

Murine atopic dermatitis responds to peroxisome proliferator-activated receptors α and β/δ (but not γ) and liver X receptor activators

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Background: Atopic dermatitis (AD) is a chronic inflammatory dermatosis now increasingly linked to mutations that alter the structure and function of the stratum corneum. Activators of peroxisome proliferator-activated receptors (PPARs) α , β/δ , and γ and liver X receptor (LXR) regulate epidermal protein and lipid production, leading to superior barrier function. Additionally, some of these activators exhibit potent antihyperplastic and anti-inflammatory activity in irritant contact dermatitis and acute allergic contact dermatitis murine models.

Objective: We evaluated the efficacy of PPAR/LXR activation in a hapten (oxazolone [Ox])–induced AD-like model (Ox-AD) in hairless mice.

Methods: Ox-AD was established with 10 Ox challenges (every other day) on the flank. After the establishment of Ox-AD, twice-daily topical application with individual PPAR/LXR activators was then performed for 4 days, with continued Ox challenges every other day. The efficacy of topical PPAR/LXR activators to reduce parameters of Ox-AD was assessed physiologically, morphologically, and immunologically.

Results: Certain topical activators of PPAR α , PPAR β/δ , and LXR, but not activators of PPAR γ , reversed the clinical dermatosis, significantly improved barrier function, and increased stratum corneum hydration in Ox-AD mice. In addition, the same activators, but again not PPAR γ , largely reversed the immunologic abnormalities in Ox-AD mice, including the increased T_H2 markers, such as tissue eosinophil/mast cell density, serum thymus and activation-related chemokine levels, the density of chemoattractant receptor–

homologous molecule expressed on T_H2-positive lymphocytes (but not serum IgE levels), and reduced IL-1 α and TNF- α activation, despite ongoing hapten challenges.

Conclusion: These results suggest that topical applications of certain activators/ligands of PPAR α , PPAR β/δ , and LXR could be useful for the treatment of AD in human subjects. (J Allergy Clin Immunol ■■■■;■■■:■■■-■■■.)

Key words: Atopic dermatitis, barrier function, liver X receptor, mouse model, peroxisome proliferator-activated receptor α , β/δ , and γ , T_H2 cells

Although long considered a primary immunologic disorder, atopic dermatitis (AD) exhibits prominent abnormalities in permeability barrier function that we and others have suspected play a role in disease pathogenesis.^{1–4} Notably, even the uninvolved skin of atopic subjects exhibits abnormal water permeability.^{5–7} These suspicions have been confirmed by recent molecular genetic investigations, which have identified a strong association between inherited mutations in the gene that encodes the corneocyte structural protein, filaggrin, and AD.^{8–10} Moreover, AD is characterized by a deterioration in other epidermal protective functions, including stratum corneum (SC) cohesion,¹¹ antimicrobial defense,¹² and decreased SC hydration,^{6,7} which further complicates disease management.

Despite compelling evidence for a primary barrier-based abnormality, therapy for human AD is still largely directed at downstream immunologic abnormalities. Although topical glucocorticoids can be beset with unacceptable side effects, topical immunomodulators are only moderately effective and could result in long-term risks.^{13,14} Thus there is a strong need for alternate therapies that are not only safe and effective but also directed at correcting the barrier dysfunction that drives AD.

Activators of peroxisome proliferator-activated receptors (PPARs) α , β/δ , and γ and liver X receptor (LXR) α/β display potent, largely positive effects on epidermal structure and function, including upregulation of filaggrin.¹⁵ Moreover, they display substantial anti-inflammatory activity in murine models of both irritant and acute allergic contact dermatitis,^{16,17} and they potently reverse epidermal hyperplasia and normalize epidermal differentiation in hyperproliferative murine disease models.¹⁸ Because the endogenous activators of these receptors are naturally occurring lipids that can be generated within the epidermis (eg, free fatty acids, eicosanoids, and oxygenated sterols), these nuclear hormone receptors could represent key regulators of epidermal homeostasis.¹⁵ Because human AD exhibits primary abnormalities in

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Abbreviations used

AD:	Atopic dermatitis
CRTH2:	Chemoattractant receptor–homologous molecule expressed on T _H 2
LXR:	Liver X receptor
Ox:	Oxazolone
PCNA:	Proliferating cell nuclear antigen
PPAR:	Peroxisome proliferator–activated receptor
SC:	Stratum corneum
TARC:	Thymus and activation-related chemokine
TEWL:	Transepidermal water loss

epidermal barrier function, resulting in downstream epidermal hyperplasia, aberrant differentiation, and T_H2-dominant reactions, the PPAR/LXR activators, in theory, possess a profile of activity that suggests potential utility in AD. We recently described a hapten-induced AD-like model that recapitulates a large spectrum of the epidermal and immunologic abnormalities of AD in human subjects,¹⁹ including a prominent T_H2 infiltrate. Hence we evaluated here several PPAR/LXR activators in this model, identifying which classes of agents demonstrate apparent clinical benefit and the extent to which these activators reverse the structural, functional, and immunologic abnormalities in affected mice. Our results show that certain activators of LXR α / β , PPAR α , and PPAR β / δ display broad efficacy, whereas PPAR γ activators exhibited little activity in this AD model.

METHODS**Animals and materials**

Female hairless mice (hr/hr), aged 6 to 8 weeks, were purchased from Charles River laboratories (Wilmington, Mass) and fed mouse diet (Ralston-Purina Co, St Louis, Mo) and water *ad libitum*. WY14643 (PPAR α activator), clofibrate (PPAR α activator), T0901317 (LXR activator), 22(R)-hydroxycholesterol (LXR activator), clobetasol propionate, and oxazolone (Ox) were purchased from Sigma Chemical Co (St Louis, Mo). GW7647 (PPAR α activator), GW0742 (PPAR β / δ activator), GW1929 (PPAR γ activator), and GW3965 (LXR activator) were purchased from TOCRIS Bioscience (Ellisville, Mo). Ciglitazone (PPAR γ activator) was purchased from Cayman Chemical (Ann Arbor, Mich). GW1514 (PPAR β / δ activator) was a gift from Dr Timothy Willson (GlaxoSmithKline, Triangle Park, NC). Rabbit anti-mouse antibody against the prostaglandin D receptor chemoattractant receptor–homologous molecule expressed on T_H2 (CRTH2)/DP2 was from Cayman Chemical. Goat anti-mouse antibody against IL-1 α was purchased from Santa Cruz Biotechnology (Santa Cruz, Calif). Rabbit anti-human antibody against CD3 was purchased from Dako (Glostrup, Denmark). Biotinylated goat anti-rabbit IgG antibody and biotinylated horse anti-goat antibody were purchased from Vector Laboratories (Burlingame, Calif). Biotinylated mAb against proliferating cell nuclear antigen (PCNA) was purchased from CalTag Laboratories (Burlingame, Calif).

Experimental protocols and functional studies

All animal procedures were approved by the Institutional Animal Care and Use Committee of the San Francisco Veterans Administration Medical Center and performed in accordance with their guidelines. Animals were sensitized with a single topical treatment with 50 μ L of 1% Ox. After 1 week, they were treated topically with 60 μ L of 0.1% Ox to both flanks once every other day for an additional 4 weeks (total of 12 challenges). After the 10th challenge, when the phenotype of AD-like, chronic allergic dermatitis was established, the therapeutic effects of activators of nuclear hormone receptors were performed as follows: 1 hour after the 11th challenge, twice-daily application of activators (20 μ L) of 10 mmol/L WY14643, 10 mmol/L GW7647, 1 mmol/L clofibrate, 4

mmol/L GW1514, 10 mmol/L GW0742, 10 mmol/L ciglitazone, 10 mmol/L GW1929, 10 mmol/L T0901317, 10 mmol/L GW3965, 10 mmol/L 22(R)-hydroxycholesterol, and 0.05% clobetasol propionate in vehicle (propylene glycol/ethanol, 7:3) were performed for 4 days; the 12th challenge with Ox was performed 1 hour before the first application of the activator or vehicle on that day. Topical clobetasol, a superpotent, class 1 topical glucocorticoid, with proven efficacy in human AD, served as a positive control, whereas another Ox-AD group was treated with vehicle alone. Basal transepidermal water loss (TEWL) was measured with an electrolytic water analyzer (Meeco, Warrington, Pa), and SC hydration, assessed as capacitance, was measured with a Corneometer CM820 (Courage-Khazaka, Cologne, Germany), as described previously.²⁰ SC surface pH was measured with a flat, glass-surface electrode from Mettler-Toledo (Giessen, Germany) attached to a pH meter (PH900, Courage-Khazaka), as described previously.²¹ These physiologic measurements were performed immediately before the 11th challenge and 48 hours after the 12th challenge with Ox. Skin samples were collected 48 hours after the 12th challenge with Ox (day 5). Blood samples were collected immediately before the 11th challenge with Ox and 48 hours after the 12th challenge (ie, after liposensor treatments).

Immunohistochemistry

Immunohistochemical staining for CRTH2 and IL-1 α was performed as described previously.²² Briefly, 5- μ m paraffin sections were incubated with the primary antibodies overnight at 4 °C. After 3 washes, sections were incubated with the secondary antibody for 30 minutes. Staining was detected with the ABC-peroxidase kit from Vector Laboratories. To detect proliferating cells by means of PCNA staining, 5- μ m paraffin sections were incubated with the biotinylated mAb against the Ki-67 antigen overnight at 4 °C, and staining was detected by using the ABC-peroxidase method. Sections were examined with a light microscope from Carl Zeiss (Jena, Germany), and digital images were captured with AxioVision software (Carl Zeiss Vision, Munich, Germany).

Quantitative morphology

The densities of CRTH2⁺ cells, eosinophils assessed in hematoxylin and eosin–stained sections, and mast cells detected by means of toluidine blue staining in an area of 220 μ m $\times$ 170 μ m were counted in more than 15 fields in the dermis of each sample. The thickness of epidermal nucleated layers was measured with AxioVision software (Carl Zeiss Vision) in hematoxylin and eosin–stained sections; measurements were performed in more than 15 fields at intervals of 100 μ m in each sample. The number of PCNA⁺ cells observed within a 50- μ m length of epidermis was counted in more than 10 sites for each sample. Data are reported as the means $\pm$ SEMs.

Serum IgE and thymus and activation-related chemokine measurements

Blood samples were collected from mice tails before and at the end of the therapeutic protocols described above. Serum IgE and thymus and activation-related chemokine (TARC) concentrations were determined by means of ELISA with a mouse IgE quantitation kit from Bethyl Laboratories (Montgomery, Tes) and Quantikine for mouse CCL17/TARC immunoassay from R&D Systems (Minneapolis, Minn), according to the manufacturer's instructions.

Electron microscopy

Skin biopsy specimens of both vehicle- and Ox-treated mice were fixed in Karnovsky fixative overnight and postfixed with either 0.25% ruthenium tetroxide or 1% aqueous osmium tetroxide containing 1.5% potassium ferrocyanide, as described previously.²³ Ultrathin sections were examined with an electron microscope (Zeiss 10A; Carl Zeiss, Thornwood, NY) operated at 60 kV.

Zymographic assessment of enzyme activity

Serine protease activity was assessed in freshly obtained skin samples by means of *in situ* zymography, as described previously.²⁴ Five-micrometer frozen sections were incubated with BODIPY-FI0–casein for 2 hours at 37 °C.

After 3 washes with 1% Tween-20, sections were counterstained with propidium iodide and examined with the confocal microscope, as above.

Statistical analysis

The 2-tailed Student *t* test was used to determine significant differences between 2 groups. A further ANOVA was calculated, followed by an α -corrected *post hoc* test (Bonferroni), when 3 or more groups were compared. Data are expressed as means $\pm$ SEMs.

RESULTS

Macroscopic and histologic response of Ox-AD to liposensor activators

We showed previously that sensitization of hairless mice with the hapten Ox, followed by 10 challenges over 20/21 days, leads to a dermatosis with global epidermal and immunologic features of human AD (Ox-AD mice).¹⁹ Here we applied 2 or 3 different activators of PPAR α , PPAR β/δ , PPAR γ , and LXR twice daily for 4 days to Ox-AD mice with established dermatitis (ie, having received 10 prior challenges). Ox-AD mice also continued to receive regularly scheduled Ox challenge doses (twice every other day) during the treatment phase. Ox-AD mice treated with 2 different synthetic activators of LXR (but not the endogenous activator 22rOH-cholesterol), 2 different synthetic ligands of PPAR α (but not clofibrate), and 2 different synthetic ligands of PPAR β/δ showed grossly (clinically) improved dermatitis, despite ongoing Ox challenges (Fig 1, A, and see Fig E1, in this article's Online Repository at www.jacionline.org for clinical effects of other activators). Likewise, dermatitis cleared in clobetasol-treated mice, but cutaneous atrophy (telangiectasia and fine wrinkling) became evident by day 5 of treatment. In contrast, 2 different, synthetic PPAR γ activators (ciglitazone and GW1929) largely failed to improve symptoms in Ox-AD mice (Fig 1, A, and see Fig E1).

The macroscopic (clinical) response to the activators was mirrored by changes in histology. Although Ox-AD mice treated with vehicle alone showed both prominent epidermal hyperplasia and a dense inflammatory infiltrate, the histologic appearance of LXR α/β , PPAR α , and PPAR β/δ activator-treated (but not PPAR γ -treated) Ox-AD mice improved (Fig 1, B), with the greatest reductions in both epidermal hyperplasia and inflammation occurring in mice treated with the 2 synthetic LXR activators (TO901317 and GW3965) and with the 2 synthetic PPAR α activators (WY14643 and GW7647) and the 2 synthetic PPAR β/δ activators (GW1514 and GW0742). (Quantitative data on epidermal hyperplasia and PCNA immunostaining are shown in Fig 1, C and D, and Fig E2, in this article's Online Repository at www.jacionline.org.) However, Ox-AD mice treated with the PPAR γ activator ciglitazone demonstrated no decrease in either epidermal thickness or inflammation, whereas in contrast, clobetasol-treated mice showed the greatest reduction in inflammation and epidermal thickness (Fig 1, C), which is consistent with the clinically apparent atrophy produced by this drug (compare with Fig 1, A). Together, these results show that activators of LXR α/β , PPAR α , and PPAR β/δ normalize or improve epidermal hyperplasia and histologic evidence of inflammation in the established dermatitis of Ox-AD mice.

PPAR/LXR activators normalize epidermal structure and function

Ox-AD mice, like human subjects with AD, display characteristic abnormalities in epidermal structure and function.¹⁹

Although barrier function deteriorates, as indicated by increased TEWL rates, SC hydration decreases and surface pH increases, approaching neutrality, as in human AD. Therefore we next assessed whether the PPAR/LXR activators reverse these functional abnormalities in Ox-AD mice. In parallel with the clinical and histologic findings, Ox-AD mice treated with the 2 synthetic LXR α/β ligands (but not 22rOH-cholesterol), 2 of the 3 PPAR α ligands (but not clofibrate), and the 2 PPAR β/δ activators demonstrated significant reductions in both TEWL (Fig 2, A) and surface pH (Fig 2, C, and Fig E3 in this article's Online Repository at www.jacionline.org for functional data for additional ligands). Although the changes in TEWL were largest in Ox-AD mice treated with LXR, PPAR α , and PPAR β/δ activators, only the LXR activators and 1 PPAR α activator (GW7647) significantly increased SC hydration (toward normal; Fig 2, B, and see Fig E3). Pertinently, clobetasol also normalized barrier function, increased SC hydration, and decreased SC pH, despite the above-described evidence of cutaneous atrophy (Figs 1 and 2 and see Fig E2). In contrast, PPAR γ activators improved none of the functional parameters (Fig 2 and see Figs E2 and E3). These results demonstrate that certain PPAR/LXR activators, but not PPAR γ activators, normalize or improve epidermal function in Ox-AD mice.

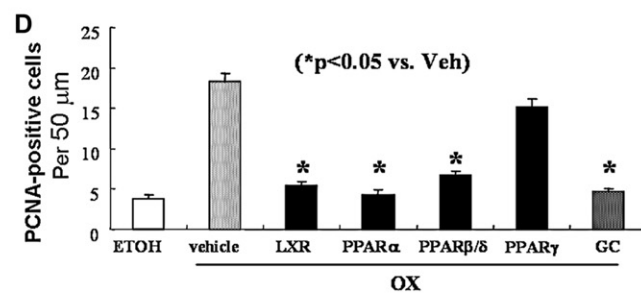
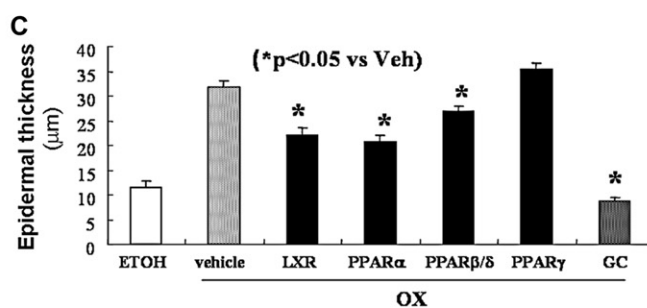
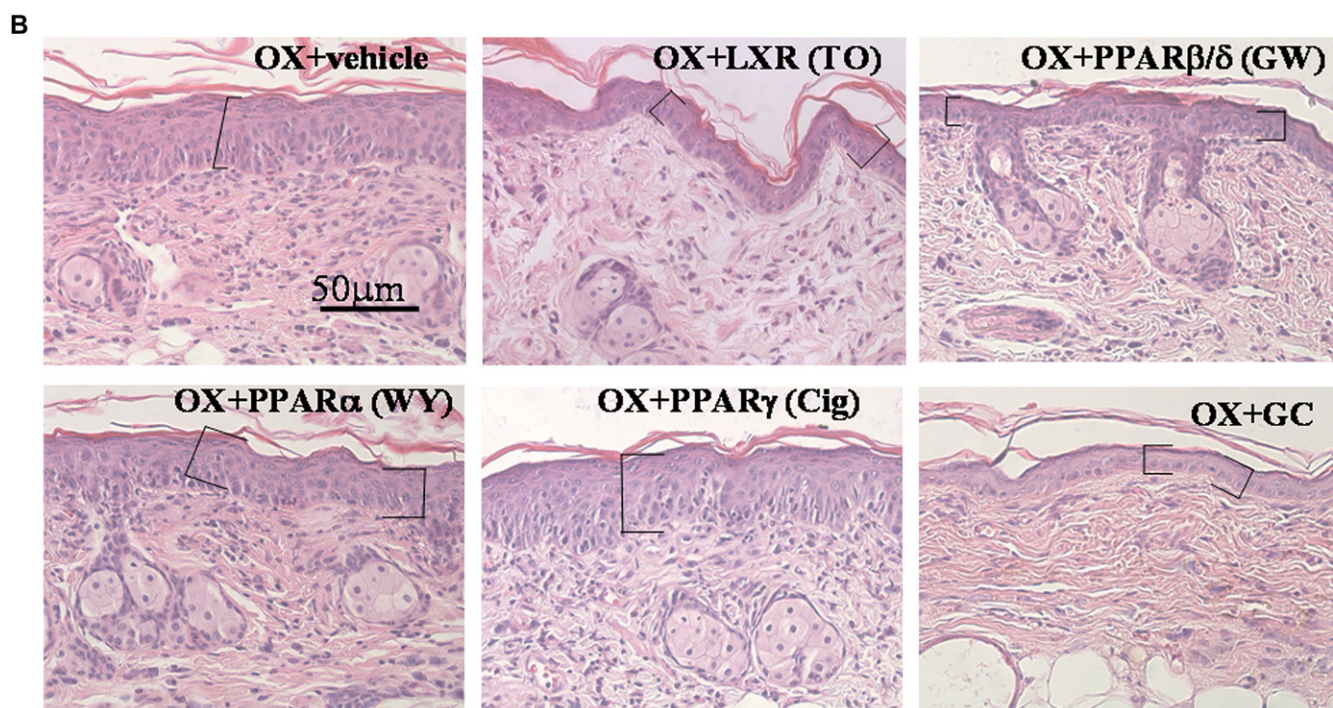
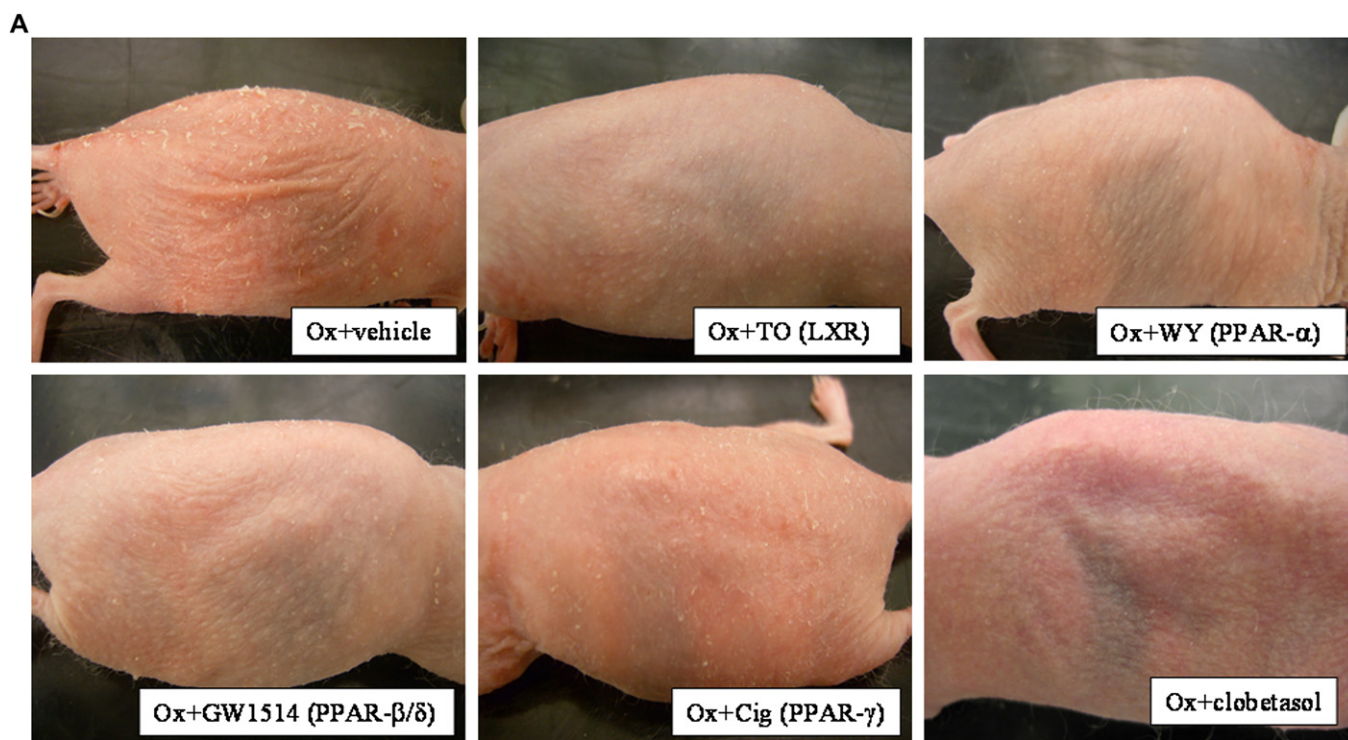
The structural basis for the permeability barrier abnormality in Ox-AD mice (and in human AD) in part reflects a failure of lamellar bodies to undergo complete exocytosis,²⁵ resulting in a paucity of extracellular lamellae.^{19,26} The further selective decrease of ceramides in human AD has been ascribed either to diminished activity of Cer-generating enzymes (ie, β -glucocerebrosidase and acidic sphingomyelinase)^{27,28} or to accelerated destruction of Cer precursors.^{29,30} Our prior studies support the first mechanism (ie, that a pH-induced increase in serine protease activity could account for abnormal lamellar bilayers in Ox-AD mice both by degrading lipid-processing enzymes and by blockade of lamellar body secretion).^{24,31} Thus we assessed whether the PPAR/LXR ligands normalized (1) lamellar body secretion and (2) the postsecretory processing of secreted ceramide precursors (Fig 3).

Treatment with the synthetic LXR activator (TO901317) restored normal quantities of secreted lamellar body contents to the SC interstices (Fig 3, E, vs Fig 3, C and D), and these secreted contents then demonstrated a normal sequence of progressive maturation into mature lamellar bilayers (Fig 3, B, vs Fig 3, A).

Both the LXR activator (TO901317) and the PPAR α activator (WY14643), which provoked the greatest decreases in SC pH (compare with Fig 2, C), reduced serine protease activity, whereas the LXR activator further restricted activity to a narrow but intense band at the stratum granulosum-SC interface (Fig 4, data for the WY compound are not shown). The reduction of serine protease activity normalized the lamellar body secretion,³¹ providing a biochemical basis for the restoration of normal lamellar membrane structures by certain liposensor activators.

PPAR/LXR activators decrease inflammation, including T_H2 immunophenotype, in Ox-AD mice

Prior studies have shown that LXR α/β , as well as PPAR α , PPAR β/δ , and/or PPAR γ , activators improve inflammation in several different models of inflammatory dermatoses (ie, psoriasisiform hyperplasia and irritant contact dermatitis) and hapten (Ox)-induced, acute allergic contact dermatitis¹⁵; however, at least



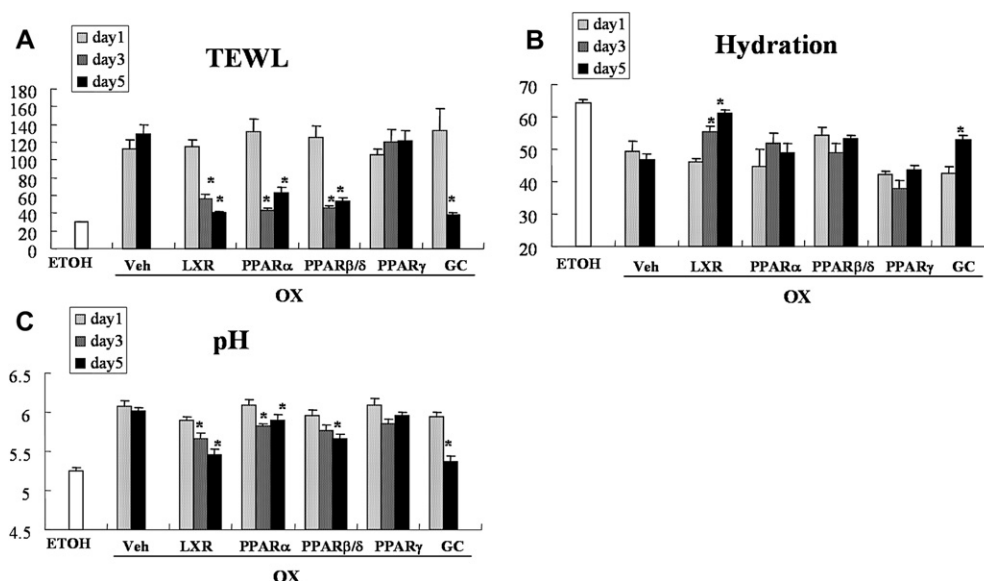


FIG 2. Ligands of LXR, PPAR α , PPAR β/δ , and clobetasol normalize epidermal function. Epidermal barrier function assessed as TEWL (A), surface pH (C), and SC hydration (B) was measured, and topical applications of the LXR, PPAR α , PPAR β/δ , and PPAR γ ligands, as well as the glucocorticoid (GC) clobetasol propionate and the vehicle (Veh), were performed as described in the Methods section. ETOH, Control group normal mice. Each experiment was repeated twice, and representative data are displayed. For TEWL and SC hydration, there were 24 measurements from 5 different animals in each group; for pH, there were 16 measurements from the same animals. * $P < .05$ (day 1 vs either day 3 or day 5).

some of the anti-inflammatory effects of PPAR γ activators were receptor independent because they occurred even in PPAR γ -deficient (knockout) mice.³² Therefore we next assessed whether the PPAR/LXR activators improve inflammatory/immunologic parameters in Ox-AD mice, assessing various immune end points 5 days after twice-daily, liposensor activator treatment in parallel with ongoing Ox challenges. We first assessed 2 general indicators of inflammation (ie, tissue eosinophilia and mast cell density/degranulation). The LXR α (T0901317), PPAR α (WY16463), PPAR β/δ (GW1514), and PPAR γ (ciglitazone) activators, as well as the superpotent topical glucocorticoid clobetasol, each decreased tissue eosinophilia levels significantly (LXR and PPAR γ ; Fig 5, A). Likewise, the same activators (except the PPAR γ activator) decreased mast cell density and the extent of mast cell degranulation (except the PPAR γ activator; Fig 5, B). These observations were confirmed by quantitative assessment in randomized coded micrographs (Fig 5, C and D). Together, these results show that certain PPAR α , PPAR β/δ , and LXR activators decrease both cutaneous eosinophilia and mast cell density in Ox-AD mice.

We next assessed more certain parameters of T_H2-mediated inflammation in PPAR/LXR-treated Ox-AD mice by using serum TARC levels and the density of CRTH2⁺ cells as markers for T_H2 immune status. The LXR α / β (T0901317), PPAR α

(WY16463), and PPAR β/δ (GW1514) activators, as well as clobetasol, significantly reduced the levels of both of these markers (Fig 6, A-C). In contrast, a PPAR γ activator (ciglitazone) reduced neither CRTH2 cell density nor serum TARC. Yet neither the topical PPAR/LXR activators nor topical clobetasol significantly reduced serum IgE levels in Ox-AD mice (Fig 6, D). Together, these results show that the PPAR/LXR activators exhibit potent anti-inflammatory activities against specific components of the immune response in Ox-AD mice.

PPAR/LXR activators decrease generation of IL-1 α and TNF- α

Barrier disruption leads to increased production of epidermal cytokines, a proinflammatory mechanism linked to the barrier-initiated (outside-inside) pathogenesis of AD.³³ We next assessed whether PPAR/LXR treatment decreased cytokine generation in parallel with improved barrier function in Ox-AD mice. Constitutive levels of IL-1 α and TNF- α were very low in normal epidermis, but immunostaining increased in both the epidermis and dermis of Ox-AD mice treated with vehicle alone or the PPAR β/δ activator GW1514 (see Fig E4 in this article's Online Repository at www.jacionline.org). Although PPAR/

FIG 1. A, PPAR α , PPAR β/δ , and LXR activators reverse murine Ox-AD. Gross appearance after applications of ligands for LXR (10 mmol/L T0901317), PPAR α (10 mmol/L WY16463), PPAR β/δ (4 mmol/L GW1514), and PPAR γ (10 mmol/L ciglitazone) and glucocorticoid (GC; 0.05% clobetasol). As a vehicle control (Ox+ vehicle), propylene glycol and ethanol (7:3) alone was applied. B, Histologic appearance after treatment with ligands for LXR, PPAR α , PPAR β/δ , and PPAR γ and the GC clobetasol (hematoxylin and eosin staining). C, Quantitative changes in epidermal hyperplasia in LXR, PPAR α , PPAR β/δ , and PPAR γ ligands and the GC clobetasol. Treated mice were assessed in coded, randomized micrographs (see the Methods section). D, PCNA⁺ cells counts (per 50 μ m) were quantitated as described in the Methods section. ETOH, Normal skin in which ethanol was applied instead of Ox; Veh, vehicle. There were 30 measurements each from 3 separate samples for epidermal hyperplasia assessment and 22 to 27 measurements each for PCNA assessment.

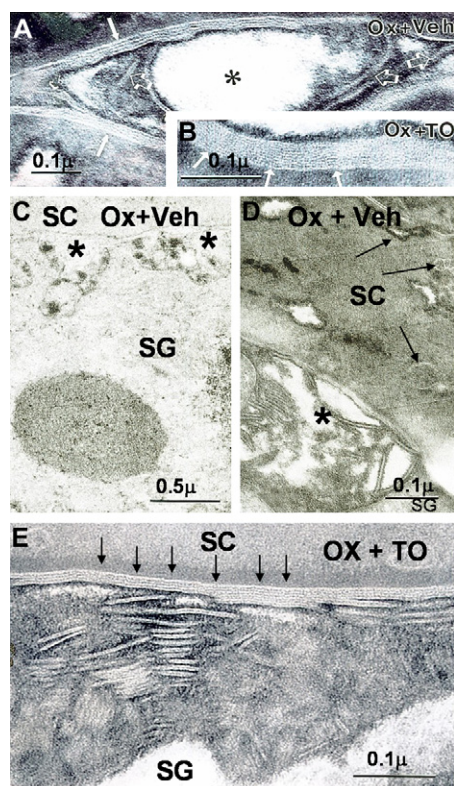


FIG 3. Topical LXR Ligand normalizes lamellar body secretion and lamellar bilayer structure in Ox-AD mice. SC of Ox plus vehicle (Veh)-treated skin sites demonstrates incomplete formation of lamellar bilayers (A, open arrows and asterisks), whereas Ox plus LXR ligand (TO; TO901317)-treated sites display both normal quantities of secreted lipid (E) and normal organization of lamellar bilayers (B and Fig 3, E, arrows). Decreased lamellar body secretion is indicated by a paucity of lamellae at the stratum granulosum (SG)-SC interface (C, asterisks). As a result of decreased secretion, abundant lamellar body contents remain entombed in corneocytes (D, arrows). Fig 3, A, B, D, and E, Ruthenium tetroxide postfixation; Fig 3, C, osmium tetroxide postfixation. Mag bars (Fig 3, A = 0.2 μ m; Fig 3, B = 0.1 μ m; Fig 3, C = 0.5 μ m; Fig 3, D = 0.1 μ m; Fig 3, E = 0.1 μ m).

LXR activators of all the receptors tested other than PPAR β/δ , including in this case the PPAR γ activator (ciglitazone), reduced IL-1 α and TNF- α immunostaining, the LXR activator (TO901317) was most effective and comparable with clobetasol (see Fig E4). These results show that topical applications of certain PPAR/LXR activators reduce epidermal cytokine production.

DISCUSSION

We show here that certain activators of the liposensor subclass of class II nuclear hormone receptors, in particular those for LXR, PPAR α , and PPAR β/δ , improve multiple parameters of the AD-like dermatosis in a hapten-induced mouse model. A very recent study also showed that a PPAR α activator prevented the emergence of inflammation in another murine model of AD.³⁴ Because the present model recapitulates virtually all of the known clinical, structural, functional, lipid biochemical, and immunologic abnormalities of human AD,¹⁹ their efficacy suggests that these agents might hold promise for the treatment of human AD. Interestingly, not all of the activators were effective; that is, neither the LXR activator, 22rOH-cholesterol, nor the PPAR α activator clofibrate

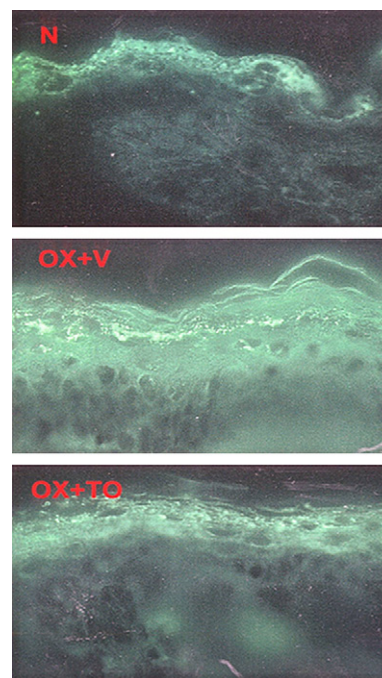


FIG 4. Topical LXR ligand reduces serine protease activity. Serine protease activity was assessed zymographically in frozen sections of normal plus ethanol vehicle (N), Ox plus vehicle (V), and Ox plus the LXR ligand TO901317 (TO).

demonstrated benefits. The apparent lack of benefit of 22rOH-cholesterol could also be because this naturally occurring compound could be metabolized further into inactive species. Alternatively, at an applied dose of 10 mmol/L, 22rOH-cholesterol could act as a bulk lipid that destabilizes extracellular lamellar bilayers.³⁵ Moreover, neither of the 2 PPAR γ activators displayed broad efficacy, despite a recent report that a PPAR γ activator, rosiglitazone, displays some anti-inflammatory activity in human AD.³⁶ Moreover, it remains possible that PPAR γ activators could be effective when administered systemically rather than topically. Furthermore, it remains to be determined whether results from this AD murine model, which might not be a strict analogue of human AD, necessarily will predict efficacy for human AD. For example, many cases of human AD are of a different cause (eg, inherited filaggrin deficiency) and can be complicated by colonizing microbial pathogens, which further aggravate the barrier abnormality in human AD³⁷ but are not known to exacerbate dermatitis in the murine analogues. Hence empiric testing of individual activators of these receptors will be needed to determine whether the activators are delivered transdermally and agents are of optimal benefit.

PPARs and LXR activators likely improve barrier function through at least 2 parallel mechanisms: stimulation of epidermal differentiation and lipid production.¹⁵ Because increased epidermal lipid production likely generates additional endogenous activators of these nuclear hormone receptors, this process can be viewed as a type of feed-forward mechanism that coordinately regulates generation of both the corneocytes and the extracellular matrix of the SC.

According to the outside-inside view of disease pathogenesis,^{37,38} inherited abnormalities in proteins important for the barrier predispose to the development of AD. Conversely,

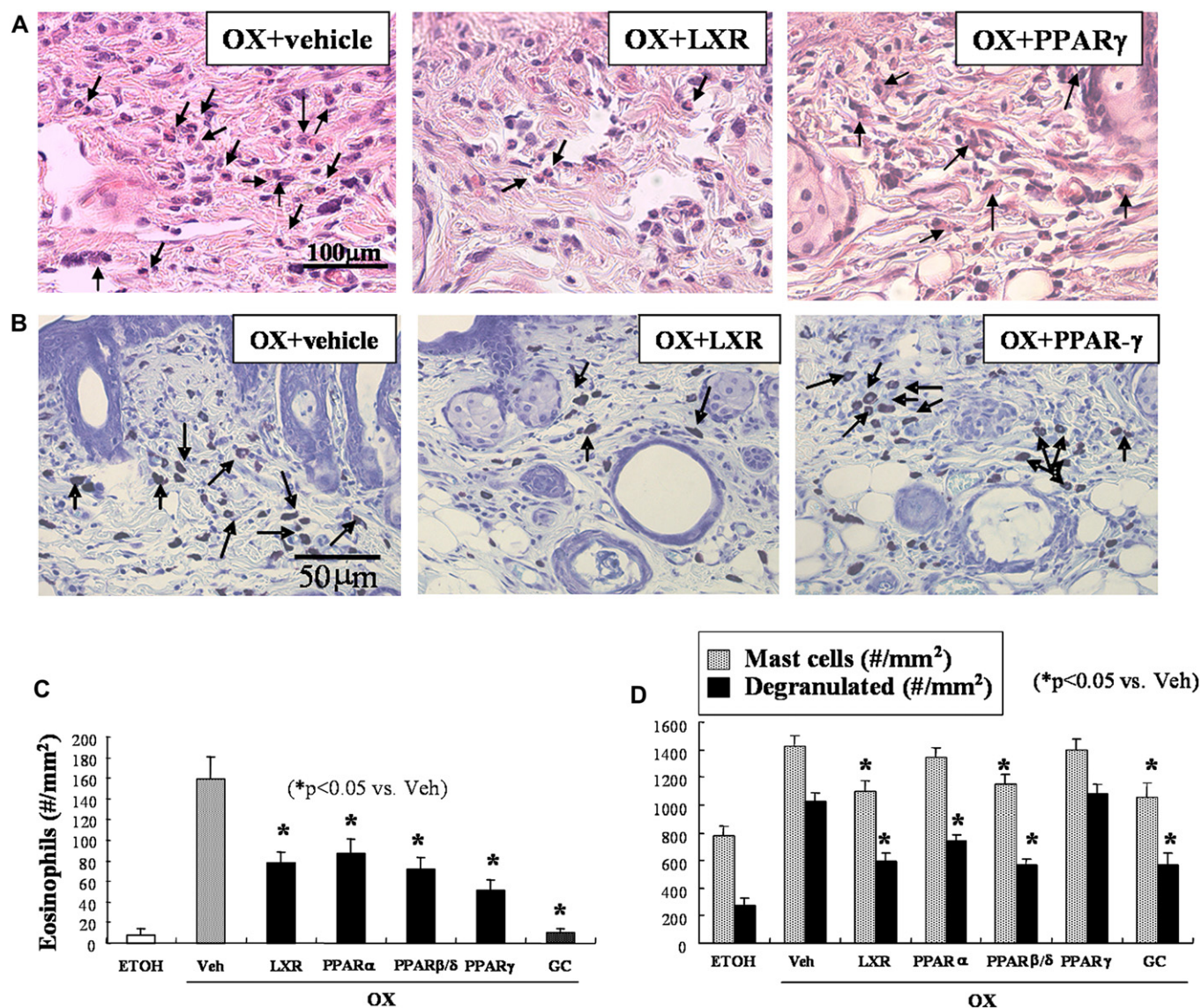


FIG 5. Topical PPAR/LXR activators decrease tissue eosinophilia and mast cells in Ox-AD mice. Eosinophils were stained in paraffin-embedded specimens with hematoxylin and eosin (**A**, arrows), whereas mast cells were stained with toluidine blue (**B**, arrows). **C** and **D**, Quantitation of tissue eosinophils (Fig 5, **C**) and mast cells numbers (and degranulated mast cell numbers) per square millimeter (Fig 5, **D**) was performed as described in the Methods section.

normalization of barrier function would, in turn, reduce the 2 major drivers of inflammation in AD.

First, cytokine generation, originating from perturbed corneocytes, as a signal that upregulates homeostatic repair mechanisms, should decrease. Indeed, our results show that both IL-1 α and TNF- α levels decrease after PPAR and LXR activator treatment.

Although the second mechanism is self-evident, it has not yet been experimentally verified; that is, improved permeability barrier function would simultaneously reduce the transdermal penetration of proinflammatory xenobiotics, including haptens and microbial pathogens. Of course, there is evidence in other cell types that certain PPARs, particularly PPAR δ activators, exert anti-inflammatory effects on macrophages and T cells.¹⁵ However, whether this mechanism was operative in these studies was not assessed. Pertinently, both topical retinoids and 1,25

dihydroxyvitamin D₃ analogues aggravate human AD (perhaps by activating epidermal proinflammatory cytokines³⁹ or by further aggravating the barrier abnormality). Notably, they also accentuate disease expression in the Ox-AD model (Y. Hatano and P. M. Elias, unpublished observations). The lack of efficacy of retinoids and vitamin D analogues in human AD is readily explicable by a comparison of the divergent activities shown by synthetic retinoids, vitamin D analogues, and PPAR/LXR on epidermal structure and function (see Table E1 in this article's Online Repository at www.jacionline.org). Notably, both retinoids and vitamin D analogues impair barrier function and stimulate epidermal proliferation *in vivo*, whereas retinoids (but not vitamin D analogues) also impede epidermal differentiation. Furthermore, it should be noted that the predicted activity profile of PPAR/LXR (and retinoids/vitamin D analogues) will be

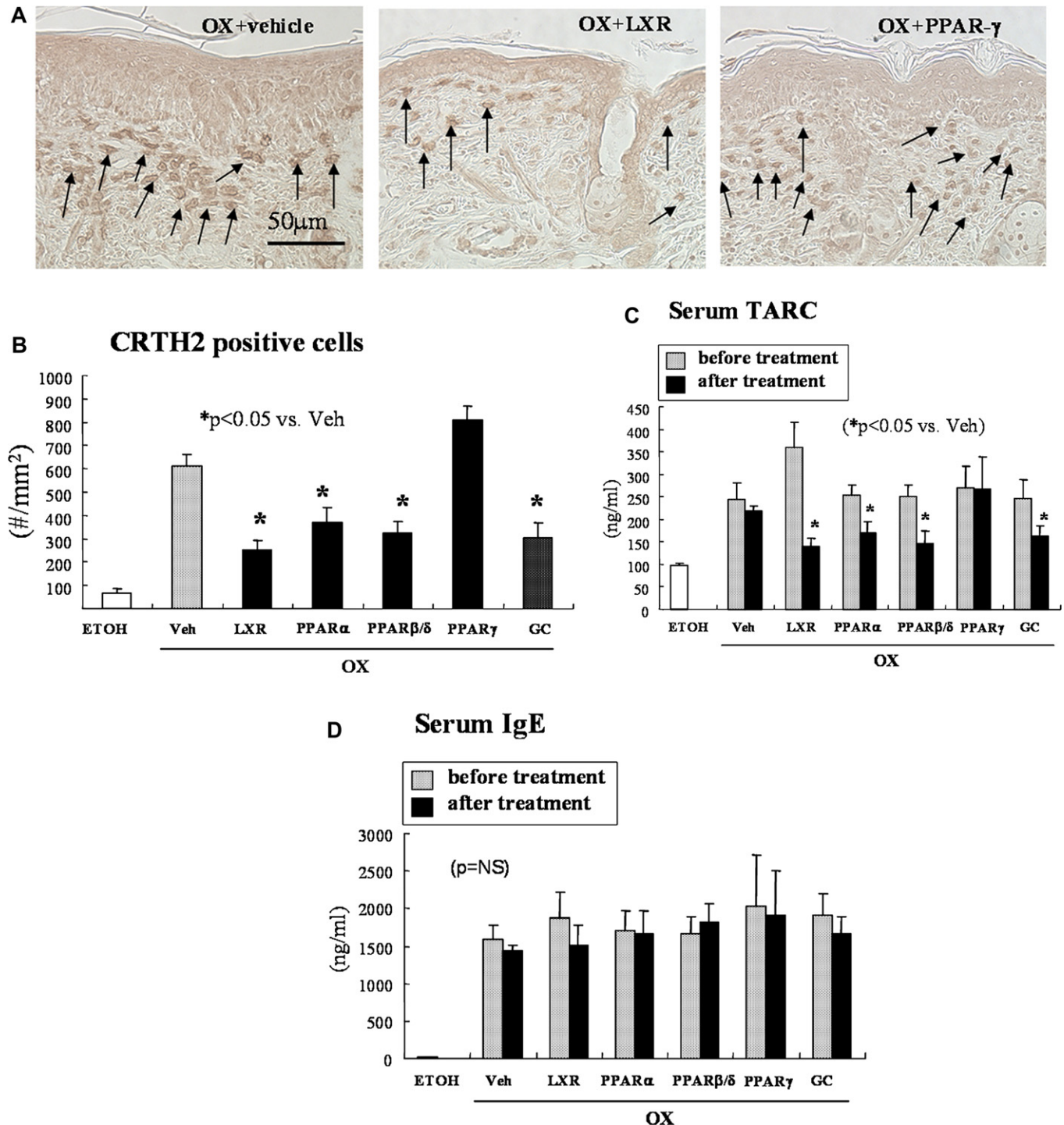


FIG 6. PPAR α , PPAR β/δ , and LXR ligands normalize T_H2 inflammation in Ox-AD mice. Skin samples and blood were collected as described in the Methods section for CRTH2 immunostaining (**A**), CRTH2⁺ cell counts (**B**), serum TARC measurements (**C**), and IgE measurements (**D**). ETOH, Ethanol-treated sites in normal mice, as control for Ox treatment. CRTH2, n = 30; TARC, n = 6-7; IgE, n = 3-5.

dependent on the expression levels of their respective receptors, as well as their heterodimerizing partner, retinoic X receptor, which could independently influence ligand activity in patients with AD.

A final key question remains. AD certainly is not the only dermatosis that is attributed to inherited mutations that alter

barrier function. At least 3 other disorders (ie, epidermolytic hyperkeratosis caused by K1/K10 mutations, loricrin keratoderma, and transglutaminase 1-deficient lamellar ichthyosis) all display barrier abnormalities but no known propensity toward AD. Could the more coherent SC in these disorders restrict antigen access?

Because these studies demonstrate broad and potent anti-inflammatory properties in yet another murine disease model (ie, hapten-induced AD), the PPAR/LXR activators could be effective in a variety of other dermatologic settings. Yet these studies did not examine all of the anti-inflammatory mechanisms by which these agents could work. Although they could exert direct effects on leukocytes and macrophages, as has been shown for some of the liposensor agents in other clinical settings,¹⁵ our studies suggest alternatively or additionally that the liposensor activators reduce inflammation by first normalizing permeability barrier function. As a result of, or at least in parallel with, a return of barrier function to normal, the amplitude of cytokine generation decreased in response to treatment with the liposensor activators. Likely, the downstream signal cascade that follows cytokine production subsequently decreases, which should, in turn, decrease the downstream signaling of chemokines and adhesion molecules that lead to inflammation (the outside-inside paradigm for the pathogenesis of inflammatory dermatoses).^{1,2,4} Because all of the liposensor activators (except PPAR γ) improved barrier function in Ox-AD mice, normalization of barrier function alone could account for decreased inflammation by means of downregulation of the cytokine cascade, as has been shown for other approaches that correct barrier function followed by a decrease in cytokine signalling.^{26,40,41} If the principal anti-inflammatory mechanism is secondary to restored barrier function, then it is interesting to speculate whether topical therapy might be substantially more effective than systemic therapy with these same agents. Yet reduction in inflammation also could improve barrier function³⁴ because a vicious cycle is operative in the pathogenesis of AD.^{38,41,42}

We thank Ms Joan Wakefield for superb editorial assistance.

Key Messages

- Certain activators of PPARs and LXR display beneficial effects in an animal model of AD.
- Thus PPAR α , PPAR β/δ , and LXR activators could prove useful for the treatment of AD in human subjects.

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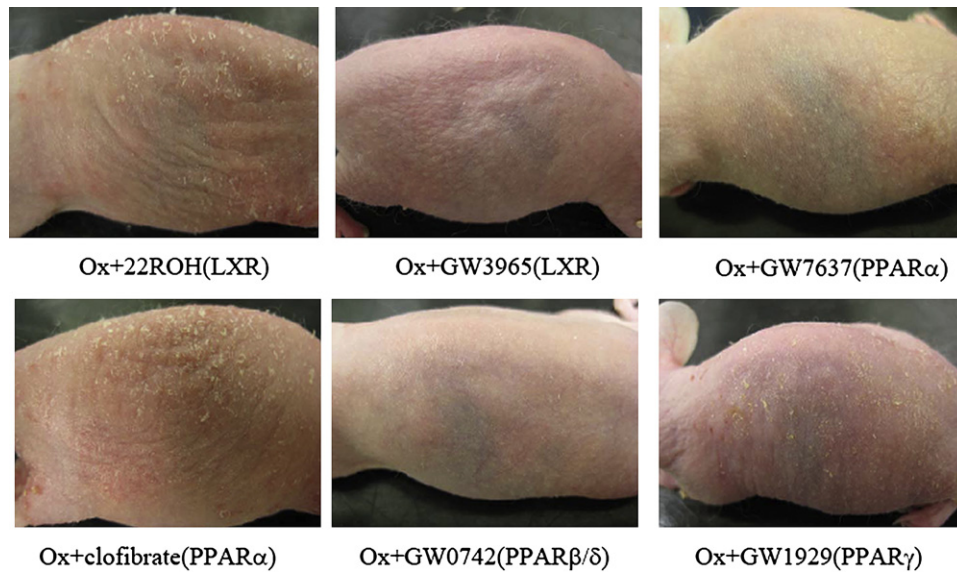


FIG E1. Clinical efficacy of additional PPAR and LXR activators (see the legend of Fig 1). This figure shows clinical (visible) changes in Ox-AD mice after topical application of several additional PPAR/LXR activators: LXR (22rOH-cholesterol and GW3965), PPAR α (clofibrate and GW7637), PPAR β/δ (GW0742), and PPAR γ (GW1929).

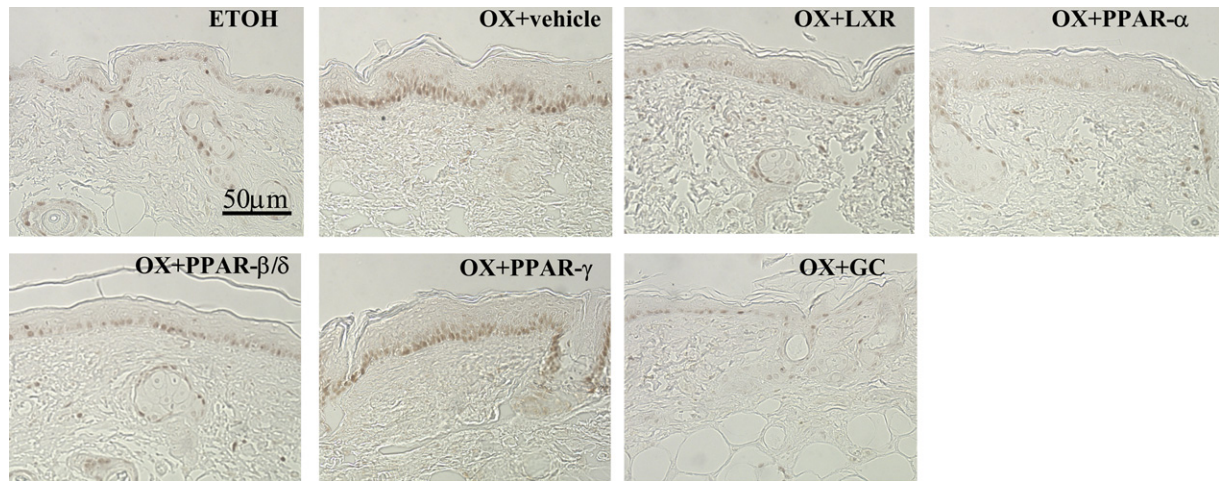
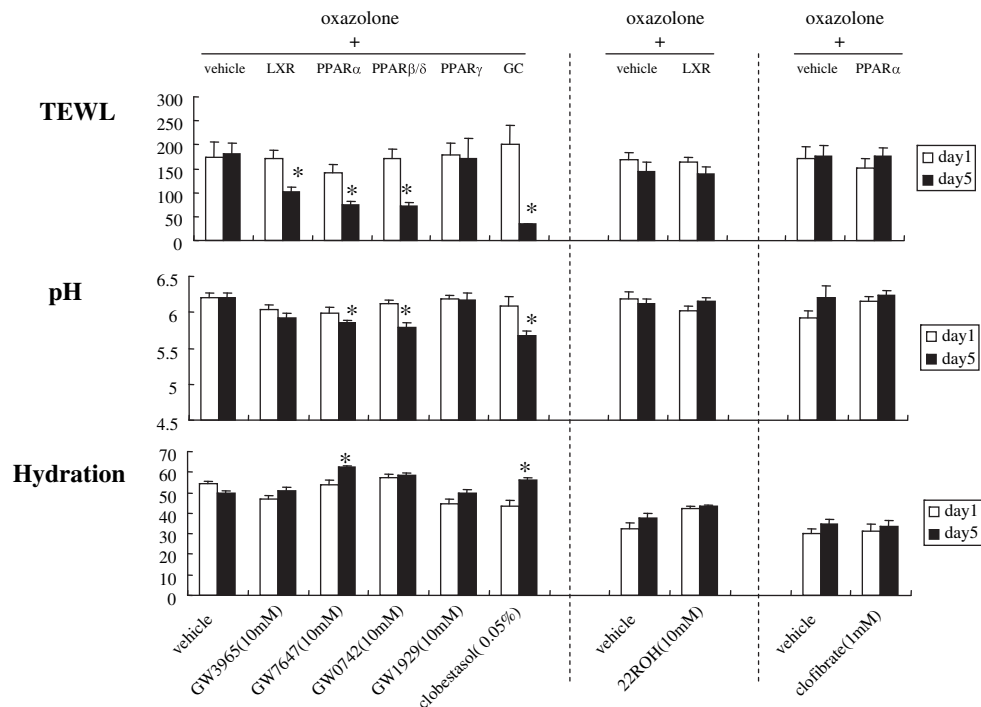


FIG E2. Liposensor activators, except PPAR γ , decrease PCNA immunostaining. Five-micrometer paraffin sections were incubated with antibody against the Ki-67 antigen, followed by incubation with a secondary antibody and ABC-peroxidase staining (see the Methods section for details). *ETOH*, Normal skin treated with ethanol alone; GC, the glucocorticoid clobetasol 0.05%; Veh, propylene glycol/ethanol (7/3 vol).



LXR: GW3965, 22ROH PPARα: GW7647, clobetasol PPARβ/δ: GW0742 PPARγ: GW1929 Clobetasol: GC

FIG E3. Functional data for additional PPAR and LXR activators (see the legend of Fig 2). This figure shows functional changes in Ox-AD mice after topical applications of several additional PPAR/LXR activators.

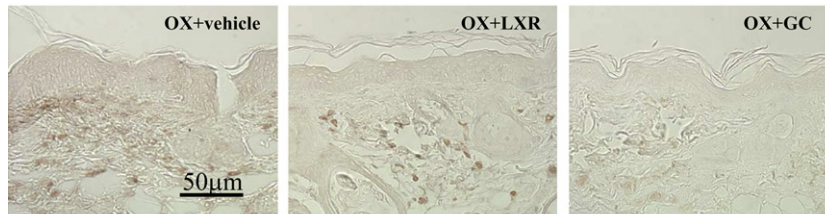
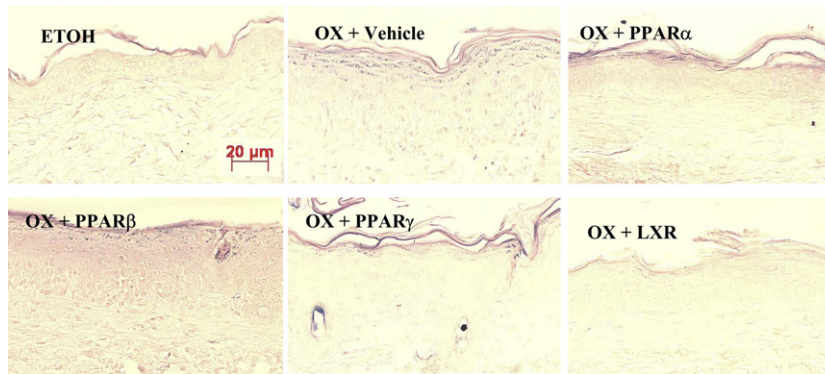
A: IL-1 α **B: TNF α** 

FIG E4. Normalization of barrier function decreases epidermal cytokine production. Samples were harvested for IL-1 α and TNF immunostaining. The LXR ligand (TO901317) decreased IL-1 α and TNF immunostaining in both the epidermis and dermis to an extent comparable with that seen after treatment with a superpotent glucocorticoid (GC), clobetasol, which served as a positive control. **A**, IL-1 α . **B**, TNF- α .

TABLE E1. Comparative effects of retinoids, vitamin D analogues, and liposensors

Receptor class	Ligands (examples)	Permeability barrier homeostasis	Epidermal proliferation	Epidermal differentiation	Inflammation	Fetal barrier development
RAR	Isotretinoin	↓	↑	↓	—	—
VDR	1,25 (OH) ₂ vitamin D3 calcipotriol	↓	↓	↑	↓	—
PPAR α	Clofibrate, WY14643	↑	↓	↑	↓	↑
PPAR β/δ	GW1514	↑	(↑)	↑	↓	↑
PPAR γ	Ciglitazone prostaglandin J2	(↑)	↓	↑	↓*	—
LXR	TO901317	↑	↓	↑	↓	↑

RAR, Retinoic acid receptor; VDR, vitamin D receptor.

↓, Decreases or worsens.

↑, Increases.

—, Comparatively little net change.

*Some anti-inflammatory effects are not receptor-dependent.