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Liver X Receptor Agonists Increase Airway Reactivity in a Model of Asthma via Increasing Airway Smooth Muscle Growth

Mark A. Birrell,* Jorge De Alba,* Matthew C. Catley,* Elizabeth Hardaker,* Sissie Wong,* Michael Collins,* Deborah L. Clarke,* Stuart N. Farrow,‡ Timothy M. Willson,‡ Jon L. Collins,‡ and Maria G. Belvisi*$

The liver X receptors (LXRα/β) are orphan nuclear receptors that are expressed in a large number of cell types and have been shown to have anti-inflammatory properties. Nuclear receptors have previously proved to be amenable targets for small molecular mass pharmacological agents in asthma, and so the effect of an LXR ligand was assessed in models of allergic airway inflammation. LXR agonist, GW 3965, was profiled in rat and mouse models of allergic asthma. In the Brown Norway rats, GW 3965 (3–30 mg/kg) was unable to reduce the bronchoalveolar lavage eosinophilia associated with this model and had no impact on inflammatory biomarkers (eotaxin and IL-1β). The compound did significantly stimulate ABCA-1 (ATP-binding cassette A1) mRNA expression, indicating that there was adequate exposure/LXR activation. In the mouse model, the LXR ligand surprisingly increased airway reactivity, an effect that was apparent in both the Ag and nonchallenged groups. This increase was not associated with a change in lung tissue inflammation or number of mucus-containing cells. There was, however, a marked increase in airway smooth muscle thickness in both treated groups. We demonstrated an increase in contractile response to exogenous methacholine in isolated airways taken from LXR agonist-treated animals compared with the relevant control tissue. We corroborated these findings in a human system by demonstrating increased proliferation of cultured airway smooth muscle. This phenomenon, if evidenced in man, would indicate that LXR ligands may directly increase airway reactivity, which could be detrimental, especially in patients with existing respiratory disease and with already compromised lung function. The Journal of Immunology, 2008, 181: 4265–4271.

The liver X receptors (LXR)3 are part of the nuclear receptor superfamily and exist in two forms known as LXRα and LXRβ. These receptors are encoded by two separate genes, Nr1h3 and Nr1h2, respectively (1). LXRs form obligate heterodimers with another type of nuclear receptor, RXR, and regulate cholesterol and lipid homeostasis. Originally it was thought that LXRs were orphan receptors until the discovery of endogenous ligands: oxysterols, including 22(R)-hydroxycholesterol, 24(S)-hydroxycholesterol, and 24(S),25-epoxycholesterol (1–4). LXR directly controls the transcription of several genes involved in the cholesterol efflux pathway including ATP-binding cassette A1 (ABCA-1) and G1 (5–8). The nuclear receptors LXRα and LXRβ have been implicated in the control of cholesterol and fatty acid metabolism, and in the intestine ligand activation of LXR/ RXR heterodimers dramatically reduces dietary cholesterol absorption, an effect postulated to be mediated by ABCA-1 (6). An LXRα/β agonist has been shown to reduce the development of atherosclerosis, a disease in which high cholesterol/fatty acid levels are thought to be involved, in two murine models (9). In a similar murine model of atherosclerosis, the same authors later found that two LXRα/β agonists reduced inflammatory gene expression. They suggested that this result indicates that LXR agonists may reduce atherosclerosis not only by promoting cholesterol efflux, but also by acting to limit the production of inflammatory mediators in the artery wall (10). This antiinflammatory action of LXR agonist has been also shown in two murine models of contact dermatitis (11).

Similarly, in various other in vitro systems LXR agonists have been shown to be anti-inflammatory (12–17). It seems clear from most of the published studies that there is a rationale for an LXR agonist to be an effective anti-inflammatory agent. Indeed, there have been several recent reviews on this topic (18, 19).

Asthma is a disease of the airways that manifests as symptoms such as shortness of breath, airway obstruction, and cough (20). Cardinal features of the disease are airway eosinophilia, an increase in inflammatory mediators and increase bronchoconstrictor responses to spasmogens, otherwise known as airway hyperreactivity (AHR) (20). It is thought that chronic allergic airway inflammation is central to the pathogenesis of this disease. As yet, there is no information on the impact of LXR ligands in allergic inflammation. Hence, the aim of this study was to determine the effect of a selective LXR agonist, GW 3965 (15), on the allergic response in preclinical models of asthma. The first study examines the effect of the LXR agonist in our in-house, fully characterized rat model of allergic inflammation, and the second study utilizes a murine model of asthma that exhibits AHR.

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‡Abbreviations used in this paper: LXR, liver X receptor; ABCA-1, ATP-binding cassette A1; AHR, airway hyperreactivity; ASM, airway smooth muscle; HASM, human airway smooth muscle; MCh, methacholine.

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Materials and Methods

**Effect of LXReβ agonist, GW 3965, on airway inflammation in the Ag-driven preclinical Brown Norway rat allergic model**

To determine the impact of the LXR agonist on Ag-induced airway inflammation, we employed our fully characterized rat preclinical allergic model (21). Male Brown Norway rats (200–225 g) were sensitized on days 0, 14, and 21 with OVA (100 μg, i.p.) administered with alum. On day 28 the rats were then challenged with aerosolized LPS-free, sterile, saline, or OVA (10 mg/ml; i.n.) in chambers for 30 min. There were two protocols employed for assessment of the impact of GW 3965 in this modeling system. Part of the first one was designed to demonstrate that the ligand actually activated the LXR in this modeling system by measuring a marker of this receptor activation, ABCA-1 mRNA expression (10). Additionally, we wanted to determine the impact of the agonist on Ag-induced inflammatory mediator production. In a previous study we have shown that in this model there is an increase in inflammatory cytokines 6 h after challenge (21), and therefore this time point was chosen for sampling. In an arm of the study the rats were orally dosed with vehicle (0.5% methylcellulose and 0.2% Tween 80 in distilled water, 2 ml/kg) or GW 3965 (3, 10, and 30 mg/kg) 1 h before and 2 and 4 h after challenge. The anti-inflammatory positive control, budesonide (3 mg/kg), was included and dosed at the same times. Six hours after challenge the animals were culled and the lungs were collected and stored at −80°C. ABCA-1 gene expression was assessed using real-time TaqMan RT-PCR as described previously (22). Before utilizing this assay, it was validated for multiplexing with the internal control, 18S, using the criteria detailed by the manufacturer (Applied Biosystems). The expression was calculated as a ratio of this internal assay control, and the data are expressed as fold difference from vehicle dosed/vehicle challenged samples. Ag-induced cytokine release was measured in the lung tissue as described in Birrell et al. (23) and is expressed as picograms per milligram of lung tissue total protein.

The second arm of the study was designed to determine the effect of the compound on Ag-induced eosinophilia. Parallel groups of rats were sensitized, challenged, and dosed (with the addition of another dose 8 h after challenge) as above. Twenty-four hours after challenge the rats were culled and airway eosinophilia was assessed (21).

**Effect of LXReβ agonist, GW 3965, in an Ag-driven preclinical murine model of asthma**

In an attempt to determine the impact on another key feature of asthma, AHR (absent in our rat model), we employed our in-house mouse model of asthma (24). The methods are described in Birrell et al. (24). Briefly, male BALB/C mice were sensitized on days 1 and 14 with OVA (10 μg, i.p.) administered with alum. On days 21–26 the mice were challenged with aerosolized LPS-free, sterile, saline, or OVA (10 ml of 50 g/L in Perspex chambers) at an airways reactivity to inhaled mannitol (MCh) determined was quantified and divided by the basement membrane length to obtain a smooth muscle area-to-basement membrane ratio. Measurements performed on each lung section were averaged and the resulting values used for comparison between groups.

All counts in this study were performed with the investigator blinded to treatment.

**Growth factor mRNA expression in the lung after LXR agonist treatment**

For assessment of mRNA expression, total cellular RNA was isolated from mouse lungs using TRI Reagent (Sigma-Aldrich) following the manufacturer’s instructions. RNA were reverse transcribed to cDNA using the SuperArray RT2 First Strand kit (Tebu-bio Laboratories) following the manufacturer’s instructions. Amplification of the cDNA and detection of target PCR product were conducted in an ABI Prism 7000 sequence detection system (Applied Biosystems), using the SuperAarray Custom RT2 Profiler PCR Array (Tebu-bio Laboratories) following the manufacturer’s instructions. The targets measured included: VEGF, PDGFα, FGFR2, FGF18, TGF-β1, TGF-β3, and EGF. We also assessed α-actin as a positive control. Results were analyzed using sequence detection software from Applied Biosystems, and the relative amount of target gene transcript was normalized to the amount of 18S internal control transcript. The data were then converted from the error signal format in the raw data to fold change 2−ΔΔct and then arbitrarily multiplied by 10^x to calculate the values into whole numbers. The data were then compared with levels in the saline/vehicle control group and are presented as fold increase over this group.

**Effect of LXR agonist on human airway smooth muscle proliferation**

To demonstrate relevance in a human system and that these findings were not just particular to GW 3965, we examined the impact of two structurally distinct LXR agonists on the proliferation rate of cultured primary airway smooth muscle. We included FCS-stimulated cells for comparison.

Tracheal rings from either lung or heart and lung transplantation donors were dissected under sterile conditions in HBSS (in mM) NaCl (118), KCl (5.9), MgSO4 (1.2), CaCl2 (2.5), Na2HPO4 (1.2), NaHCO3 (25.5), and glucose (5.6). The epithelial cells were then removed by passing through the trachea. Two tracheal rings were prepared from each trachea and suspended for isometric tension recordings in 10 ml organ baths (Linton Instrumentation) containing Krebs-Henseleit solution (pH 7.4) maintained at 37°C and bubbled constantly with 95% O2/5% CO2. The isometric tension was measured using force displacement transducers (model FT-03; Grass Instruments) connected to a data acquisition system (Biopac Systems MP100 workstaton) operating on a Windows PC using AcqKnowledge software (Biopac Systems). The tissues were allowed to equilibrate at a resting tension of 800 mg for at least 1 h with washing every 45 min with a maximal response to MCh (10−4 M) was obtained to ensure that all tissues used in the assessment were reacting within preestablished, standard criteria. After a resting period and a basal return to washout, a cumulative concentration response to MCh (10−9 to 10−2.5 M) was conducted on each tissue. The response to MCh was then calculated in ng tension using the AcqKnowledge software.

Measurement of airway smooth muscle thickness was performed following an immunohistochemistry protocol modified from Leung et al. (27). Insulated formalin-fixed lung tissues were processed and wax embedded. Four-micrometer sections of paraffin-embedded tissue were dewaxed and rehydrated. Intrinsic peroxidase activity was blocked by incubating the sections with 3% hydrogen peroxide (20 min). Non specific binding was reduced with 10% normal horse serum (Vector Laboratories) in PBS containing 0.1% BSA and 0.025% Tween 80 for 20 min. Sections were then rinsed and a primary anti-smooth muscle actin mAb (1/1000 dilution; clone I44; Sigma-Aldrich) diluted in PBS containing 0.1% BSA and 0.025% Tween 80 was applied for 1 h at room temperature. Sections were then incubated with a secondary biotinylated horse-adsorbed horse antimouse IgG (1/500; Vector Laboratories) for 30 min, followed by detection with a Vectastain Elite ABC kit for mouse IgG (Vector laboratories; PK6102). The staining was revealed using the diaminobenzidine (which stains brown; Sigma-Aldrich) procedure counterstaining with Mayer’s hematoxylin. Tissues incubated with a control IgGa (1/20,000 from goat serum; Sigma-Aldrich I5256) instead of the primary antisem were used as negative controls.

Image processing was performed using an Axioplan microscope (Zeiss) fitted for both transmitted light and fluorescence imaging. Images were captured using an Axiocam digital camera (Zeiss) at maximum sensitivity and analyzed using a KS300 image analysis system (Carl Zeiss MicroImaging).

Airways of similar perimeter (<1000 μm; n = 3) were randomly chosen across each lung section. Basement membrane was outlined and the length determined. The area of the smooth muscle layers was quantified and divided by the basement membrane length to obtain a smooth muscle area-to-basement membrane ratio. Measurements performed on each lung section were averaged and the resulting values used for comparison between groups.

All counts in this study were performed with the investigator blinded to treatment.
sodium pyruvate (1 mM), L-glutamate (2 mM), nonessential amino acids (1/1003), and the antimicrobial agents detailed above.

The human airways smooth muscle (HASM) cell suspension was placed in a tissue culture flask (75 cm²) containing 6 ml of supplemented DMEM and allowed to adhere (12 h) at 37°C in 5% CO2/air. The culture medium was replaced after 4–5 days (12 ml) and thereafter every 3–4 days. When the cells reached confluence (10–14 days) and demonstrated a typical "hill and valley" appearance and positive immunostaining for α-actin (95%), they were seeded onto 6-well plates (Costar) at an initial density of 20,000 cell per well for proliferation experiments. At 60% confluence, the cells were growth arrested by being placed in DMEM containing apotransferrin (5 μg/ml), insulin (1 μM), ascorbate (100 μM), and

FIGURE 1. Ag-sensitized male Brown Norway rats were treated with vehicle or LXR agonist around challenge, and samples were taken 6 (A–C) or 24 h (D) later. ABCA-1 mRNA gene expression was measured in the lung tissue using multiplexing real-time RT-PCR and expressed as fold difference from vehicle-challenged control (A). Eotaxin (B) and IL-1β (C) levels in the lung tissue were determined by ELISA. Airway lumen eosinophilia is shown in Fig. 1D. Statistical analysis was performed on the absolute data using a t test with a Mann-Whitney posttest for single comparisons and a Kruskal-Wallis test with a Dunn’s posttest for multiple comparisons. †, Significant (p < 0.05) differences from nonstimulated control group; *, significant (p < 0.05) difference from stimulated control group (mean ± SEM, n = 8).

FIGURE 2. Sensitized male mice were challenged with vehicle or Ag once a day for 6 days. They received vehicle or budesonide (3 mg/kg, p.o., b.i.d) throughout the challenging phase. Airway reactivity was monitored using noninvasive lung function, and airway reactivity was assessed via increase aerosolized doses of MCh. Statistical analysis was performed on the absolute data using t test with a Mann-Whitney posttest for single comparisons and a Kruskal-Wallis test with a Dunn’s posttest for multiple comparisons. †, Significant (p < 0.05) differences from nonstimulated control group; *, significant (p < 0.05, # = p value of 0.05) difference from stimulated control group (mean ± SEM, n = 8).

FIGURE 3. Sensitized male mice were challenged with vehicle or Ag once a day for 6 days. They received vehicle or GW 3965 (3, 10, or 30 mg/kg, p.o., b.i.d) throughout the study. Airway reactivity was monitored using noninvasive lung function, and airway reactivity was assessed via increase aerosolized doses of MCh. Shown are all doses of the compound at a single aerosolized dose of MCh (4 μg/ml). Statistical analysis was performed on the absolute data using t test with a Mann-Whitney posttest for single comparisons and a Kruskal-Wallis test with a Dunn’s posttest for multiple comparisons. †, Significant (p < 0.05) differences from nonstimulated control group and * indicates significant (p < 0.05, # = p value of 0.05) difference from stimulated control group (mean ± SEM, n = 8).
BSA (0.1% (w/v)) for 24 h. Twenty-four hours after the cells were growth arrested, HASM cells were treated in serum-free medium with either vehicle (0.1% DMSO) or increasing concentrations of the LXR agonists GW 3965 and T 1317 (10^{-9} to 10^{-6} M) for 72 h at 37°C. A positive control of 3% serum was also included. Cells were then washed with PBS, trypsinized, and resuspended in DMEM containing 10% FCS. Cell numbers were determined using a Bright-Line hemacytometer (Sigma-Aldrich).

### Results

#### Effect of LXR agonist, GW 3965, on airway inflammation in the Ag-driven preclinical Brown Norway rat allergic model

Treatment with the GW 3965 caused a dose-related, statistically significant increase in ABCA-1 mRNA expression in the lung tissue (Fig. 1A). This indicated that in this model system the ligand is actually activating the LXR, suggesting that the dosing regimen used is appropriate and that this ligand is effective in rodents. Although we clearly demonstrate activation of the LXR in this preclinical model, the selective ligand failed to significantly affect Ag-induced inflammatory mediator production or airway eosinophilia (Fig. 1, B–D). The positive control employed in this study, budesonide, significantly inhibited the Ag-induced increase in IL-1 and eotaxin production and the increase in airway lumen eosinophilia (Fig. 1, B–D).

#### Effect of LXR agonist, GW 3965, in an Ag-driven preclinical murine model of asthma

Fig. 2 clearly shows that exposure to Ag has caused a significant increase in reactivity to inhaled MCh in comparison with the vehicle-challenged controls. The study is further validated by the

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**Statistical analysis**

Specifics on numbers and statistical analyses performed are presented in the figure legends.

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**Results**

**Effect of LXR agonist, GW 3965, on airway inflammation in the Ag-driven preclinical Brown Norway rat allergic model**

Treatment with the GW 3965 caused a dose-related, statistically significant increase in ABCC1 mRNA expression in the lung tissue (Fig. 1A). This indicated that in this model system the ligand is actually activating the LXR, suggesting that the dosing regimen used is appropriate and that this ligand is effective in rodents. Although we clearly demonstrate activation of the LXR in this preclinical model, the selective ligand failed to significantly affect Ag-induced inflammatory mediator production or airway eosinophilia (Fig. 1, B–D). The positive control employed in this study, budesonide, significantly inhibited the Ag-induced increase in IL-1β and eotaxin production and the increase in airway lumen eosinophilia (Fig. 1, B–D).

**Effect of LXR agonist, GW 3965, in an Ag-driven preclinical murine model of asthma**

Fig. 2 clearly shows that exposure to Ag has caused a significant increase in reactivity to inhaled MCh in comparison with the vehicle-challenged controls. The study is further validated by the
observation that the clinically relevant positive control, budesonide, has significantly attenuated the Ag-induced response (Fig. 2). Interestingly, the LXR agonist appeared to cause a dose-related, significant increase in airway reactivity to MCh (Fig. 3). This increase in reactivity was apparent at most of the challenging doses of MCh (Fig. 4). Furthermore, this increase in airway reactivity is evident in the vehicle-challenged, agonist-dosed group, indicating that the effect is independent of the allergic response (Fig. 4).

In an attempt to determine how the LXR agonist is causing this apparent increase in AHR, we examined the inflammatory status of the lung tissue and the level of mucus present. Fig. 5 demonstrates that Ag challenge significantly increases both the inflammatory status of the mouse airways and the number of mucus-containing epithelial cells. As expected, steroid treatment significantly decreased the cellular inflammation and the mucus content (Fig. 5). The LXR agonist, however, did not appear to have any impact on these indices, suggesting they are not the reason for the increase in AHR observed (Fig. 4).

To examine this phenomenon more closely and to confirm the enhanced responsiveness seen previously, we repeated the study and measured the responsiveness of the airways ex vivo. Fig. 6 demonstrates that the ex vivo upper airways from the animals treated with the LXR ligand respond to a greater extent in comparison with the control animals. As the most likely explanation for this is an increase in airway smooth muscle (ASM) mass, we measured this in the lung samples. Fig. 7 shows that the Ag challenge has caused a significant increase in ASM thickness, expressed as a ratio of the basement membrane layer, compared with the vehicle-challenged animals. Treatment with the glucocorticoid has significantly attenuated this increase (Fig. 7). Treatment with the LXR agonist caused an increase in ASM thickness in both the vehicle and Ag-challenged group (compared with the respective non-compound-treated control group), which is likely to partly explain the increase in airway responsiveness in vivo and ex vivo (Fig. 7). Fig. 8 contains representative images of the staining observed in the treatment groups. It can be clearly seen that the LXR ligand increases the thickness of ASM.

In an attempt to determine how the LXR agonist induced the increase in ASM thickness, we used gene array technology to measure the levels of mRNA expression of a number of growth factors. In the lung samples taken at the completion of the study there appeared to be no increase in mRNA levels for VEGFα, PDGFα, FGF1, FGF2, FGF18, TGF-β1, TGF-β3, and EGF (data not shown). There was an increase in mRNA for α-actin (vehicle dosed/saline challenged, 13,818 arbitrary units (a.u.); vehicle.
dosed/OVA challenged, 12,783 a.u.; GW3965 dosed/OVA challenged, 16,783 a.u.), which would suggest the technique worked correctly.

While the data in the preclinical allergic model is very compelling, we wanted to see if parallel data could be observed in a human-based system, so we determined the effects of the LXR agonist GW 3965 on HASM proliferation. Additionally, we wanted to confirm that the findings were linked to agonist activity at LXR and not specific to GW 3965, so we profiled a structurally different agonist T1317 in parallel. GW 3965 (10⁻⁹ to 10⁻⁶ M) and T1317 (10⁻⁹ to 10⁻⁷ M) caused a concentration-related increase in cell proliferation (Fig. 9).

Discussion

The liver X receptors (LXRα/β) are orphan nuclear receptors that are expressed in a large number of cell types. In addition to playing a role in control of cholesterol and fatty acid metabolism, endogenous (oxysterols) and synthetic (GW 3965) LXR agonists have been shown to have anti-inflammatory properties. Treatment with LXR ligands has shown beneficial effects in preclinical models of atherosclerosis, which is thought to be via their impact on cholesterol levels and their anti-inflammatory properties (10). Chronic allergic airway inflammation is thought to be central to the pathogenesis of asthma. It is thought that by inhibiting this chronic allergic inflammation disease, symptoms would be attenuated; therefore, the aim of this study was to determine whether activation of these purported anti-inflammatory nuclear receptors would be effective in preclinical models of asthma. In our characterized Brown Norway rat model of asthma we were able to demonstrate that the LXR ligand activated LXRs, suggesting that the dosing regimen used was appropriate and confirming that this ligand activated rodent LXRs. Although we clearly demonstrate activation of the LXR in this preclinical model, the compound failed to significantly affect Ag-induced inflammatory mediator production or airway eosinophilia. This study demonstrated a “typical” inflammatory phenotype that could be inhibited by the positive control compound, the glucocorticoid receptor agonist budesonide. We have previously shown the same LXR ligand to attenuate innate, LPS-induced airway inflammation in a rat model using a similar dosing regimen (28). This may suggest that although activation of this receptor may have anti-inflammatory properties in some preclinical rodent models, it does not modify allergic inflammation.

Although the ligand failed to affect allergic inflammation, we wanted to examine its effect on another cardinal feature of asthma, AHR. To perform this assessment we switched to our mouse model of asthma that exhibits AHR, as this element of the “disease” phenotype is missing from our rat model. Surprisingly, while the murine model was shown to be performing normally (i.e., we observed AHR to an inhaled bronchopasmic agent in the mice challenged with Ag, which was attenuated with steroid treatment), with the LXR ligand there was an increase in airway responsiveness. Furthermore, the ligand caused an increase in airway reactivity in the non-Ag-challenged group. This would suggest that the increase in AHR observed with LXR agonist treatment may not be related to the allergic response. Further evidence for this comes from the fact that the ligand did not appear to modulate cellular burden or the extent of mucus production in the lung tissue in either the vehicle or Ag-challenged groups. Having appeared to rule out an increase in cellular recruitment and mucus production as a cause for the LXR agonist-induced AHR, we wanted to examine the possible role of the next most likely candidate: ASM. ASM can undergo hyperplasia and/or hypertrophy, which can lead to structural changes in the airway wall and may contribute to the development of persistent airway obstruction and increased non-specific AHR in chronic severe asthma (29). Therefore, we repeated the study and measured the responsiveness of isolated trachea to exogenous MCh and also measured the thickness of the ASM in histologically prepared lung tissues. In this repeat of the study we were able to demonstrate AHR in ex vivo samples taken from LXR agonist-dosed animals and an increase in ASM thickness. These findings would strongly suggest that the observed AHR is due to an increase in ASM mass, which may not always be related to the allergic status of the airway. It also corroborates the compound-induced AHR observed using enhanced pause. Furthermore, this phenomenon may not necessarily be restricted to airway smooth muscle. However, we cannot rule out the possibility that the AHR is not related to an increase in ASM thickness but is a result of a direct effect on ASM function. We did attempt to determine whether there was an associated increase in growth factors using array technology; however, at the time the samples were collected, we could not detect any increase in the candidate “re-modeling” gene expression levels of those assessed. This might suggest that the increase in ASM was not due to an up-regulation of these growth factors or that we did not measure them at the correct time point or the correct site. When using the arrays, we did detect an increase in α-actin mRNA expression, which corroborated the increase in ASM measured. While we do not know how the LXR ligand is causing this effect, we think that this is a very interesting finding. It could be possible that an endogenous altered LXR response may be responsible for AHR observed in a subset of the general population. It is conceivable that people with AHR could have a greater LXR signal possible through a number of mechanisms, such as increased receptor number, endogenous ligands, receptor signaling, or a decrease in endogenous negative control of the receptor. Normally an increase in airway responsiveness might not result in clinical symptoms, but it could with the addition of underlying allergic airway inflammation. As positive effects of LXR ligands have been demonstrated in other chronic disease models such as atherosclerosis, it seems likely that they are being progressed to the clinic. Therefore, it is very important to make others aware of these findings so they can carefully consider this information before designing and embarking on any clinical trial protocols with ligands of this sort.

While the data in the preclinical allergic model are very compelling, we also wanted to determine whether parallel data could be observed in a human-based system. Therefore, we determined the effects of the LXR agonist GW 3965 on HASM proliferation. Additionally, we wanted to confirm that the findings were linked to the activation of LXR and not specific to GW 3965, so we profiled a structurally different agonist T1317 in parallel. The data from these primary human cell-based assays suggest the observed effect in the model is not peculiar to the mouse and that it is not restricted to GW 3965 but to other LXR agonists. This result does appear to be at odds with a recent publication by Delvecchio et al. in which they show data suggesting that T1317 inhibits airway smooth muscle cell proliferation (30). The reason for this difference is not known, but it could be due to the fact that we treat nonstimulated cells whereas they use much higher concentrations of ligand, which are in excess of the reported IC₅₀ and they stimulate them with platelet-derived growth factor. Similarly, Blaschke et al. has published that LXR agonist can block the proliferation of vascular smooth muscle cells in vitro in a rodent model (31), which might suggest a difference between the role of LXR in airway and vascular smooth muscle.

In conclusion, we have demonstrated that LXR ligands appear not to affect allergic inflammation but that they cause AHR in preclinical airway models. These results, if they are apparent in humans, would indicate that LXR ligands may directly increase
AR, which could be detrimental, especially in patients with existing respiratory disease and with already compromised lung function.

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