Genetic Deletion of β -Arrestin-2 and the Mitigation of Established Airway Hyperresponsiveness in a Murine Asthma Model

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Abstract

β -Arrestin-2 (β arr2) is a ubiquitously expressed cytosolic protein that terminates G protein-coupled receptor signaling and transduces G protein-independent signaling. We previously showed that mice lacking β arr2 do not develop an asthma phenotype when sensitized to, and challenged with, allergens. The current study evaluates if an established asthma phenotype can be mitigated by deletion of β arr2 using an inducible Cre recombinase. We sensitized and challenged mice to ovalbumin (OVA) and demonstrated that on Day (d) 24 the allergic asthma phenotype was apparent in uninduced β arr2 and wild-type (WT) mice. In a second group of OVA-treated mice, tamoxifen was injected on d24 to d28 to activate Cre recombinase, and OVA aerosol challenge was continued through d44. The asthma phenotype was assessed using lung mechanics measurements, bronchoalveolar lavage cell analysis, and histological assessment of mucin and airway inflammation. Compared with their respective saline-treated controls, OVA-treated WT mice and mice expressing the inducible Cre recombinase displayed a significant asthma phenotype at d45. Whereas tamoxifen treatment had no significant effect on the asthma phenotype in WT mice, it inhibited β arr2

expression and caused a significant reduction in airway hyperresponsiveness (AHR) in Cre-inducible mice. These findings suggest that β arr2 is actively required for perpetuation of the AHR component of the allergic asthma phenotype. Our finding that β arr2 participates in the perpetuation of AHR in an asthma model means that targeting β arr2 may provide immediate and potentially long-term relief from daily asthma symptoms due to AHR irrespective of inflammation.

Keywords: β -arrestin-2; asthma mitigation; airway hyperresponsiveness; β -arrestin-2-floxed mice

Clinical Relevance

Our finding that β -arrestin-2 participates in the perpetuation of airway hyperresponsiveness in an asthma model means that targeting β -arrestin-2 may provide immediate and potentially long-term relief from daily asthma symptoms due to airway hyperresponsiveness irrespective of inflammation.

Asthma is a chronic inflammatory disease characterized by airway inflammation, hyperresponsiveness, and remodeling. The development of asthma involves a set of complex interactions among antigen and multiple immune and lung structural cell

types; these interactions are orchestrated spatially and temporally (1). The perpetuation of asthma is less well understood but involves a continued inflammatory response initiated by a positive feedback loop among, at least,

airway epithelial cells, Th2 cells, and eosinophils that may or may not be sustained by allergens (1, 2). Ongoing allergen exposure results in the production of IL-13 and other cytokines that lead to asthma exacerbations and perpetuation of

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the disease. However, drug exacerbation (3), aberrant functional regulation of immune cells (4), IgE antibodies (5), or airway structural cells (4, 6) can also sustain airway remodeling and thus the progression of the disease (1, 2). For many patients with asthma, however, signs and symptoms of the disease are usually sustained or worsen over time. Current asthma therapies are aimed at reducing the frequency of asthma exacerbations and the magnitude of associated symptoms. Therapeutic mitigation of ongoing asthma pathogenesis can be studied using murine models of chronic allergen exposure, but this is relatively unexplored compared with acute models that examine asthma development (7). Because β -arrestin-2 (β arr2) has been shown to play a role in asthma phenotype development, we examine here the role for β arr2 in perpetuating the asthma phenotype.

β arr2, a ubiquitously expressed member of the arrestin family of proteins, is a multifunctional cytosolic protein that translocates and binds to agonist-activated cell surface receptors, most prominently G protein-coupled receptors (GPCRs) (8). When β -arrestin binds the receptor, it sterically prevents G protein coupling and thus desensitizes G protein-dependent signaling. Additionally, arrestins act as signal transducers by scaffolding GPCRs to a wide array of intracellular signaling intermediates, including MAPKs, NF- κ B, and other signaling molecules (8). Thus, β -arrestins can both terminate and initiate cell signaling pathways. Importantly, β arr2-dependent signaling has been shown to promote cellular chemotaxis and antiapoptosis, implicating β arr2 in inflammation/metastasis (9) and propelling the investigation of its role in the study of cancer (10–14), atherosclerosis (15), and inflammatory disorders, including asthma.

We previously showed that expression of β arr2 is required for development of the asthma phenotype in mice (16). Our subsequent work has revealed that β arr2-mediated regulation of inflammatory cell migration and airway smooth muscle (ASM) cell contractile function underlies its role in promoting the asthma phenotype. β arr2 positively regulates inflammatory cell migration to the lung in a murine model of allergic airway inflammation. In particular, lung migration of CD4⁺ T cells and eosinophils is impaired in β arr2-knockout (KO) mice (16, 17). Additionally, our

Figure 1. (A) Strategy for β -arrestin-2 (β arr2) gene (*Arrb2*) conditional inactivation and use of conditional β arr2 knockout mice in allergen-induced asthma mitigation. To generate the conditional knockout *arrb2* allele, coding exon 2 of *arrb2* was flanked by loxP sequences. The loxP-flanked exon 2 was excised from *arrb2* by tamoxifen-inducible Cre recombinase. Tamoxifen binding to the estrogen receptor–Cre recombinase fusion protein (CreERT2) translocated Cre activity into the nucleus to excise exon 2. Expression of CreERT2 was driven by the Rosa26 promoter, which allowed ubiquitous and constitutive expression of the gene. Removal of exon 2 by Cre recombinase resulted in an early termination and frame-shift in β arr2 gene transcription and translation, leaving a predicted residual 18-amino-acid peptide. The asterisk indicate a stop codon in the mRNA and the position where protein translation stops. (B) Schematic illustration of the asthma induction protocol using uninduced β arr2 (ui- β arr2) and wild-type (WT) mice in the Day (d)24 experiment. The mice were sensitized by ovalbumin (OVA) injections (intraperitoneal [i.p.]) on d0 and d14. From d21 to d23, the mice were challenged daily with OVA aerosol. Saline control groups were injected with saline i.p. (100 μ l) and exposed to saline aerosol on those days. Asthma phenotype was assessed on d24. (C) Schematic illustration of the asthma mitigation protocol using induced β arr2 mice in the d45

in vitro data show that CCR4-mediated CD4⁺ Th2 cell chemotaxis is significantly inhibited when β arr2 is absent (16). Recently we have shown that β arr2-dependent signaling transduces the proinflammatory effects in the airway mediated by activated protease-activated receptor-2 (18). β arr2 also plays an important role in regulating airway smooth muscle contractile tone by constraining β_2 -adrenoreceptor (β_2 AR)-activated, G protein-mediated cAMP accumulation and consequent ASM cell relaxation (19). Thus, β arr2 contributes to the development of allergic asthma through activation or desensitization of cell signaling in multiple cell types that regulate a variety of functions. It is likely that perpetuation of the asthma phenotype is also regulated through many of these same β arr2-mediated functional pathways, but this hypothesis remains unexplored.

In the current study we examined the role for β arr2 in perpetuating the established asthma phenotype. We generated mice having the β arr2 gene flanked by loxP sites (β arr2^{flx/flx}) and bred them to mice that use the global promoter Rosa-26 (R26) to express the fusion protein between estrogen receptor and Cre recombinase (R26-CreERT2). The CreERT2 fusion is inactive until bound by tamoxifen, which then allows nuclear translocation and the action of Cre to inactivate the β arr2 gene. These conditional β arr2 mice (R26-CreERT2/ β arr2^{flx/flx}), referred to as uninduced β arr2 or ui- β arr2) allowed us to delete β arr2 expression after the asthma phenotype had developed at d24 but before the chronic asthma phenotype was assessed at d45. Using acute and chronic ovalbumin (OVA) sensitization and challenge (S/C) models, we evaluated the asthma phenotype at d24 and d45 through lung mechanics measurements, analysis of bronchoalveolar lavage cells, and histological assessment of mucin and airway inflammation.

Materials and Methods

Animals

All animal experimental protocols were approved by the Institutional Animal Care and Use Committee at Duke University

Medical Center and were performed in accordance with the standards established by the US Animal Welfare Acts. Only male mice were used. The wild-type (WT) mice used as controls were Cre-negative littermates (β arr2^{flx/flx}) of the R26-CreERT2/ β arr2^{flx/flx} experimental mice.

Targeting Vector for Conditional β arr2 KO Mice

Recombineering technique (20, 21) was used to generate the targeting vector whereby an 11.4-kb fragment of mouse genomic DNA containing β arr2 (*arrb2*) was cloned into the PL253 plasmid. Two LoxP cassettes were inserted into the *arrb2* gene flanking coding Exon 2 (Figure 1A) (details are provided in the online supplement).

Ovalbumin Sensitization and Challenge

As shown in Figure 1B (for the d24 experiment) and Figure 1C (for the d45 experiment), mice were sensitized by intraperitoneal injections of 10 μ g OVA adsorbed to 2 mg alum on d0 and d14. For both the d24 and d45 experiments, starting on d21, animals were challenged with OVA (1% wt/vol in saline) aerosol for 1 hour according to the schedule in Figure 1C (22). For the d45 experiment alone, tamoxifen (Sigma-Aldrich, St. Louis, MO) was injected intraperitoneally at 75 mg/kg every day for 5 days starting at d24 when the allergic asthma phenotype was established. All mice were assessed for asthma phenotype 24 hours after the final OVA challenge (details are provided in the online supplement).

Airway Responsiveness Measurement

Airway hyperresponsiveness (AHR) was measured using the forced oscillation technique as described previously (22, 23) and in the online supplement. In brief, Newtonian resistance was calculated in anesthetized mice after administration of increasing doses of intravenous methacholine (MCh). To calculate the provocative concentration of MCh that would cause a doubling of baseline resistance (PC₁₀₀), the absolute value Newtonian resistance dose-response data

were fit to the exponential growth function ($y = a \cdot e^{kx}$) using nonlinear regression analysis. PC₁₀₀ was calculated by log-linear interpolation (24) (details are provided in the online supplement).

Bronchoalveolar Lavage Fluid Analysis

Bronchoalveolar lavage fluid was used to determine total cell counts (18). For differential cell counts, cytocentrifuged lavage preparations were stained using a Hema-3 staining kit (Fisher Scientific, Kalamazoo, MI), and 200 cells were classified using standard morphologic criteria (details are provided in the online supplement) (17).

Lung Histology and Semiquantification of Inflammation

Paraformaldehyde-fixed, paraffin-embedded lung sections were stained with hematoxylin and eosin or periodic acid Schiff for evaluation of cellular inflammation or airway mucin content, respectively, using a four-tiered semiquantitative scoring method (details are provided in the online supplement) that was performed in a blinded manner (17, 18).

Western Blot Analysis

Whole cell lysates were prepared, and equal amounts of total protein (25 μ g) were loaded for Western blot analysis (details are provided in the online supplement). β -Arrestins were detected with the polyclonal antibody A2CT, which has a 10-fold higher affinity for β arr2 over β arr1.

Statistical Analysis

The data were presented as mean $\pm$ SEM for each treatment group. Group comparisons were conducted using Student's *t* test or ANOVA as appropriate, and $P < 0.05$ or $P = 0.05$ was considered statistically significant. A one-tailed *t* test was used for PC₁₀₀ comparisons as noted in the figure legends.

Results

Tamoxifen-Induced Cre Recombination of β arr2 Gene

We successfully generated mice that globally express inducible Cre recombinase and

Figure 1. (Continued). experiment. The mice were sensitized and challenged with OVA (or saline) as in the d24 experiment. To activate CreERT2 and inactivate *arrb2*, the mice were injected with tamoxifen (75 mg/kg, i.p.) for 5 consecutive days (d24–d28). After tamoxifen administration, the mice were challenged with OVA aerosol on d28, d29, d30, d35, d36, d37, d42, d43, and d44. Asthma phenotype was assessed on d45. mRNA, messenger RNA.

where Exon 2 of the β arr2 gene is loxP-flanked (ui- β arr2) (Figure 1A).

OVA S/C Up-Regulates Lung β arr2 Expression

We initiated the d24 and d45 saline or OVA S/C experiments (Figures 1B and 1C) in ui- β arr2 mice and WT controls. Saline-treated ui- β arr2 mice expressed lung levels of β arr2 comparable to those in WT mice at d24 (Figure 2A) and d45 (Figure 2B). OVA S/C treatment resulted in a significant increase in lung, but not spleen, β arr2 expression on d24 (Figure 2A; see Figure E1A in the online supplement) and d45 (Figure 2B; Figure E1B), which was comparable in ui- β arr2 and WT mice.

Asthma Phenotype Assessment at d24

To ensure that β arr2 was deleted only after an allergic asthma phenotype was achieved, we performed the d24 experiment (Figure 1B). On d24, AHR, as assessed by PC₁₀₀, was not different between WT and ui- β arr2 mice for either the saline- or OVA-treated condition (Figure 3A), indicating that the presence of the (uninduced) Cre-ERT2 gene does not affect the airway response to MCh in control or OVA-S/C treated mice. The OVA-induced decline in PC₁₀₀ evident in both genotypes (relative to respective saline controls) at d24 illustrates that mice were more sensitive to bronchoconstrictor and thus had developed AHR. Airway resistance values, from which PC₁₀₀ values were calculated, are shown in Figure E2. OVA-treated WT and ui- β arr2 mice showed comparable levels of lung eosinophils, which were significantly increased relative to their respective saline-treated controls (Figure 3B). The histologic evaluation of lung tissue demonstrated significant peribronchiolar/perivascular inflammation and mucin content that was comparable in WT and ui- β arr2 OVA-S/C mice compared with saline-treated controls (Figures 3C and 3D). As expected, the collective data show that OVA S/C produced a robust asthma phenotype that was comparable in WT and ui- β arr2 mice on d24.

Time Course of Tamoxifen-Induced β arr2 Depletion in OVA-Treated ui- β arr2 Mice

We evaluated the time course of tamoxifen-induced β arr2 depletion in OVA-treated ui- β arr2 mice by collecting lung tissue at

Figure 2. Changes in lung β arr2 protein level in the ui- β arr2 and WT mice after saline or OVA treatment on d24 (A) and d45 (B). β -actin served as a protein loading control in all the immunoblots. Representative immunoblots of β arr2 protein in mouse lungs (upper panel) on d24 (A) and d45 (B) after OVA sensitization and challenge (S/C). The bar graphs show the corresponding average densitometric quantification of tissue β arr2 protein levels. Open bars: Saline-treated ui- β arr2 and WT mice; solid bars: OVA S/C ui- β arr2 and WT mice ($n = 3-6$ randomly selected lung samples from each genotype/treatment group were used for immunoblots). Representative blots are shown. * $P < 0.05$ compared with saline-treated mice.

Figure 3. Asthma phenotype assessment on d24 for mice after OVA S/C (d24 experiment). (A) Airway hyperresponsiveness (AHR) was assessed in terms of provocative concentration (PC₁₀₀) of methacholine (MCh). The dose–response data were fit to the exponential growth function ($y = a \cdot e^{kx}$) using nonlinear regression analysis. The dose of MCh that would cause a 100% increase in (or doubling of) baseline resistance was calculated by log-linear interpolation and plotted for each treatment/genotype group. (B) Bronchoalveolar lavage differential cell count. Bronchoalveolar lavage total inflammatory cells (B, upper panel), eosinophils (B, lower left panel), and lymphocytes (B, lower right panel) per milliliter were counted to evaluate inflammation. (C and D) Histological semiquantification of lung inflammation. Inflammatory cell recruitment into the airway subepithelial space (C) and epithelial mucin content (D) were analyzed by scoring stained lung sections for cellular inflammation and airway mucin, respectively, in a blinded fashion. The scores were standardized by dividing with the number of airways counted. Open bars: Saline-treated ui-βarr2 and WT mice; solid bars, OVA S/C ui-βarr2 and WT mice ($n = 3–8$ for all the experiments). * $P < 0.05$ compared with respective saline-treated WT and ui-βarr2 mice. H&E, hematoxylin and eosin; PAS, periodic acid Schiff.

d34, d38, and d45 (Figures E3A and E3B; Figure 4). Lung βarr2 expression was significantly decreased at d34 (Figure E3A) and almost completely depleted by d38 (Figure E3B) and d45 (Figure 4).

Effect of βarr2 Depletion on Asthma Phenotype at d45

The time course of βarr2 depletion showed that lung βarr2 expression was barely detectable on d38 (Figure E3B). Thus, we performed the d45 experiment to evaluate

whether or not tamoxifen-induced βarr2 depletion was associated with changes in asthma characteristics on d45. Similar to results from d24 (Figure 3A), on d45 AHR, as assessed by PC₁₀₀, was not different between WT and ui-βarr2 mice for either the saline- or OVA-treated condition (Figure 5A), indicating no effect of the Cre-ERT2 on AHR at d45. The OVA-induced decline in PC₁₀₀ illustrates that mice (both WT and ui-βarr2) were more sensitive to bronchoconstrictor and thus had developed

AHR at d45 (Figure 5A). Tamoxifen treatment had no significant effect on AHR in OVA-treated WT mice but caused a significant increase in PC₁₀₀ in OVA-treated i-βarr2 mice (Figure 5A). In fact, PC₁₀₀ in OVA-treated i-βarr2 mice returned to levels observed in the d45 experiment saline control mice (ui-βarr2) and were significantly different from those of WT-OVA/tamoxifen-treated mice (Figure 5A). Thus, βarr2 depletion in OVA S/C-treated mice significantly alleviated

Figure 4. Changes in lung β arr2 protein level with time in ui- β arr2 and WT mice after OVA and tamoxifen treatment in the d45 experiment. β -actin served as a protein loading control in all the immunoblots. Representative immunoblot of β arr2 protein in mouse lung (upper panel) on d45 after OVA S/C plus tamoxifen treatment. The lower panel shows the average densitometric quantification of tissue β arr2 protein levels on d34, d38, and d45 for OVA S/C plus tamoxifen treatment. Bars: OVA S/C and tamoxifen-treated WT and i- β arr2 mice ($n = 3-6$ randomly selected lung samples from each genotype/treatment group were used for immunoblots). Representative blots are shown. * $P < 0.05$ compared with OVA S/C and tamoxifen-treated WT mice.

AHR, the hallmark feature of allergic asthma. The absolute resistance values, from which PC_{100} was calculated, are shown in Figures E4A and E4B.

As expected, total cells and eosinophils in bronchoalveolar lavage fluid were significantly and comparably elevated in ui- β arr2 and WT mice on d45 after OVA challenge (Figure 5B). Similarly, bronchoalveolar lavage lymphocytes were elevated in OVA S/C-treated mice, and these levels were not different between genotypes despite the fact that only ui- β arr2 mice showed a statistically significant increase over saline-treated controls (Figure 5B). In WT and i- β arr2 mice, OVA/tamoxifen treatment was associated with reductions in total bronchoalveolar lavage cells and eosinophils (Figure 5B),

suggesting a mild antiinflammatory effect of tamoxifen. It appears that loss of β arr2 caused an additional decline in bronchoalveolar lavage cells because total cells and eosinophils were significantly reduced compared with OVA treatment in only the i- β arr2 mice. However, there was no statistically significant difference in any bronchoalveolar lavage cell type between OVA/tamoxifen-treated WT and i- β arr2 mice. Tamoxifen + OVA treatment did not cause any significant change in histological assessment of airway inflammation (Figure 5C) or mucin content (Figure 5D) compared with groups treated with OVA only, suggesting that loss of β arr2 was associated with minimal impact on the inflammatory phenotypes at d45.

Discussion

Our earlier work demonstrated that β arr2 expression is required for asthma phenotype development in mice (16). Our present study extends those findings by demonstrating that β arr2 is also required for allergen-induced perpetuation of AHR in a murine model of asthma. In human asthma, AHR, as assessed by PC_{20} , is widely accepted as a good indicator of disease state and is used to track disease progression (23, 25). Thus, mitigation of AHR is a key therapeutic goal of asthma treatment. If translatable, our current findings point to β arr2 as an important therapeutic target.

The mechanism by which β arr2 influences AHR may be through its role as a G protein signal terminator and/or as a transducer of GPCR signaling. One of the causes of restricted airflow in asthma is bronchoconstriction, which is reversed by agonist-induced activation of β 2AR-mediated airway smooth muscle relaxation (26). We have previously shown that β arr2, acting in its classical role as a G protein signal terminator, desensitizes and constrains β 2AR signaling/function in both human and murine airway smooth muscle (19, 27). Furthermore, administration of exogenous β 2AR agonist reduces MCh-induced constriction significantly more in naive β arr2 KO mice than in control mice. Although in the present study no exogenous β 2AR agonist was administered, hypotension associated with MCh-induced bradycardia will cause a baroreflex increase in circulating epinephrine, the endogenous ligand for β 2ARs. Thus, it is plausible

that the loss of β arr2 expression in i- β arr2 mice allowed enhanced epinephrine-induced opposition to MCh-induced constriction, resulting in reduced AHR. Similarly, β arr2 may also constrain the bronchoprotective effect of circulating epinephrine (whether basal or stimulated), which tempers MCh-induced bronchoconstriction. Additionally, the impact of β arr2-mediated constraint of β 2AR-induced ASM relaxation may be even more pronounced in our OVA S/C model where β arr2 expression is elevated. Taken together, the alleviation of AHR in OVA S/C i- β arr2 mice may be a result of loss of β arr2-mediated constraint of β 2AR-induced bronchorelaxation or bronchoprotection.

There is good evidence to suggest that β arr2-dependent signaling downstream of β 2ARs and chemokine receptors is proinflammatory in asthma (16–18, 22). β arr2 promotes the chemotaxis of lymphocytes and eosinophils to the airways (1, 16, 17, 28). Therefore, we expected β arr2 depletion to mitigate inflammation in the i- β arr2 mice; however, no clear mitigation of inflammation was observed. One possible explanation for the persistence of inflammation is that insufficient time elapsed between β arr2 gene deletion and assessment of inflammation. The mitigation of airway inflammation in murine models is associated with migration of regulatory T cells to airways (29) and egress of effector T cells from lung to lymph nodes, processes that may take weeks after allergen challenge ends (1, 30, 31). The unanticipated delay in, and the incompleteness of, β arr2 deletion may have been a result of interference by OVA-induced stimulation of β arr2 transcription versus tamoxifen-induced Cre-mediated recombination of the β arr2 gene because it is highly unlikely that existing β arr2 protein could persist in live cells for 3 weeks. Because our study focused on a single assessment time point (d45) and because substantial loss of β arr2 expression was not observed until d38, only 7 days were available for mitigation of inflammation. Future studies may need to use a revised tamoxifen dosing schedule and/or extend the protocol beyond 45 days.

It is unlikely that loss of the AHR phenotype in i- β arr2 mice is a result of differences in the inflammatory phenotype. First, neither tamoxifen treatment nor β arr2 deletion was associated with a decline in inflammation assessed histologically,

Figure 5. Asthma phenotype assessment on d45 for OVA S/C mice treated with tamoxifen in the d45 experiment. (A) AHR was assessed in terms of PC₁₀₀ of MCh comparing saline versus OVA treatments or OVA plus tamoxifen treatments. The dose-response data were fit to the exponential growth function ($y = a \cdot e^{bx}$) using nonlinear regression analysis. The dose of MCh that would cause a 100% increase in (or doubling of) baseline resistance was calculated by log-linear interpolation and plotted for each treatment/genotype group. (B) Bronchoalveolar lavage differential cell count. Bronchoalveolar lavage total cells (B, top panel), eosinophils (B, left panel), and lymphocytes (B, right panel) per milliliter were counted to evaluate inflammation. (C and D) Histological semiquantification of lung inflammation. Inflammatory cell recruitment into the airway subepithelial space (C) and the epithelial mucin content (D) were analyzed by scoring stained lung sections for cellular inflammation and airway mucin, respectively, in a blinded fashion. The scores were standardized by dividing with the number of airways counted. Open bars: OVA S/C WT and ui-βarr2 mice; solid bars: OVA S/C WT and ui-βarr2 mice; shaded bars: OVA S/C and tamoxifen-treated WT and i-βarr2 mice ($n = 6-9$ for all the experiments). * $P < 0.05$ or $P = 0.05$ compared with respective saline-treated control mice. † $P < 0.05$ compared with respective OVA-treated (no tamoxifen) mice. § $P < 0.05$ compared with OVA/tamoxifen-treated WT mice.

which is arguably a more indicative measure than BAL cell counts. Consistent with other reports that describe effects of estrogen receptor activation on allergic inflammation (32–34), tamoxifen treatment alone appeared to have a mild anti-inflammatory effect on BAL cell counts in the present study. However, tamoxifen had no effect on peribronchiolar cell numbers, airway mucin density, or AHR. Additionally, despite the observation that eosinophils were significantly reduced in tamoxifen-treated i-βarr2 mice but not in similarly treated WT mice, the absolute number of bronchoalveolar lavage

eosinophils was not different between the two genotypes, making it difficult to ascribe differences in lung mechanics to changes in lung inflammatory cells. In summary, βarr2 deletion did not mitigate airway inflammation in this study. Thus, it is unlikely that mechanisms of inflammation underlie the reduction in AHR observed in OVA S/C-treated i-βarr2 mice.

A large clinical study of patients with asthma has shown that AHR can be dissociated from the presence of inflammatory cells in the airways (35). Similar dissociation has also been observed in murine models of airway diseases

(36–38). Animal models with prolonged allergen challenge have shown that once the allergen challenge was discontinued, AHR declined faster compared with airway changes such as collagen deposition, hyperplasia of airway smooth muscle and goblet cells, and tissue eosinophilia (39). Consistently, in the present study we found a significant decline in AHR, but not perivascular/peribronchiolar airway inflammation or mucus metaplasia, after βarr2 deletion. The likely explanation for the alleviation of AHR in our study is that βarr2-mediated constraint of β2AR-induced bronchodilation/

bronchoprotection was reduced in the absence of β arr2 expression. However, we cannot rule out the possibility that β arr2-dependent signaling itself may promote AHR through an as yet unknown signaling pathway that is unapparent in naive mice (19) but activated in OVA S/C mice.

Airway remodeling, a feature of chronic asthma, is represented by airway wall thickening, subepithelial fibrosis, and goblet cell hyperplasia (40). These structural changes can affect AHR in human asthma (41). In murine asthma models, chronic OVA exposure and associated airway remodeling has been reported to cause a progressive decrease in AHR (22, 42, 43). We did not assess airway remodeling in the current study because few of the aforementioned airway remodeling features would have manifested by d45.

The OVA model used in our study has the drawback that an adjuvant must be

administered to sensitize mice to the OVA protein. However, the strengths of the model, such as similarity to human asthma regarding immunoglobulin isotype switching, cytokine milieu, bronchoconstrictor-induced AHR, and development of some aspects of airway remodeling, strongly advocate for the use of this OVA-induced allergic airway asthma model. Furthermore, we have previously shown that the proasthma role of β arr2 is not unique to the OVA model because β arr2 is required for asthma phenotype development using IL-13 or OVA plus protease models (17, 18). In summary, our data show that β arr2 deletion results in a significant alleviation of AHR, clearly indicating that β arr2 plays a significant role in perpetuating AHR in allergic asthma. Although targeting the β arr2 gene did not mitigate the inflammatory component of asthma,

examination of the inflammatory phenotype at a more distant time point might provide more illuminating results. Nevertheless, targeting β arr2 may provide immediate and potentially long-term relief from daily asthma symptoms due to the AHR effect alone. Further investigation into the role for β arr2 in cell-specific targets using this new conditional model will provide valuable insights to delineate the pathophysiology of AHR in asthma. ■

Author disclosures are available with the text of this article at www.atsjournals.org.

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