Exon skipping of FcεRIβ eliminates expression of the high-affinity IgE receptor in mast cells with therapeutic potential for allergy

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Edited by K. Frank Austen, Brigham and Women’s Hospital, Boston, MA, and approved October 26, 2016 (received for review May 27, 2016)

Allergic diseases are driven by activation of mast cells and release of mediators in response to IgE-directed antigens. However, there are no drugs currently available that can specifically down-regulate mast cell function in vivo when chronically administered. Here, we describe an innovative approach for targeting mast cells in vitro and in vivo using antisense oligonucleotide-mediated exon skipping of the β-subunit of the high-affinity IgE receptor (FcεRIβ) to eliminate surface high-affinity IgE receptor (FcεRI) expression and function, rendering mast cells unresponsive to IgE-mediated activation. As FcεRIβ expression is restricted to mast cells and basophils, this approach would selectively target these cell types. Given the success of exon skipping in clinical trials to treat genetic diseases such as Duchenne muscular dystrophy, we propose that exon skipping of FcεRIβ is a potential approach for mast cell-specific treatment of allergic diseases.

Asthma and related allergic diseases affect up to 1 in 10 people in developed countries, and about 10% of patients with asthma cannot be controlled with current therapeutic approaches. The most widely used therapies for asthma rely upon dampening the inflammatory response with either oral or inhaled glucocorticosteroids and relaxing the constricted airway smooth muscle cells with β-adrenoreceptor agonists. However, high doses of steroids when needed for severe asthma are associated with undesirable side effects and inhaled β-adrenoreceptor agonists can increase the risk of death from asthma if not used in combination with glucocorticosteroids. It is suggested that β-adrenoreceptor agonists may promote underlying inflammation that could contribute to the airway wall remodeling seen in asthma (1). Other clinical approaches that aim for more long-term alleviation of symptoms, including desensitization with incremental increases in dose of allergen or hypo-sensitization to induce immune tolerance, have proven beneficial for some, but not all, patients, and serious adverse effects can occur (2).

In addition to asthma, other allergic diseases have similar treatment strategies and also have an unmet clinical need. An example is atopic dermatitis (AD), which like asthma, is also a multifactorial disease with complex pathophysiology, remains incurable, and affects 10–20% of children in the United States (3). The two major symptoms of AD are inflammatory lesions and severe pruritus. Both the prevalence and the economic burden of AD are increasing worldwide. Symptomatic treatment with topical and/or systemic glucocorticosteroids or calcineurin inhibitors are still considered the gold standard. However, due to the known adverse effects of these drugs, prescription rates are low and patient compliance is often poor (3). Thus, there is still a need for new therapeutic approaches with a better safety profile.

An approach for intervention in allergic inflammation that we have pursued is based on the finding that the gene loci 11q12-q13 are strongly linked to allergy and asthma susceptibility and that the membrane spanning 4A (MS4A) gene family is clustered around 11q12-q13 (4–7). Moreover, the genes MS4A11 (encoding the protein CD20) and MS4A42 (encoding the protein FcεRIβ (β-subunit of the high-affinity IgE receptor)) are associated with the activation and proliferation of B cells (8) and mast cells (9–11), respectively, and are therefore considered potential candidates for the linkage of these genetic regions with allergy. In particular, FcεRIβ contributes to IgE-dependent mast cell signaling by trafficking the FcεRI receptor complex to the cell surface and amplifying FcεRI-induced signaling (12, 13). The first transmembrane domain of FcεRIβ is required for trafficking the receptor complex (14), whereas the C-terminal immunoreceptor tyrosine-based activation motif (ITAM) amplifies signaling (15). Thus, a report that polymorphisms in MS4A42 were associated with development of asthma gained interest (16), but studies into the functional consequence of mutations in MS4A42 did not appear to affect the function of FcεRIβ (17). However, we have identified the expression of a truncated isoform of FcεRIβ (t-FcεRIβ) that lacks exon 3 of MS4A42, which encodes the first two transmembrane domains of FcεRIβ (10). This truncated isoform does not traffic to the plasma membrane (9, 10) and appears to function in intracellular rather than plasma membrane signaling (9). Therefore, our hypothesis is that perturbation of MS4A42 splicing could lead to disproportionate expression of the t-FcεRIβ isoform at the expense of full-length (FL) FcεRIβ isoform and thus perturb trafficking of the FcεRI receptor complex to the plasma membrane as well as mast cell responses to IgE-directed antigens.

Here, we have examined whether manipulation of MS4A42 splicing favors t-FcεRIβ formation, disrupts FcεRI expression and signaling, and has functional consequences. We identified that forced expression of t-FcεRIβ using antisense oligonucleotide (AON)-mediated exon skipping of MS4A42 exon 3 eliminated expression of FcεRI in

Significance

We identified an innovative use for the technique of antisense oligonucleotide-mediated exon skipping to specifically target and down-regulate IgE receptor expression in mast cells. Exon skipping is typically used as part of personalized medicine, where a mutant exon is skipped after sequencing the patients’ affected genes. Our approach, however, targets a nonmutated gene and an exon that is critical for surface IgE receptor expression. It does not require a personalized approach with genetic sequencing or multiple iterations of oligonucleotides that would require clinical trials. Furthermore, the diseases to be treated with this technology are ideal for local delivery of the oligonucleotides by aerosols or topical cream formulations.

Author contributions: G.C. conceived research; G.C., W.B., M.A.B., and D.D.M. designed research; G.C., Y.Y., T.F., A.D., G.K.A., W.B., and M.A.B. performed research; G.C., Y.Y., T.F., G.K.A., and W.B. analyzed data; and G.C., M.A.B., and D.D.M. wrote the paper.

Conflict of interest statement: Carrying out this study resulted in the filing of US Provisional Patent Application No. 62/289,447 by G.C. and D.D.M.

This article is a PNAS Direct Submission. Freely available online through the PNAS open access option.

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This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1608520113/-/DCSupplemental.
mast cells and resulted in mast cells that were functionally unresponsive to IgE-mediated antigen challenge. Given the recent promising results of using AONs to alter splicing in diseases (for reviews, see refs. 18–20), and their success in clinical trials for Duchenne muscular dystrophy (21, 22), we propose that our results warrant further study to develop this approach as a potential mast cell-specific treatment for allergic diseases.

Results
Loss of FcεRI with FcεRIβ Exon Skipping. We first tested whether AONs could be efficiently transfected into mast cells using a control 25-mer FITC-conjugated morpholino AON in primary mouse bone marrow–derived mast cells (BMMCs). We achieved >95% efficiency in mouse BMMCs at 24 h (Fig. 1A and B) with no evidence of cytotoxicity as determined by propidium iodide staining (Fig. 1A).

The naturally occurring truncation of MS4A2 exon 3 leads to loss of the first two transmembrane domains of FcεRIβ resulting in the expression of t-FcεRIβ that does not traffic to the plasma membrane nor associate with FcεRI (9, 10). Therefore, we predicted that skipping exon 3 of MS4A2 following FcεRIβ AON treatment would result in preferential production of t-FcεRIβ instead of FL FcεRIβ as well as loss of expression of surface FcεRI, which is dependent on FL FcεRIβ (9, 12–14). We attempted to induce exon skipping with AONs designed to target Ms4a2 exon 3 at the intron-exon boundary and identified that FcεRIβ AONs dose-dependently induced exon skipping of FcεRIβ mRNA as indicated by RT-PCR compared with cells transfected with an equivalent 25-mer standard control AON (Fig. 1C).

To test whether exon skipping leads to loss of surface expression of FcεRI, we used flow cytometry to measure surface FcεRI expression in mouse BMMCs and found that it was reduced after 10 μM FcεRIβ AON treatment by 95.6 ± 0.4% (n = 5; P < 0.0001) (Fig. 1D), thus virtually eliminating FcεRI expression (efficiency of transfection was >95%). In agreement with the exon-skipping data (Fig. 1C), the effects of FcεRIβ AONs on surface FcεRI expression were also dose dependent (Fig. 1E).

Degranulation and Calcium Influx. In view of the loss of surface FcεRI expression with FcεRIβ AON transfection, we tested whether there were corresponding reductions in responses to antigen in BMMCs. There was a dose-dependent decrease in degranulation in response to DNP with increasing concentrations of FcεRIβ AON, and degranulation was eliminated with 10 μM FcεRIβ AON (Fig. 2A). One micromolar FcεRIβ AON resulted in 80% reduction in surface FcεRI expression (Fig. 1C) whereas the reduction in degranulation was lower (∼25%), although significant (Fig. 2A). A possible explanation for this finding is that the number of FcεRI receptors and signaling capacity appears to exceed the requirements for degranulation, an explanation in agreement with studies reported in RBL-2H3 mast cells (23). Although FcεRI numbers vary during the cell cycle (24) and among different mast cell types, with estimates from 130,000 in human lung mast cells (25) to ~290,000 per cell in RBL-2H3 cells (23) and 120,000–380,000 in human cord blood-derived mast cell/basophil cultures (26), it is likely that mast cells in general harbor surplus FcεRI as significant degranulation is observed with aggregation of a few hundred receptors (23).

The specificity of FcεRIβ AON treatment was next determined by its effect on thapsigargin-induced degranulation. Although FcεRI-dependent degranulation was eliminated in BMMCs, thapsigargin-induced degranulation was unaffected by FcεRIβ exon skipping (Fig. 2B). In addition, we found the same pattern for calcium mobilization with robust inhibition of the calcium signal in response to FcεRI aggregation whereas the response to thapsigargin was unaffected (Fig. 2C). As with thapsigargin, IgE-mediated calcium influx is dependent upon store-operated calcium entry (27). Therefore, FcεRIβ exon skipping appears to selectively target IgE-dependent activation without disrupting cell responses to other stimuli.

Signaling, Cytokine Release, and Migration. We next examined the effects of FcεRIβ exon skipping in BMMCs on cell signaling events that regulate both degranulation and de novo cytokine synthesis and in particular whether residual weak signals that fail to stimulate degranulation were still sufficient to induce synthesis of cytokines. As expected from the diminished calcium signal, we found no significant FcεRI-mediated phosphorylation of PLCγ1 with FcεRIβ AON transfection, and unlike FcεRI-mediated activation, thapsigargin does not induce phosphorylation and acts independently of PLCγ (Fig. 3A and B). Phosphorylation of AKT and ERK are more distal signals than PLCγ phosphorylation, but phosphorylation of both of AKT and ERK were also markedly

Fig. 1. Transfection of AONs induce exon skipping and loss of surface FcεRI expression. (A) Density dot plots of FITC positivity (x axes) versus propidium iodide positivity (y axes) of mock-treated BMMCs (Top) and FITC morpholino AON-transfected BMMCs (Bottom). (B) Transfection efficiency data displayed as histogram with mock-treated (red line) versus FITC AON-treated (blue line) BMMCs. (C) Transfection of Ms4a2 exon 3-targeted AONs (FcεRIβ AON) induces exon skipping in mouse BMMCs. Qualitative RT-PCR bands correspond to full-length (FL) FcεRIβ and t-FcεRIβ (exon 3 truncation), shown with arrows as determined by DNA size markers. (D) Flow-cytometric analysis of surface FcεRIα expression in BMMCs 48 h after transfection with either the standard control AON (Standard Con AON, blue lines) or FcεRIβ AONs (red lines). Black line is the isotype control. Data are representatives of five experiments. (E) Loss of surface FcεRI expression with FcεRIβ AON transfection is dose dependent. Combined data from five experiments. Data are the mean ± SEM. ****P < 0.0001.
Fig. 2. Transfection of FcRIγ AONs eliminates mast cell responsiveness to IgE. (A) Transfection of FcRIγ AON in mouse BMMCs results in a dose-dependent reduction in degranulation in response to antigen challenge. Data are the mean ± SEM from five experiments. (B) Degranulation of BMMCs in response to either antigen (DNP; Left) or thapsigargin (Right). Standard control AON is in blue, and FcRIγ AON is in red. (C) Ratios of calcium signaling was performed on the same cells as in B. The arrowhead denotes the time of the stimulant addition. Transfection of FcRIγ AON (red lines) eliminates both degranulation (B, Left) and the calcium response (C, Left) to antigen without affecting the degranulatory or calcium responses to thapsigargin (B and C, Right). Data are the mean ± SEM from four experiments. Statistical analyses were carried out as ANOVA with Tukey's posttest, comparing each DNP concentration to the corresponding DNP concentration with 0 μM AON (A) or FcRIγ AON to Std Con AON for each concentration (B) or time point (C). *P < 0.05, **P < 0.01, ****P < 0.0001.

Reduced by FcRIγ exon skipping (Fig. 3 A, C, and D). In contrast to PLCγ1, the AKT and ERK pathways are activated by thapsigargin, but neither AKT nor ERK phosphorylation was affected by FcRIδ exon skipping (Fig. 3 A, C, and D).

Low-level activation of mast cells can result in the production of cytokines without evidence of acute signaling events. One such example is IgE alone, which did not elicit rapid phosphorylation of PLCγ1, AKT, or ERK (Fig. 3 A–D), but did cause release of the cytokine GM-CSF after 6 h (Fig. 3E). Nevertheless, this release, as well as that induced by IgE plus antigen to induce FcRI aggregation, was blocked by FcRIγδ exon skipping without affecting thapsigargin-induced GM-CSF release in BMMCs (Fig. 3E).

We also examined the effects of exon skipping on antigen-mediated BMMC migration (28). Standard control AON-treated BMMCs that were sensitized with IgE migrated toward antigen (DNP-BSA), but FcRIγ AON-treated BMMCs did not (Fig. 3F). However, BMMC migration mediated through KIT, the receptor for stem cell factor (SCF), was not reduced and if anything it was enhanced by FcRIγδ exon skipping, although not to a statistically significant extent (Fig. 3F). Collectively, these data indicate that FcRIγδ exon skipping selectively and substantially abrogates FcRI-dependent responses in BMMCs.

Survival and Proliferation. In addition to the classical view that IgE binding to FcRI on mast cells primes mast cells for activation by bivalent/multivalent antigens, it is now recognized that IgE binding to FcRI leads to activation of mast cells to release prosurvival cytokines that maintain viability of the cells (29–31). In the absence of external supporting cytokines, mast cells rapidly undergo apoptosis, which is suppressed by the addition of IgE. Therefore, elevation of tissue IgE during allergic diseases, such as the lung in asthma or the skin in AD, could contribute to increased mast cell numbers by promoting cytokine release and mast cell survival. We therefore tested whether FcRIγδ exon skipping would also eliminate the prosurvival effect of IgE on mast cells. BMMCs were deprived of the culture growth-promoting cytokine, IL-3, for 24 h after transfection with AONs. Over the course of 72 h, IgE almost completely protected mast cells from cell death and apoptosis after withdrawal of IL-3 in control AON-treated BMMCs (Fig. S1). However, treatment of BMMCs with FcRIγ AON, which resulted in loss of surface FcRI expression (Fig. S1), eliminated the protective effect of IgE after IL-3 withdrawal (Fig. S1). The apparent decrease in FcRI expression in the presence of IgE we believe reflects the decreased affinity of MAR-1 for FcRI once it is occupied by IgE (32). Taken together, FcRIγδ exon skipping could potentially suppress the prosurvival effect of elevated IgE in vivo and thus the increase in mast cell population as well as reduce IgE-dependent degranulation in allergic disease.

We next examined the effects of FcRIγδ exon skipping (day 0) on mast cell proliferation in the presence of IL-3, while monitoring both surface FcRI expression (Fig. S2) and proliferation using a CellTrace Violet dilution assay (Fig. S2). The majority of the proliferation occurred between day 5 and day 7 with a population of cells appearing with diluted CellTrace dye at day 7 (Fig. S2). There was no difference in proliferation in the absence or presence of FcRIγδ exon skipping at either 5 or 7 d. There was a population of BMMCs treated with FcRIγ AON that began to express FcRI on the surface at day 7, despite all of the cells at day 5 being negative for surface FcRI (Fig. S2), suggesting that these cells were regaining FcRI expression. Gating the populations of cells based on surface FcRI expression and plotting CellTrace Violet fluorescence demonstrated that the cells expressing surface FcRI were the cells that had proliferated (Fig. S2). These data most likely indicate that, although overall proliferation is not affected by FcRIγ AON treatment, BMMCs that do proliferate dilute the FcRIγ AON between daughter cells, thus reducing exon-skipping efficacy.

In Vivo Passive Cutaneous Anaphylaxis. As proof-of-concept of therapeutic utility, we sought evidence that the FcRIγδ exon-skipping approach is effective in vivo, having in mind the possibility of...
delivering FcRIβ AONs locally to affected tissues in allergic diseases such as allergic rhinitis, asthma, or atopic dermatitis. Localized administration of FcRIβ AON would take advantage of its specificity toward FcRI-mediated responses and restrict targeting to mast cells in the affected tissue. Therefore, we used the well-established model of passive cutaneous anaphylaxis (PCA) to test the efficacy of localized delivery of the AONs by means of the Vivo-Morpholino AONs. With this approach, we observed a partial but substantial reduction in PCA reaction with administration of FcRIβ AON compared with control AON (Fig. 4A). Examination of total RNA isolated from skin adjacent to the injected ears by qualitative RT-PCR revealed that the administration of FcRIβ AON had resulted in exon skipping in vivo (Fig. 4B). The efficiency of exon skipping in vivo was postchallenge, performed histological analysis of the ears with the partial reduction in the PCA reaction compared with near-complete block of degradation in BMMCs (Fig. 2). We also note that, in this PCA model, as in cell culture, FcRI receptors are saturated with DNP-specific IgE, whereas in a naturally occurring allergic disease only a minor fraction of receptors may be occupied by an allergen-specific IgE and thus could be more susceptible to FcRIβ exon-skipping therapy.

In Vivo Allergic Dermatitits Model. As further evidence of therapeutic potential for FcRIβ AON administration, we used an established allergic dermatitis model using tolenuenedisocyanate (TDI) to sensitize the mice as described in Materials and Methods. FcRIβ AON or standard control AON were injected into the ears either after sensitization and before TDI challenge (Fig. S3) or with once-weekly injections for 4 wk during sensitization to demonstrate improvement over time (Fig. 5). Using these models, we were able to assess both the immediate and late-phase allergic responses. Both the immediate response at 1 h postchallenge and the late-phase response at 24 h postchallenge were markedly reduced after weekly injections of FcRIβ AON compared with standard control AON (Fig. 5A). At 24 h postchallenge, we performed histological analysis of the ears for inflammation by assessing cellular infiltrate and edema in the H&E-stained skin sections and identified a significant reduction in inflammation with FcRIβ AON treatment (Fig. 5B and C). We also measured inflammatory cytokines in the ear skin 24 h postchallenge and found markedly reduced levels with FcRIβ AON treatment compared with standard control AON-treated mice (Fig. 5D–G). Similar results were observed if FcRIβ AON treatment was administered after the mice were sensitized (Fig. S3). Although the efficacy was less than in mice receiving five injections. Taken together, these data indicate that FcRIβ AON treatment reduces both early and late-phase inflammatory responses in a TDI-induced allergic dermatitis model and that repeated administration of FcRIβ AONs could have prophylactic potential for treating allergic inflammation.

Proof-of-Principle in Human Mast Cells. To confirm that the findings in our mouse models translate to humans, we next tested whether exon skipping of FcRIβ resulted in reduced FcRI expression and IgE-dependent degranulation in human mast cells. The human MS4A2 gene contains AT-rich regions in the splicing donor or acceptor sites at the intron–exon or exon–intron boundaries of exon 3 reducing RNA binding affinity of AONs. Thus, for human mast cells, we could achieve exon skipping, but with less efficiency than with BMMCs. Transfection of LAD-2 cells with an FcRIβ AON resulted in around 75% exon skipping as demonstrated by reduction in FL FcRIβ mRNA assessed with quantitative RT-PCR (Fig. 6A). This degree of exon skipping resulted in a significant reduction in surface FcRI expression of ~50% (Fig. 6B). We next confirmed that the reduction in FcRI surface expression with FcRIβ AON transfection resulted in reduced IgE-dependent degranulation by cross-linking biotinylated IgE with streptavidin and identified a significant reduction in IgE-dependent degranulation (Fig. 6C) without observing any change in thapsigargin-induced degranulation (Fig. 6D). Taken together, these data demonstrate that FcRIβ AONs act comparably in human mast cells as they do in mouse mast cells, although further refinement and screening of human constructs is required to achieve optimal exon skipping in human mast cells.

Discussion

Current treatments for allergic diseases generally rely upon neutralizing the effects of inflammatory mediators or dampening of the inflammatory response with steroids. A more desirable approach would be to suppress release or production of inflammatory mediators by targeting the principal effector immune cells. Targeting mast cell FcRI signaling pathways has been suggested given the preeminent role played by this cell and receptor in allergic disease. However, the same kinases and signaling molecules are used by other receptors in various immune cells, and targeting these signaling molecules is unlikely to achieve the required specificity. Targeting FcRI directly by inhibiting binding of IgE to FcRI is problematic because cross-linking FcRI with anti-FcRI antibodies mimics activation by antigen-inducing anaphylactic degranulation. An approach with some clinical success is the use of omalizumab, a humanized anti-IgE antibody that binds to the Fc fragment of IgE, blocking its binding to FcRI (for review, see ref. 33). Because omalizumab binds to IgE directly, it does not activate mast cells or interact with FcRI. However, a potential limitation is that other cells, such as dendritic cells (33–35), express a trimeric form of FcRI (lacking FcRIβ expression) that functions in antigen presentation where IgE binding to trimeric FcRI may have immunoregulatory functions to dampen allergic responses (34–36).

For the purpose of achieving selective targeting of mast cells and avoiding the limitations of previous therapies, the FcRIβ subunit appeared to be one promising candidate. Tetrameric FcRI contains α and β subunits associated with dimeric γ subunits, whereas trimeric FcRI consists of just the α and dimeric γ subunits (12, 33, 37). Importantly, expression of tetrameric FcRI is restricted to mast cells and basophils, whereas trimeric FcRI that lack FcRIβ are expressed on dendritic and other cell types (12, 33–35). In addition to being exclusively located in mast cells and basophils, FcRIβ is critically required for trafficking of FcRI to the cell surface in mast cells (9). Although FcRIβ has been considered as a mast cell-specific target in recent years (9, 10, 12, 38), the problem was how to effectively target FcRIβ. Delivery of siRNA or shRNA to silence FcRIβ is not totally effective in mast cells even with use of lentiviral delivery techniques and results in 50–80% reduction of protein expression (9, 39). We therefore
considered the innovative approach of forcing the cell to produce an alternative splice variant of FcεRIβ (t-FcεRIβ) that does not associate with FcεRIα or FcεRIγ. The current study indicates that this approach works efficiently by eliminating surface FcεRI expression and function, rendering mast cells completely unresponsive to IgE. This has significant implications on not only immediate mast cell activation and the wheal and flare reaction, but also on cytokine release and the late-phase allergic response, which we demonstrate is also reduced in an allergic dermatitis model. Importantly, we demonstrate thus increasing the efficacy of the drug. We are currently examining affected tissues by reducing mast cell recruitment and survival, induced exon skipping of FcεRIβ survival and chemoattractant factors for mast cells (28, 40), AON-mediated altered splicing of FcεRIβseeking to develop a proof-of-concept for further development of AON-mediated altered splicing of FcεRIβ as a therapeutic approach for allergic diseases.

Our studies provide important proof-of-concept data for developing AON-induced exon skipping of FcεRIβ as a therapeutic and/or preventative treatment for allergic diseases. Our in vitro and in vivo mouse studies demonstrate surprisingly potent efficacy in vitro with a “knockout” effect and a more variable, but still impressive effectiveness in vivo with some mice exhibiting no histological evidence of inflammation 24 h after TDI challenge in the allergic dermatitis model. Although we demonstrate that efficient exon skipping can be achieved in skin mast cells in vivo, a limitation is that effective delivery of AONs into mast cells in skin is limited by diffusion of drug within the skin and the surface area of skin that can be infiltrated by intradermal injection. Systemically administered AONs distribute poorly into the skin and topical administration would be more desirable delivery mechanism for skin disorders. The AON system that we describe here is probably best suited for topical application. To develop this approach further, we need to identify effective methods of delivery over larger surface areas of skin and into other tissues of interest such as the airways. An additional limitation is that the sequence of the human MS4A2 gene is less conducive to exon skipping than the mouse Ms4a2. Although the experimental dermatis model illustrated the full potential of the AON approach in mice, it is apparent that more refined AON configurations might be needed to achieve this in humans.

Despite these challenges, we believe that this approach has the potential for clinical application. AON-mediated exon skipping is emerging as a promising gene therapy approach with the advantage that it does not alter the genome and is reversible with cessation. Exon skipping has been effectively used for rare genetic defects where frameshift mutations in genomic DNA lead to loss of protein function (for reviews, see refs. 18–20). Indeed, two new drug applications for the AON drugs Drisapersen (BioMarin Pharmaceutical) and Eteplinsen (Sarepta Therapeutics) to treat Duchenne muscular dystrophy have been filed with the Food and Drug Administration. However, exon skipping is predominantly used for rare genetic disorders that require gene sequencing, a personalized gene therapy approach, and separate clinical trials and approvals for each exon targeting AON, causing a burden on development. We have devised an AON approach to alter splicing of FcεRIβ with the potential of treating allergic conditions such as asthma and atopic dermatitis, which are amenable to inhaled or topical treatments, respectively, and not associated with a specific genetic defect, thus eliminating the need for a personalized approach. Our data indicate that this could be a viable approach for developing AONs with limited off-target effects.
treatment of allergic diseases and a mast cell-specific therapeutic strategy that, we believe, has the potential to translate into the clinic.

Materials and Methods

For additional information on methods, see SI Materials and Methods.

Experiments on mice carried out at NIH were conducted under a protocol approved by the Animal Care and Use Committee at National Institute of Allergy and Infectious Diseases (NIAID), NIH. BM-MCs were developed from bone marrow obtained from femurs of C57BL6J mice (The Jackson Laboratory), as previously described (41).

Transfection of Mast Cells with AONs. Transfection was achieved using the Nucleofector II and Cell Line Kit V (Lonza) as described (9), and program X-001 was used. AONs were purchased from Gene-Tools LLC.


Allergic Dermatitis TDI Model. Female 6-wk-old BALB/cAnN were purchased from Charles River Laboratories. All aspects of the current study were conducted in accordance with the Animal Care and Use Program of the North Carolina State University (Institutional Animal Care and Use Committee Protocol No. 13-111-8).

ACKNOWLEDGMENTS. We thank Dr. Ana Olivera (NIAID, NIH) for discussions and Sarah Ehling (Department of Molecular Biomedical Sciences, North Carolina State University) for technical assistance. Financial support was provided by the Division of Intramural Research of National Institute of Allergy and Infectious Diseases and National Heart, Lung, and Blood Institute within the NIH, and from departmental start-up funds (to G.C.) (Department of Molecular Biomedical Sciences, College of Veterinary Medicine, North Carolina State University).