type 2 immune response as evident in ΔdblGata mice.
In the context of food-induced allergic responses, IL-9 is important for mast cell recruitment resulting in intestinal mastocytosis and driving intestinal mast cell maturation (132, 141). The source of IL-9 in the intestine was recently shown to be MMC9 cells (52). MMC9s are capable of producing abundant amounts of IL-9 and, therefore, are the principal IL-9 produces in the intestine (52). Elevated levels of MMC9 directly correlate with increasing severity of anaphylaxis. Pathologically, Il9 and mast cell specific transcripts were found at elevated levels in patients with atopic dermatitis who had also developed food allergy, further supporting the claim that MMC9 cells are pivotal in the crosstalk between skin sensitization and development of food allergy (52). Further, adoptive transfer of MMC9 cells is capable of reconstituting IgE-mast cell dependent phenotype (52). Consistent with these findings, we observe increased numbers of MMC9s in ∆dblGata mice simultaneous with more severe allergic responses. Increased intestinal mastocytosis and mast cell activation can also be attributed to the elevated levels of IL-9 secreting cells. We hypothesis that due to the hand-in-hand nature of CD4+ TH2 cells and MMC9s, increased frequency of one promotes the increase of the other and together they regulate the severity of the response. Previously, we have reported no change in ILC2 numbers during the induction of food allergy (52). Although ILC2 cells have a role in food allergies, their overall frequency is not of consequence. Consistent with this, we note that a reduction in ILC2 cell numbers does not appear to alter the allergic response in ∆dblGata mice. In fact, the heightened response is in spite of the lower ILC2 levels. We hypothesize that while the ILC2 - CD4+ TH2 interaction can augment the TH2 response, it is not critical for it.

Eosinophils, while part of the immune repertoire, have been implicated in inflammatory responses such as eosinophilic esophagitis and asthma (147-149). In the context of food allergy, eosinophils were observed to infiltrate the large intestine in mice with allergy induced diarrhea (150). A possible interaction between eosinophils and mast cells leading to mast cell degranulation has also been described (151-153). However, it was also shown that eosinophils are not required for the induction of allergic diarrhea with the use of IL-5/eotaxin-1 double deficient mice (126) leading to contrasting reports in the
involvement of eosinophils in food allergic responses. In this study, we have successfully induced IgE-mast cell dependent responses in mice lacking eosinophils, the ∆dblGata mutants (120). It appears that eosinophils are not necessary for the development of food allergy and the subsequent anaphylaxis reactions.

In conclusion, mice with diminished GATA-1 function reveal a complex role for GATA-1 in MC maturation and functionality. In vitro, reduced GATA-1 activity leads to impairment of MC maturation and IgE-mediated degranulation supporting an important role for GATA-1 in MC function. However, in vivo analyses reveal that the MC-intrinsic GATA-1 requirement in maturation and degranulation does not necessarily impact IgE-MC-dependent immune responses due to associated MC-extrinsic role for GATA-1 in heightened CD4⁺ Th2-immunity. These studies reveal the complexity of immune signaling pathways with the existence of compensatory processes that can independently alter cell intrinsic requirements via cell extrinsic processes.

Further studies need to be conducted to elucidate the specific mechanism through which reduced GATA-1 expression impacts the development of IgE-mediated responses. Since the ∆dblGata is a global knockout, it is not possible to discern if the observed responses are due to effects on MCs or CD4⁺ Th2 cells. One possible experiment to examine this is to employ an adoptive bone marrow transfer model where bone marrow from ∆dblGata mice are transferred intravenously into irradiated WT mice under the active food allergy model. Here, the recipient WT mice will develop mast cells from ∆dblGata bone marrow while the CD4⁺ Th2 compartment will be from the WT mice, as described previously by our group (52). By observing the IgE-mediated response in recipient mice with ∆dblGata MCs, it would be possible to understand the impact on MCs alone in vivo. Examining possible differences in gene expression between WT and ∆dblGata mice is another line of investigation that could shed light on possible mechanisms. DNA microarrays, RNA-sequencing, and RT-PCR are examples of powerful tools that can be used for gene expression analysis. In addition, ChIP analysis can be employed to investigate DNA-GATA protein interactions to study if GATA-1 can induce CD4⁺ Th2 in the absence of the double GATA sequence in ∆dblGata mice. Finally, building a cytokine profile for comparison, between WT and ∆dblGata mice, in the murine food allergy model
will allow researchers to observe if $\Delta$dblGata mutation impacts the ability to produce various cytokines in different cell types and their downstream effects.
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