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 PPARα, PPARβ/δ, and LXR, but not activators of PPARγ, 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 PPARγ, 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-1α and TNF-α activation, despite ongoing hapten challenges.

Conclusion: These results suggest that topical applications of certain activators/ligands of PPARα, PPARβ/δ, and LXR could be useful for the treatment of AD in human subjects. (J Allergy Clin Immunol 2010;125:160-9.)

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 epidermal barrier function, resulting in downstream epidermal hyperplasia, aberrant differentiation, and T_H2-dominant reactions, the PPAR/LXR activators, in theory, possess

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Supported by National Institutes of Health grants AR19098, AG028492, and AI059311 and the Medical Research Service, Department of Veterans Affairs.

Disclosure of potential conflict of interest: T. M. Mauro received grant support from the National Institutes of Health. K. R. Feingold receives research support from Merck, the National Institutes of Health, and the Department of Veterans Affairs. The rest of the authors have declared that they have no conflict of interest.

Received for publication February 4, 2009; revised June 19, 2009; accepted for publication June 29, 2009.

Available online October 9, 2009.

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0091-6749/$36.00

doi:10.1016/j.jaci.2009.06.049

Abbreviations used
AD: Atopic dermatitis
CRTH: Chemoattractant receptor–homologous molecule expressed on T_H2
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

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 LXRα/β, PPARα, and PPARβ/δ display broad efficacy, whereas PPARγ 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 (PPARα activator), clofibrate (PPARα activator), T0901317 (LXR activator), 22(R)-hydroxycholesterol (LXR activator), clobetasol propionate, and oxazolone (Ox) were purchased from Sigma Chemical Co (St Louis, Mo). GW7647 (PPARα activator), GW0742 (PPARβ/δ activator), GW1929 (PPARγ activator), and GW3965 (LXR activator) were purchased from TOCRIS Bioscience (Ellisville, Mo). Ciglitazone (PPARγ activator) was purchased from Cayman Chemical (Ann Arbor, Mich). GW1514 (PPARβ/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-1α 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 μL of 1% Ox. After 1 week, they were treated topically with 60 μL 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 μL) 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-1α was performed as described previously.[22] Briefly, 5-μm 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-μm 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 μm × 170 μm 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 μm in each sample. The number of $PCNA^+$ cells observed within a 50-μm length of epidermis was counted in more than 10 sites for each sample. Data are reported as the means ± 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.