Immunity
Article

Leukotriene B4-Driven Neutrophil Recruitment to the Skin Is Essential for Allergic Skin Inflammation

Michiko K. Oyoshi,[1,7,8,*] Rui He,[1,7,8,9] Yitang Li,[2,7] Subhanjan Mondal,[2,7] Juhan Yoon,[1,7] Roshi Afshar,[3,7] Mei Chen,[4,7] David M. Lee,[4,7,10] Hongbo R. Luo,[2,7] Andrew D. Luster,[3,7] John S. Cho,[6] Lloyd S. Miller,[6,11] Allison Larson,[5,7] George F. Murphy,[5,7] and Raif S. Geha[1,7,*]

[1]Division of Immunology
[2]Department of Laboratory Medicine
Boston Children's Hospital, Boston, MA 02115, USA
[3]Division of Rheumatology, Allergy, and Immunology, Massachusetts General Hospital, Boston, MA 02114, USA
[4]Division of Rheumatology, Immunology, and Allergy
[5]Dermatopathology Program
Brigham and Women's Hospital, Boston, MA 02115, USA
[6]Division of Dermatology, Departments of Medicine, David Geffen School of Medicine, University of California Los Angeles, Los Angeles, CA 90095, USA
[7]Departments of Pediatrics, Pathology, and Medicine, Harvard Medical School, Boston, MA 02115, USA
[8]These authors contributed equally to this work
[9]Present address: Department of Immunology, Shanghai Medical College, Fudan University, Shanghai 200030, China
[10]Present address: Novartis Institutes for Biomedical Research, 4002 Basel, Switzerland
[11]Present address: Department of Dermatology, Johns Hopkins University School of Medicine, Baltimore, MD 21231, USA
*Correspondence: michiko.oyoshi@childrens.harvard.edu (M.K.O.), raif.geha@childrens.harvard.edu (R.S.G.)
http://dx.doi.org/10.1016/j.immuni.2012.06.018

SUMMARY

Scratching triggers skin flares in atopic dermatitis. We demonstrate that scratching of human skin and tape stripping of mouse skin cause neutrophil influx. In mice, this influx was largely dependent on the generation of leukotriene B4 (LTB4) by neutrophils and their expression of the LTB4 receptor BLT1. Allergic skin inflammation in response to epicutaneous (EC) application of ovalbumin to tape-stripped skin was severely impaired in *Ltb4r1*$^{-/-}$ mice and required expression of BLT1 on both T cells and non-T cells. Cotransfer of wild-type (WT) neutrophils, but not neutrophils deficient in BLT1 or the LTB4-synthesizing enzyme LTA4H, restored the ability of WT CD4^{+} effector T cells to transfer allergic skin inflammation to *Ltb4r1*$^{-/-}$ recipients. Pharmacologic blockade of LTB4 synthesis inhibited allergic skin inflammation elicited by cutaneous antigen challenge in previously EC-sensitized mice. Our results demonstrate that a neutrophil-T cell axis reliant on LTB4-BLT1 interaction is required for allergic skin inflammation.

INTRODUCTION

Eicosanoids are biologically active lipid mediators that are rapidly generated at sites of injury or inflammation. Leukotrienes (LTs) and prostaglandins are two classes of eicosanoids generated by the metabolism of arachidonic acid through the 5-lipoxygenase (5-LO) pathway and the cyclooxygenase pathway, respectively (Funk, 2001). 5-LO expression and LT generation are generally restricted to myeloid leukocytes, particularly neutrophils, eosinophils, monocytes, macrophages, and mast cells (Werz, 2002). LTs consist of leukotriene B4 (LTB4) and of cysteinyl leukotrienes (cys-LTs), which include LTC4, LTD4, and LTE4. The relative amounts of LTB4 and cys-LTs depend on the relative expression of the 5-LO distal enzymes LTA4 hydrolase (LTA4H) and LTC4 synthase (LTC4S), respectively. LTB4 is generated from innate immune cells such as neutrophils, macrophages, and mast cells in response to a variety of stimuli (Peters-Golden and Henderson, 2007). It primarily acts on myeloid leukocytes, causing activation of integrins, adhesion to endothelium walls, and chemotaxis (Goodarzi et al., 2003; Huang et al., 1998; Patcha et al., 2004).

Two receptors for LTB4 have been identified: BLT1 and BLT2. BLT1 is a high-affinity receptor for LTB4 predominantly expressed on neutrophils, macrophages, eosinophils, and activated T cells, with little expression, if any, on resting T cells. BLT2 exhibits low-affinity binding for LTB4 and is ubiquitously expressed (Friedrich et al., 2003). All LTB4 effects on both neutrophils and T cells are lost in *Ltb4r1*$^{-/-}$ mice (Tager et al., 2003; Tager et al., 2000). LTB4 has been implicated in the pathogenesis of allergic diseases (Ohnishi et al., 2008). Nocturnal concentrations of LTB4 and cys-LTs are elevated in the bronchoalveolar lavage (BAL) fluid of patients with nocturnal asthma (Wenzel et al., 1995). Zileuton, a specific inhibitor of 5-LO, has been shown to decrease LTB4 concentrations in BAL fluid, raising the possibility that its beneficial effect in asthma may involve decreased production of both cys-LTs and LTB4 (Wenzel et al., 1995). Studies on *Ltb4r1*$^{-/-}$ mice have suggested an important role of the LTB4-BLT1 pathway in the recruitment of effector CD4^{+} and CD8^{+} T cells to antigen-challenged airways

in a classical mouse model of asthma (Miyahara et al., 2005; Tager et al., 2003; Terawaki et al., 2005).

Atopic dermatitis (AD) is an inflammatory skin disease characterized by itching and scratching, dermal infiltration with T helper 2 (Th2) cells, and a systemic Th2 cell-mediated response, with elevated serum immunoglobulin E (IgE) and eosinophilia. Neutrophils from AD patients have elevated LTA4H activity (Okano-Mitani et al., 1996) and release more LTB4 in response to several stimuli (Hilger et al., 1991). LTB4 concentrations are elevated in the lesional skin of patients with AD (Fogh et al., 1989; Koro et al., 1999; Ruzicka et al., 1986; Thorsen et al., 1990). However, little is known about the role of LTB4 in AD. To this end, we have developed a mouse model of allergic skin inflammation with many similarities to AD. In this model, repeated epicutaneous (EC) sensitization of tape-stripped skin with ovalbumin (OVA) results in a Th2 cell-dominated systemic immune response characterized by dermal infiltration of $CD4^+$ T cells and eosinophils and increased local expression of Th2 cell-derived cytokines (Spergel et al., 1998; Spergel et al., 1999). We have used this model to examine the potential role of LTB4 in allergic skin inflammation. Our findings indicate that LTB4-mediated attraction of neutrophils to skin subjected to tape stripping, a surrogate for scratching, is essential for the development of allergic inflammation at cutaneous sites of antigen introduction.

RESULTS

Scratching and Tape Stripping Increase Neutrophils and Neutrophil-Derived LTB4 and BLT1 in the Skin

Mechanical injury to the skin by scratching triggers flares in patients with AD (Fleischer and Boguniewicz, 2010). We examined whether scratching triggers a cellular infiltration in the skin. There were virtually no detectable neutrophils in the dermis of unscratched skin. In contrast, neutrophils, which were easily differentiated from other inflammatory cells by their characteristic multilobed nuclei, accumulated in the dermis of scratched skin (Figures 1A and 1B). There was also an increase in the epidermal thickness of one cell layer in scratched-skin sites (Figure 1A). LTB4 is a potent chemoattractant for neutrophils, which express the LTB4 receptor BLT1 (Peters-Golden and Henderson, 2007). Levels of LTB4 and BLT1 messenger RNA (mRNA) were significantly elevated in scratched skin compared to control unscratched skin (Figures 1C and 1D).

We examined whether tape stripping, a surrogate for scratching, causes neutrophil accumulation in mouse skin. Shaved dorsal skin of C57BL/6 mice was tape-stripped six times with Tegaderm and examined 24 hr later. Histologic examination revealed that there were virtually no detectable neutrophils in the dermis of shaved, non-tape-stripped skin. In contrast, neutrophils were abundant in the dermis of tape-stripped skin (Figures 1E and 1F). There was an increase in epidermal and dermal thickness in tape-stripped skin sites compared to non-tape-stripped skin (27.24 ± 1.4 μm versus 8.04 ± 0.4 μm and 252.9 ± 11.4 μm versus 154.9 ± 11.9 μm, respectively; $n = 4$, $p < 0.001$ each). The amounts of LTB4 and BLT1 mRNA were significantly elevated in tape-stripped skin compared to non-tape-stripped skin (Figures 1G and 1H). An influx of neutrophils was also observed in ear skin 24 hr after tape stripping six times with Tegaderm, as determined by flow cytometry analysis of cell suspensions from trypsin-digested skin for the presence of $CD11b^+Gr\text{-}1^+$ cells (Figure 1I).

Neutrophils, but Not Mast Cells, Are the Source of Elevated LTB4 in Tape-Stripped Mouse Skin

Neutrophil influx, LTB4 accumulation, and expression of BLT1 mRNA in tape-stripped skin were comparable in mast cell-deficient WBB6F1/*J-Kit*^{W}/*Kit*$^{W\text{-}v}$ (*Kit*^{W}/*Kit*$^{W\text{-}v}$) mice and wild-type (WT) WBB6F1 controls (Figures 2A–2C). Neutrophil depletion in C57BL/6 mice by Gr-1 monoclonal antibody (mAb) resulted in the loss of >99% of the $CD11b^+Gr\text{-}1^+$ cells from the blood (Figure S1A available online) and strongly impaired the accumulation of neutrophils, LTB4, and BLT1 mRNA in tape-stripped ear skin, compared to treatment with isotype-control antibody (Figures 2D–2F). The role of neutrophils in the accumulation of LTB4 and BLT1 mRNA in tape-stripped skin was confirmed in mice depleted of neutrophils by treatment with cyclophosphamide (CTX) (Figures 2G–2I), which resulted in a >98% depletion of circulating blood neutrophils (Figure S1B). These results suggest that the accumulation of LTB4 and BLT1 mRNA in tape-stripped mouse skin is largely dependent on neutrophils.

Neutrophil Influx in Tape-Stripped Mouse Skin Is Largely Dependent on BLT1 and Neutrophil Production of LTB4

Kinetic analysis revealed that neutrophil accumulation in tape-stripped ear skin of WT mice peaked at 24 hr and waned by 72 hr (Figure 3A), as recently reported (Gregorio et al., 2010; Guiducci et al., 2010). There was markedly less accumulation of neutrophils in tape-stripped ear skin of *Ltb4r1*$^{-/-}$ mice compared to WT controls at all time points (Figure 3A). The residual accumulation of neutrophils in *Ltb4r1*$^{-/-}$ mice may be due to robust induction by tape stripping of mRNA for neutrophil chemoattracting chemokines, such as CXCL2, interleukin-1β (IL-1β), and IL-6; both the rapid induction (within 6 hr) of these cytokines and neutrophil influx into tape-stripped skin were blunted in mice lacking myeloid differentiation primary response gene 88 (*Myd88*) (Figures 3B and 3C). These results indicate that neutrophil influx in tape-stripped mouse skin is largely dependent on BLT1.

To examine whether BLT1 expression by neutrophils is important for their accumulation in tape-stripped skin, we compared the ability of adoptively transferred neutrophils purified from bone marrow of $CD45.2^+$ WT and *Ltb4r1*$^{-/-}$ mice to accumulate in tape-stripped ears of $CD45.1^+$ WT recipients depleted of neutrophils by CTX. Because of the short half-life of neutrophils, the recipients' ears were tape-stripped 20 hr before neutrophil transfer to allow for expression of inflammatory cytokines in injured skin. Adoptively transferred WT neutrophils, but not *Ltb4r1*$^{-/-}$ neutrophils, accumulated in recipient ears 4 hr and, to a lesser extent, 24 hr post transfer (Figure 3D). The rise in blood neutrophil count and the percentages of $CD45.2^+$ donor neutrophils in the spleens 4 hr post transfer were comparable in recipients of WT and *Ltb4r1*$^{-/-}$ neutrophils (data not shown), indicating that the general circulation of *Ltb4r1*$^{-/-}$ neutrophils was not impaired.

Neutrophil accumulation in ear skin 24 hr after tape stripping was severely diminished in *Lta4h*$^{-/-}$ mice compared to WT controls (Figure 3E) and in WT mice treated with the LTA4H inhibitor bestatin (Figure 3F). Because most of the LTB4 that accumulated in tape-stripped skin was derived from neutrophils, we examined

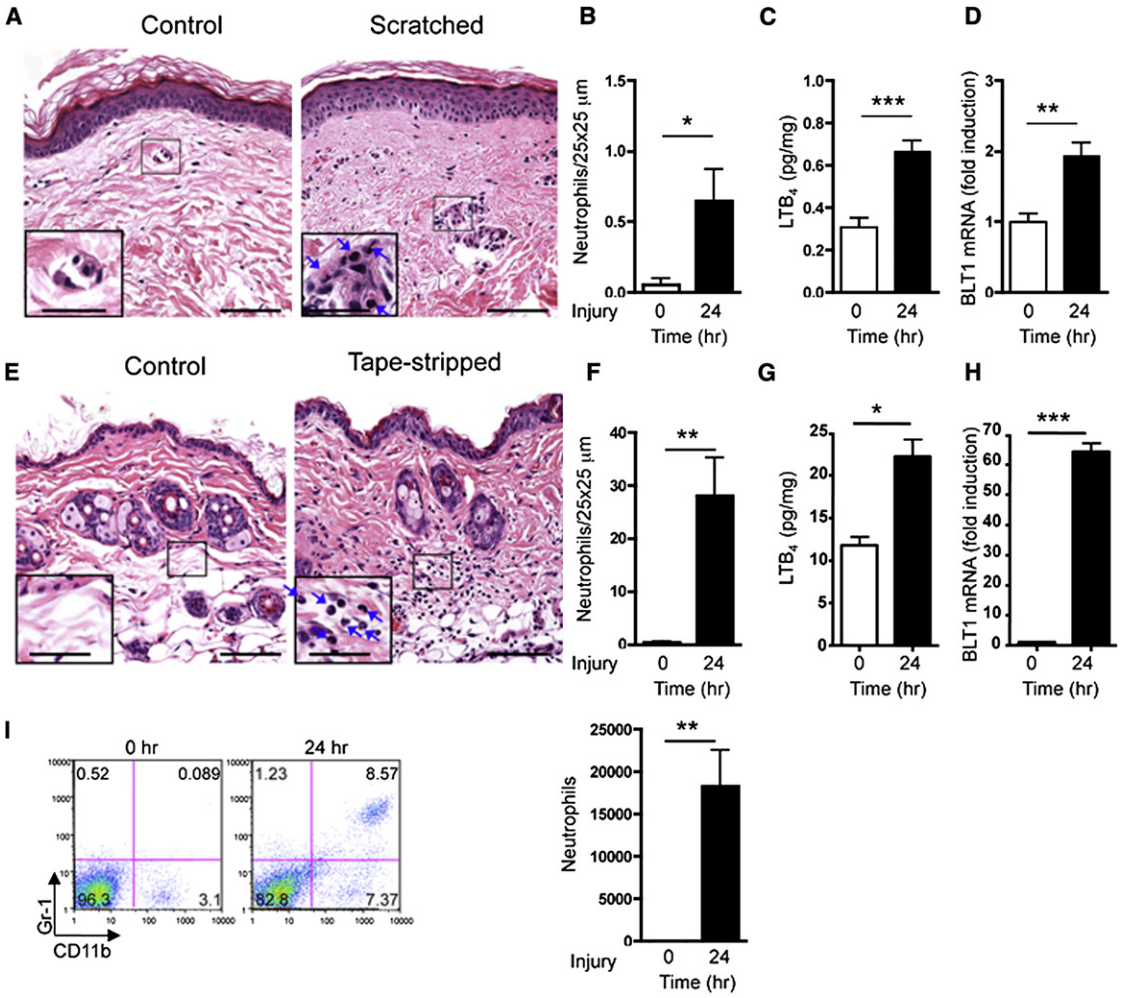

Figure 1. Mechanical Skin Injury Results in the Accumulation of Neutrophils, LTB4, and BLT1 mRNA in Human and Mouse Skin

(A–D) Representative photomicrographs (A), quantification of neutrophils (B), LTB4 concentrations (pg/mg wet skin tissue) (C), and BLT1 mRNA expression (D) in scratched and unscratched human skin (n = 2). Arrows indicate neutrophils. Magnification, 200X; inset, 400X. Scale bars represent 70 μm; inset, 35 μm. BLT1 mRNA levels are expressed as fold induction relative to unmanipulated skin.

(E–H) Representative photomicrographs (E), quantification of neutrophils (F), LTB4 concentrations (pg/mg wet skin tissue) (G), and BLT1 mRNA expression (H) in tape-stripped and unmanipulated back skin of C57BL/6 mice (n = 4).

(I) Representative flow cytometry analysis and quantification of $CD11b^{+}Gr\text{-}1^{+}$ cells in tape-stripped and unmanipulated ear skin of C57BL/6 mice (representative of four mice).

Columns and error bars represent mean and SEM, respectively. *p < 0.05, **p < 0.01, ***p < 0.001.

whether neutrophil-derived LTB4 was important for the optimal accumulation of neutrophils in tape-stripped skin. There was minimal influx of adoptively transferred $CD45.2^{+}$ *Lta4h*$^{-/-}$ neutrophils, which cannot synthesize LTB4, into tape-stripped ears of CTX-treated $CD45.1^{+}$ WT recipients, compared to $CD45.2^{+}$ WT neutrophils (Figure 3G). The percentages of $CD45.2^{+}$ donor neutrophils in the spleens were comparable in recipients of *Lta4h*$^{-/-}$ and WT neutrophils (data not shown).

Neutrophil-derived LTB4 may act in an autocrine and/or paracrine manner to promote neutrophil recruitment to tape-stripped skin. To address this issue, we performed cotransfer experiments in which *Lta4h*$^{-/-}$, *Ltb4r1*$^{-/-}$, or WT neutrophils loaded with CMTMR (5-(and-6)-(((4-chloromethyl) benzoyl) amino) tetramethylrhodamine) dye (red) were mixed with equal numbers of WT neutrophils loaded with CMFDA (5-chloromethylfluorescein diacetate) dye (green) and adoptively transferred into CTX-treated WT recipients. Four hours later, ear cells were analyzed, and the homing index of *Lta4h*$^{-/-}$ and *Ltb4r1*$^{-/-}$ neutrophils was calculated relative to that of WT neutrophils, set at 1. Coadministration of WT neutrophils partially, but not completely, restored the influx of *Lta4h*$^{-/-}$ neutrophils to tape-stripped skin (Figure 3H), indicating both paracrine and autocrine roles of neutrophil-derived LTB4 in the recruitment of neutrophils to mechanically injured skin. *Ltb4r1*$^{-/-}$ neutrophils cotransferred with WT neutrophils accumulated significantly less than *Lta4h*$^{-/-}$ neutrophils cotransferred with WT neutrophils (Figure 3H), supporting a paracrine component to the action of neutrophil-derived LTB4.

The role of LTB4 in neutrophil recruitment to the skin was not limited to mechanical injury induced by tape stripping. Treatment

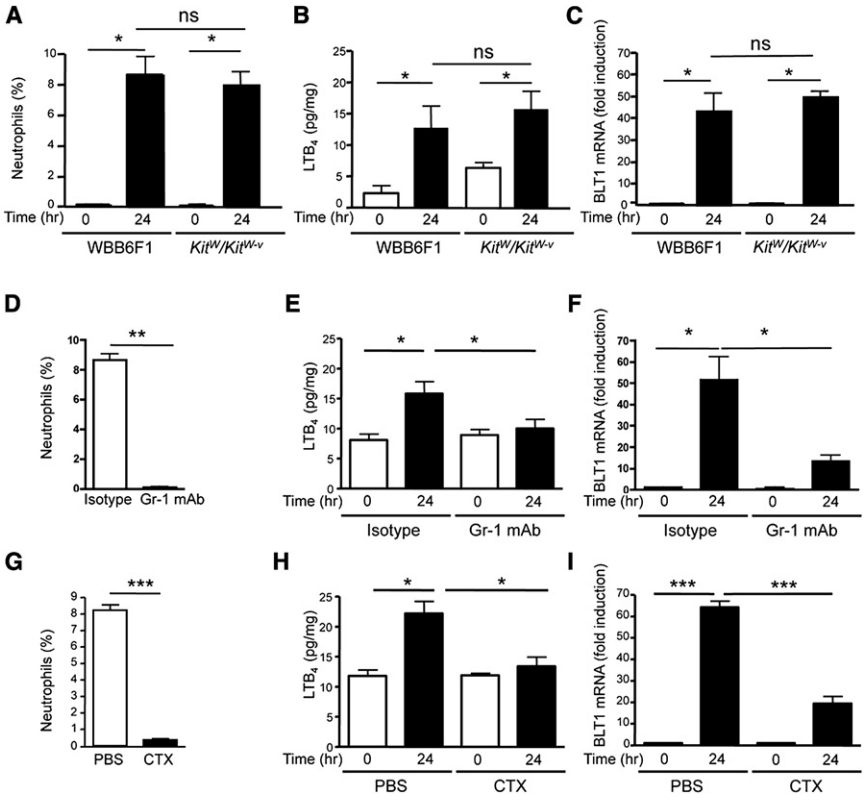

Figure 2. Neutrophils, but Not Mast Cells, Are the Source of Increased LTB4 in Tape-Stripped Skin

(A–I) $CD11b^+Gr\text{-}1^+$ neutrophils (% of total ear cells) (A, D, and G), LTB4 concentrations (pg/mg wet skin tissue) (B, E, anf H), and BLT1 mRNA expression (C, F, and I) in tape-stripped and unmanipulated ear or back skin of $Kit^W/Kit^{W\text{-}v}$ mice and WBB6F1 controls (A–C), of tape-stripped C57BL/6 mice treated with Gr-1 mAb or isotype control (D–F), and of tape-stripped C57BL/6 mice treated with CTX or vehicle (G–I). Ear skin was used for (A), (D), and (G). n = 3 per group for (A)–(I). BLT1 mRNA levels are expressed as fold induction relative to unmanipulated skin. Columns and error bars represent mean and SEM, respectively. $^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$. ns, not significant. See also Figure S1.

of mice with bestatin resulted in a significant reduction in neutrophil recruitment to sites of cutaneous infection with *S. aureus* (Figure S2A) and in increased bacterial counts and development of larger lesions at these sites (Figures S2B and S2C). Bestatin treatment also significantly inhibited the recruitment of neutrophils in response to sterile wounding of the skin (Figure S2D) and resulted in significantly larger skin lesions on day 1 post wounding (Figure S2E).

BLT1 Expression by Both T Cells and Non-T Cells Is Essential for Allergic Skin Inflammation

BLT1 expression on T cells has been shown to be important in a mouse model of antigen-driven allergic airway inflammation (Tager et al., 2003). Consistent with this finding, $Ltb4r1^{-/-}$ mice failed to develop allergic skin inflammation following EC sensitization with OVA, as evidenced by their failure to increase dermal infiltration by $CD4^+$ T cells and eosinophils and upregulate IL-4 and IL-13 mRNA expression in OVA-sensitized skin (Figures S3A and S3B). Impaired skin inflammation in $Ltb4r1^{-/-}$ mice was not due to failure to mount a systemic immune response to EC sensitization, given that their OVA-specific antibody concentrations and cytokine secretion by OVA-stimulated splenocytes were comparable to those of WT controls (Figures S3C and S3D).

To investigate the role of BLT1 expression on T and non-T cells in allergic skin inflammation, $CD4^+$ donor T cells from EC-sensitized WT and $Ltb4r1^{-/-}$ mice were examined for their capacity to transfer allergic skin inflammation to WT and $Ltb4r1^{-/-}$ recipients. OVA-stimulated $CD4^+$ splenocytes from EC-sensitized mice were adoptively transferred by intravenous (i.v.) injection to naive recipients, which were challenged with OVA on shaved and tape-stripped back skin on days 0 and 3 after transfer and examined on day 7. As previously reported in BALB/c mice (He et al., 2008), $CD4^+$ T cells from C57BL/6 WT donors EC-sensitized with OVA transferred allergic skin inflammation to WT recipients, as evidenced by significant increases in epidermal and dermal thickness, number of $CD4^+$ T cells, and IL-4 and IL-13 mRNA expression at sites of OVA challenge compared to sites of saline challenge (Figure 4 and data not shown). $CD4^+$ T cells from $Ltb4r1^{-/-}$ donors EC-sensitized with OVA failed to transfer allergic skin inflammation to WT recipients. Unexpectedly, $CD4^+$ T cells from WT donors EC-sensitized with OVA failed to transfer allergic skin inflammation to $Ltb4r1^{-/-}$ recipients (Figures 4A–4D), indicating a critical role for BLT1 expression on non-T cells in our model. As expected, $CD4^+$ T cells from $Ltb4r1^{-/-}$ donors EC-sensitized with OVA failed to transfer allergic skin inflammation to $Ltb4r1^{-/-}$ recipients. Recipients of $CD4^+$ T cells from OVA-sensitized donors failed to develop allergic skin inflammation in response to skin challenge with saline (data not shown). Transfer of $CD4^+$ T cells from saline-sensitized mice failed to elicit detectable skin inflammation in OVA-challenged skin of recipients (data not shown).

Expression of BLT1 by Neutrophils Is Important for the Development of Allergic Skin Inflammation

To determine whether BLT1 expression on neutrophils is important for the development of allergic skin inflammation, we compared WT and $Ltb4r1^{-/-}$ neutrophils for their capacity to rescue the transfer of allergic skin inflammation by OVA-activated $CD4^+$ effector T cells from EC-sensitized WT mice to $Ltb4r1^{-/-}$ recipients. Neutrophils were administered i.v. (10^7 per mouse) at days 0 and 3 of the 1-week-long epicutaneous challenge with OVA (Figure 5A). Administration of WT neutrophils rescued the ability of WT $CD4^+$ T cells to transfer allergic skin inflammation to $Ltb4r1^{-/-}$ recipients. This was evidenced by significant increases in epidermal and dermal thickness,

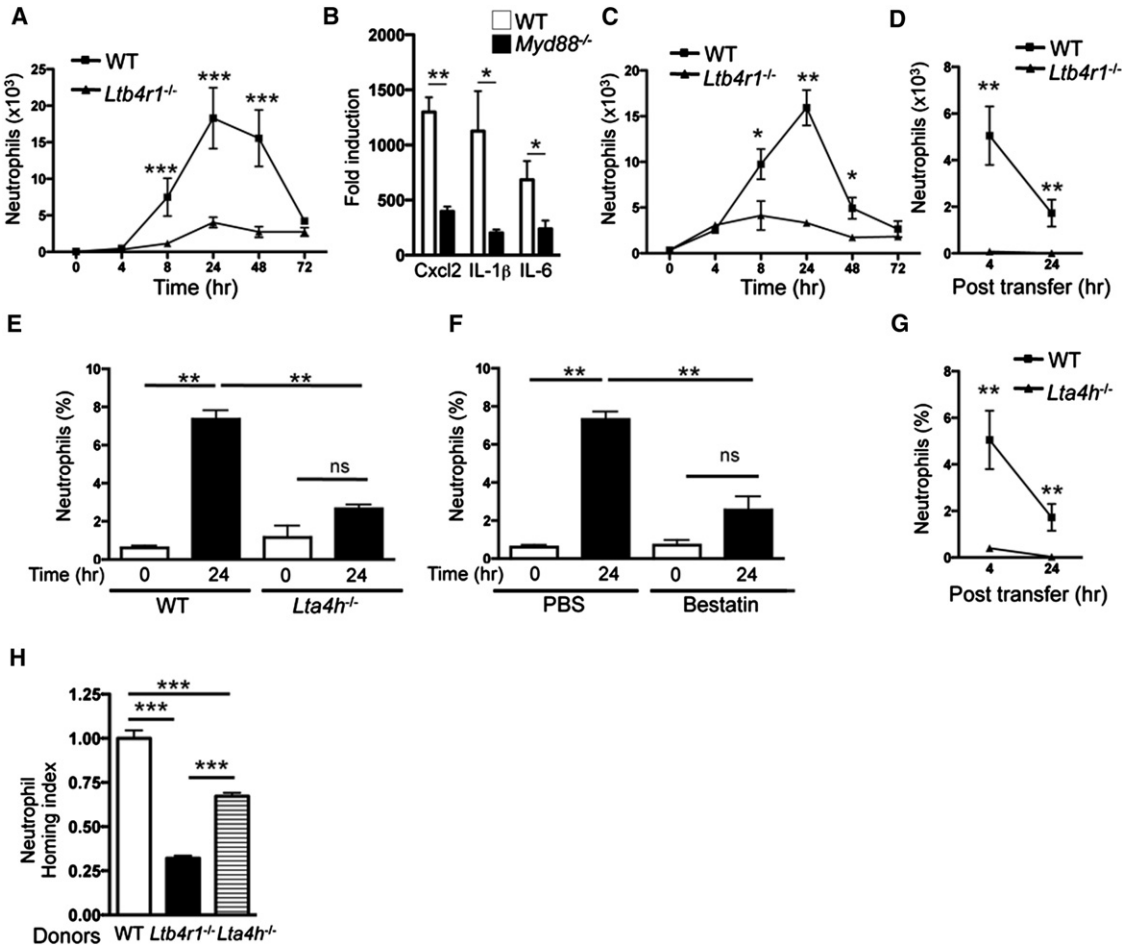

Figure 3. Neutrophils' Accumulation in Tape-Stripped Skin Is Dependent on Their Expression of BLT1 and LTA4H

(A) Number of neutrophils in WT and *Ltb4r1*$^{-/-}$ ear skin at different time points (hr) after tape stripping.

(B) Induction of CXCL2, IL-1β, and IL-6 mRNA expression 6 hr after tape stripping in the skin of WT and *Myd88*$^{-/-}$ mice on BALB/c background. mRNA levels are expressed as fold induction relative to unstripped WT skin (0 hr) (n = 4 per group).

(C) Percentages of neutrophils (% total ear cells) in ear skin of *Myd88*$^{-/-}$ mice and WT controls at different time points after tape stripping (n = 5 per group).

(D) Percentage of neutrophils (% total ear cells) from WT and *Ltb4r1*$^{-/-}$ donors in tape-stripped ears of CTX-treated WT recipients (n = 4 per group).

(E and F) Percentage of neutrophils (% total ear cells) in WT and *Lta4h*$^{-/-}$ ear skin 24 hr after tape stripping (E) and in WT mice treated with bestatin or vehicle control prior to tape stripping (F).

(G) Accumulation of neutrophils (% total ear cells) from WT and *Lta4h*$^{-/-}$ donors in tape-stripped ears of CTX-treated WT recipients (n = 4 per group).

(H) Homing index of *Ltb4r1*$^{-/-}$ and *Lta4h*$^{-/-}$ neutrophils in tape-stripped ear skin of CTX-treated WT recipients (n = 4 per group).

Experiments in (D) and (G) were performed together and thus share the same WT controls. Columns and error bars represent mean and SEM, respectively (n = 4 mice per group). *p < 0.05, **p < 0.01, ***p < 0.001. ns, not significant. See also Figure S2.

numbers of CD4$^+$ T cells in the dermis, and expression of IL-4 and IL-13 mRNA, compared to the skin of mice that received only WT CD4$^+$ T cells (Figures 5A–5D). In contrast, administration of *Ltb4r1*$^{-/-}$ neutrophils failed to rescue the ability of WT CD4$^+$ T cells to transfer allergic skin inflammation to *Ltb4r1*$^{-/-}$ recipients. These results demonstrate that BLT1 expression by neutrophils is critical for the development of allergic skin inflammation.

Accumulation of Effector T Cells to Antigen-Challenged Skin Requires BLT1 Expression by Neutrophils

The failure of *Ltb4r1*$^{-/-}$ neutrophils to rescue the ability of WT CD4$^+$ T cells to transfer allergic skin inflammation to *Ltb4r1*$^{-/-}$ recipients prompted us to directly investigate the role of BLT1 expression by neutrophils in the accumulation of antigen-specific effector CD4$^+$ T cells into cutaneous sites of antigen challenge. We examined the ability of CD4$^+$ OT-II T cells, activated with OVA peptide and antigen presenting cells (APCs) for 4 days in vitro, to accumulate into the tape-stripped ear skin of recipients following challenge with OVA and cholera toxin (CT) or, as a control, CT alone. Vα2$^+$Vβ5$^+$ WT OT-II effector T cells accumulated significantly more in antigen-challenged skin than in control-challenged ear skin of WT recipients (Figure 5E). In contrast, WT OT-II effector T cells failed to accumulate in antigen-challenged skin of *Ltb4r1*$^{-/-}$ recipients (Figure 5E), indicating that BLT1 expression by non-T cells is important for the

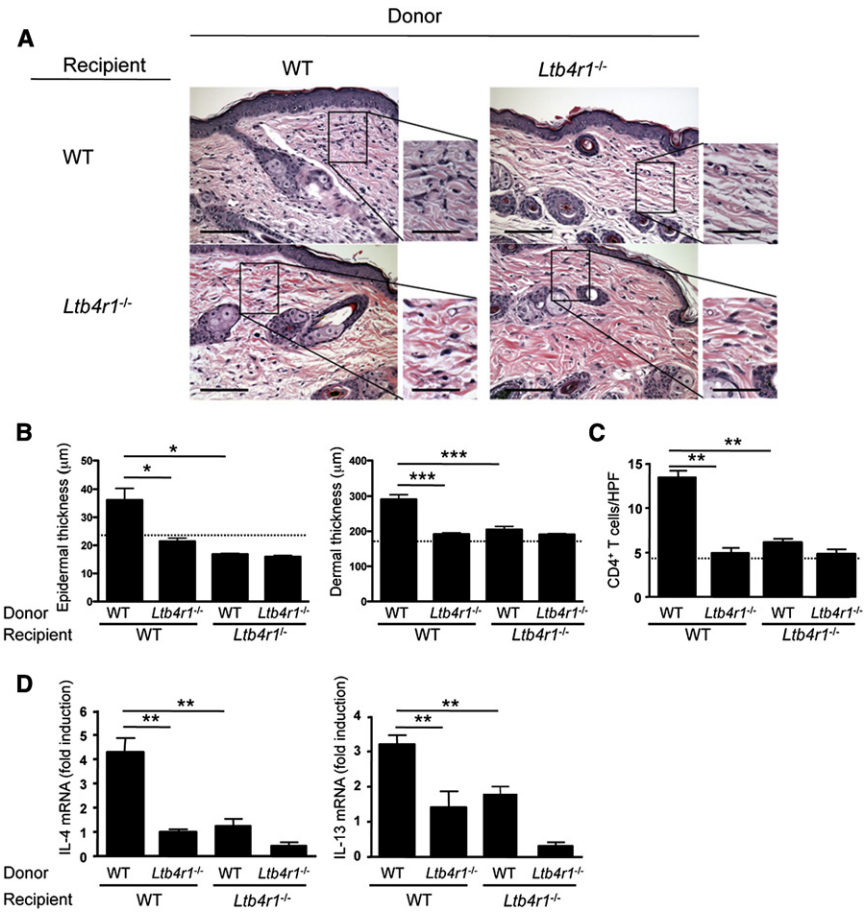

Figure 4. BLT1 Expression by Both T Cells and Non-T Cells Is Essential for Allergic Skin Inflammation

(A) Representative photomicrographs of H&E-stained sections from OVA-challenged skin of WT and *Ltb4r1*$^{-/-}$ recipients of CD4$^+$ T cells from WT or *Ltb4r1*$^{-/-}$ mice EC-sensitized with OVA. n = 5 for each of the four groups. Magnification, 200X; inset, 400X. Scale bars represent 100 μm; inset, 50 μm.

(B and C) Epidermal and dermal thickness (B), and numbers of infiltrating CD4$^+$ T cells (C). The dotted line in (B) and (C) represents the mean value for OVA-challenged skin of recipients of CD4$^+$ T cells from saline-sensitized WT donors.

(D) mRNA levels for IL-4 and IL-13 expressed as fold induction relative to OVA-challenged skin of recipients of CD4$^+$ T cells from saline-sensitized WT donors (n = 5).

Columns and error bars represent mean and SEM, respectively. *p < 0.05, **p < 0.01, ***p < 0.001. See also Figure S3.

homing of CD4$^+$ effector T cells to the skin. *Ltb4r1*$^{-/-}$ OT-II effector T cells failed to accumulate in antigen-challenged skin of WT recipients (Figure S4A), despite normal proliferation to OVA (data not shown) and normal expression of the skin homing receptor CCR4 and memory marker CD44 (Figures S4B and S4C), confirming the role of BLT1 expression in T cells' homing to the skin.

Coadministration of WT neutrophils i.v. significantly increased the accumulation of adoptively transferred WT OT-II effector T cells into antigen-challenged skin of WT recipients (Figure 5F). More importantly, it completely rescued the ability of WT OT-II effector T cells to accumulate in antigen-challenged skin of *Ltb4r1*$^{-/-}$ recipients (Figure 5F). These results demonstrate that BLT1 expression by neutrophils is critical for the accumulation of CD4$^+$ effector T cells into cutaneous sites of antigen challenge and for allergic skin inflammation.

Neutrophil-Derived LTB4 Is Important for the Development of Allergic Skin Inflammation

We next investigated whether LTB4 production by skin-infiltrating neutrophils is important for the development of allergic skin inflammation. To this purpose, we examined whether *Lta4h*$^{-/-}$ neutrophils rescued the ability of OVA-activated CD4$^+$ effector T cells from EC-sensitized WT mice to transfer allergic skin inflammation to *Ltb4r1*$^{-/-}$ recipients. Because *Lta4h*$^{-/-}$ neutrophils failed to migrate to tape-stripped skin, neutrophils from *Lta4h*$^{-/-}$ mice and WT controls were injected intradermally (i.d.) on days 0 and 3 at the site of EC challenge. Intradermal administration of WT neutrophils rescued the ability of WT CD4$^+$ T cells to transfer allergic skin inflammation to *Ltb4r1*$^{-/-}$ recipients, evidenced by significant increases in epidermal and dermal thickness, CD4$^+$ T cell infiltration in the dermis, and expression of IL-4 and IL-13 mRNA, compared to antigen-challenged skin of mice that received only WT CD4$^+$ T cells (Figures 6A–6D). In contrast, i.d. injection of *Lta4h*$^{-/-}$ neutrophils into *Ltb4r1*$^{-/-}$ recipients failed to rescue the ability of WT CD4$^+$ T cells to transfer allergic skin inflammation to *Ltb4r1*$^{-/-}$ recipients. In addition, i.d. administration of *Ltb4r1*$^{-/-}$ neutrophils, which had an intact ability to produce LTB4, caused an increase in the accumulation of OT-II cells in the EC-challenged ear skin of *Ltb4r1*$^{-/-}$ recipients, and an increase in ear scratching, comparable to those caused by i.d. administration of WT neutrophils (Figures 6E and 6F).

LTB4 induces an itch-associated scratching response in mice (Kim et al., 2007). Tape stripping induced significantly more scratching in WT mice than in *Ltb4r1*$^{-/-}$ and *Lta4h*$^{-/-}$ mice, suggesting that LTB4 is important in the scratching response to mechanical injury (Figure S5A). Mice EC-sensitized with OVA exhibited increased scratching (Figure S5B), and LTB4 concentrations were elevated in OVA-sensitized mouse skin sites (Figure S5C), as in AD skin lesions. These results suggest that generation of LTB4 by infiltrating neutrophils is critical for their ability to promote T cell-mediated allergic skin inflammation.

Pharmacologic Blockade of LTB4 Synthesis Inhibits Acute Skin Flares in Previously EC-Sensitized Mice

Cutaneous exposure to antigens provokes skin flares in AD patients (Nosbaum et al., 2010; Sicherer and Leung, 2007). Therefore, we next investigated whether administration of

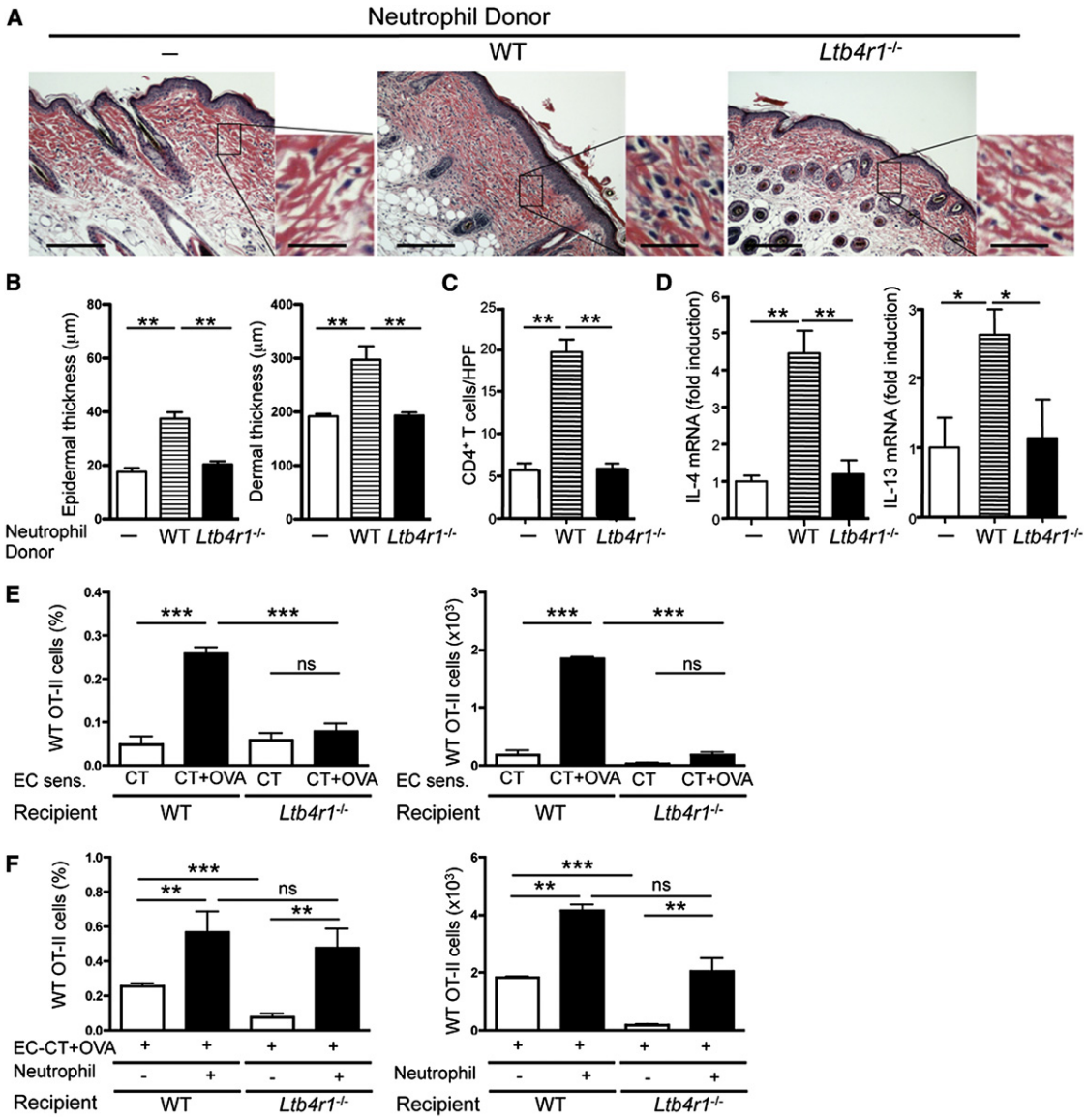

Figure 5. WT Neutrophils, but Not *Ltb4r1*$^{-/-}$ Neutrophils, Restore Allergic Skin inflammation in *Ltb4r1*$^{-/-}$ Recipients

(A) Representative photomicrographs of H&E-stained sections from OVA-challenged skin of *Ltb4r1*$^{-/-}$ recipients of CD4$^+$ T cells from OVA-sensitized WT mice transferred alone (n = 3) or with neutrophils from WT (n = 5) or *Ltb4r1*$^{-/-}$ donors (n = 4). Magnification, 100X; inset, 400X. Scale bars represent 200 μm; inset, 50 μm.

(B–D) Epidermal and dermal thickness (B), number of infiltrating CD4$^+$ T cells (C), and mRNA levels of IL-4 and IL-13 expressed as fold induction relative to recipients of CD4$^+$ T cells from OVA-sensitized WT mice transferred without neutrophils (D).

(E) Percentage (% total ear cells) and number of Vα2$^+$Vβ5$^+$ transgenic OT-II CD4$^+$ T cells from WT donors accumulating in OVA-challenged ear skin of WT and *Ltb4r1*$^{-/-}$ recipients.

(F) Effect of coadministration of WT neutrophils on the percentage (% total ear cells) and number of Vα2$^+$Vβ5$^+$ transgenic OT-II CD4$^+$ T cells from WT donors which accumulate in OVA-challenged ear skin of WT and *Ltb4r1*$^{-/-}$ recipients.

Columns and error bars represent mean and SEM, respectively (n = 3–5 mice per group). *p < 0.05, **p < 0.01, ***p < 0.001. ns, not significant. See also Figure S4.

bestatin to EC-sensitized mice prevented the development of allergic skin inflammation following cutaneous antigen challenge. C57BL/6 mice were EC-sensitized with OVA for 7 weeks, then immediately challenged by application of OVA to a previously unsensitized shaved and tape-stripped skin site on the back. Mice were treated with intraperitoneal (i.p.) injection with bestatin (0.4 mg/day) or vehicle control daily for 7 days, beginning 2 days before the 7-day challenge (Figure 7A). Mice treated with vehicle alone developed allergic skin inflammation in response to cutaneous OVA challenge, as evidenced by significantly increased epidermal and dermal thickness, dermal infiltration with CD4$^+$ T cells, and expression of Th2 cell-derived cytokines, compared to challenge with saline (Figures 7B–7E). In contrast, mice treated with bestatin failed to develop allergic skin

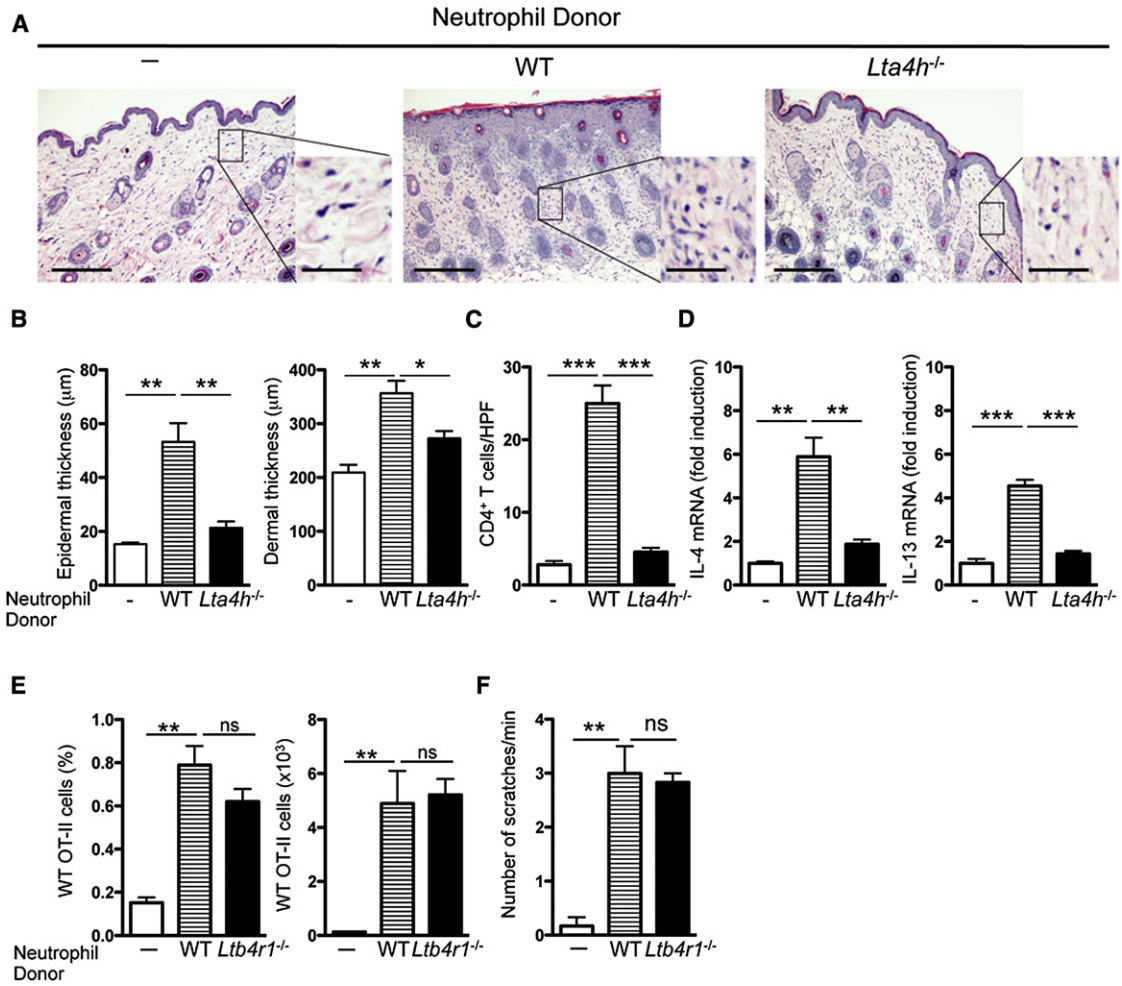

Figure 6. Intradermal Injection of *Lta4h*$^{-/-}$ Neutrophils Fails to Restore Allergic Skin Inflammation in *Ltb4r1*$^{-/-}$ Recipients

(A) Representative photomicrographs of H&E-stained sections from OVA-challenged skin of *Ltb4r1*$^{-/-}$ recipients of CD4$^+$ T cells from OVA-sensitized WT mice transferred alone or with neutrophils from WT or *Lta4h*$^{-/-}$ donors (n = 3 per group). Magnification, 100X; inset, 400X. Scale bars represent 200 μm; inset, 50 μm.

(B–D) Epidermal and dermal thickness (B), number of infiltrating CD4$^+$ T cells (C), and mRNA levels of IL-4 and IL-13 expressed as fold induction relative to recipients of CD4$^+$ T cells from OVA-sensitized WT mice transferred without neutrophils (D).

(E and F) Effect of i.d. injection of WT and *Ltb4r1*$^{-/-}$ neutrophils on the homing of WT OT-II cells to OVA-challenged skin of *Ltb4r1*$^{-/-}$ recipients (E) and on ear scratching by the recipients (F).

Columns and error bars represent mean and SEM, respectively. n = 3–4 mice per group. *p < 0.05, **p < 0.01, ***p < 0.001. See also Figure S5.

inflammation in response to cutaneous OVA challenge. These results suggest that inhibition of LTB4 production prevents allergic skin inflammation driven by cutaneous antigen exposure in previously sensitized mice.

DISCUSSION

In this study, we have identified a critical role for neutrophils in allergic skin inflammation in a mouse model with features of AD. We demonstrated that LTB4 production by neutrophils was essential for their accumulation in mechanically injured skin, for the recruitment of effector CD4$^+$ T cells to cutaneous sites of antigen exposure, and for the development of allergic skin inflammation.

Mechanical skin injury by scratching in humans and its surrogate, tape stripping in mice, caused robust infiltration of neutrophils and accumulation of LTB4 and mRNA for its receptor, BLT1, in the dermis. The slight increase in epidermal thickness in injured human and mouse skin is possibly due to the release of cytokines that promote keratinocyte proliferation, which include IL-6 (Werner et al., 2007). Accumulation of neutrophils and BLT1 mRNA was substantially greater in tape-stripped mouse skin than in scratched human skin, possibly because mouse skin is much thinner than human skin and might be more susceptible to mechanical skin injury. The relatively comparable elevation of LTB4 concentrations in tape-stripped mouse skin and scratched human skin suggests a more rapid washout of LTB4 from injured mouse skin. The baseline concentration of LTB4 may reflect a background signal intrinsic to the assay used and/or be derived from resident cells in the skin and may have not been biologically active, given that neutrophils were not detected in unmanipulated skin. Studies in *Lta4h*$^{-/-}$

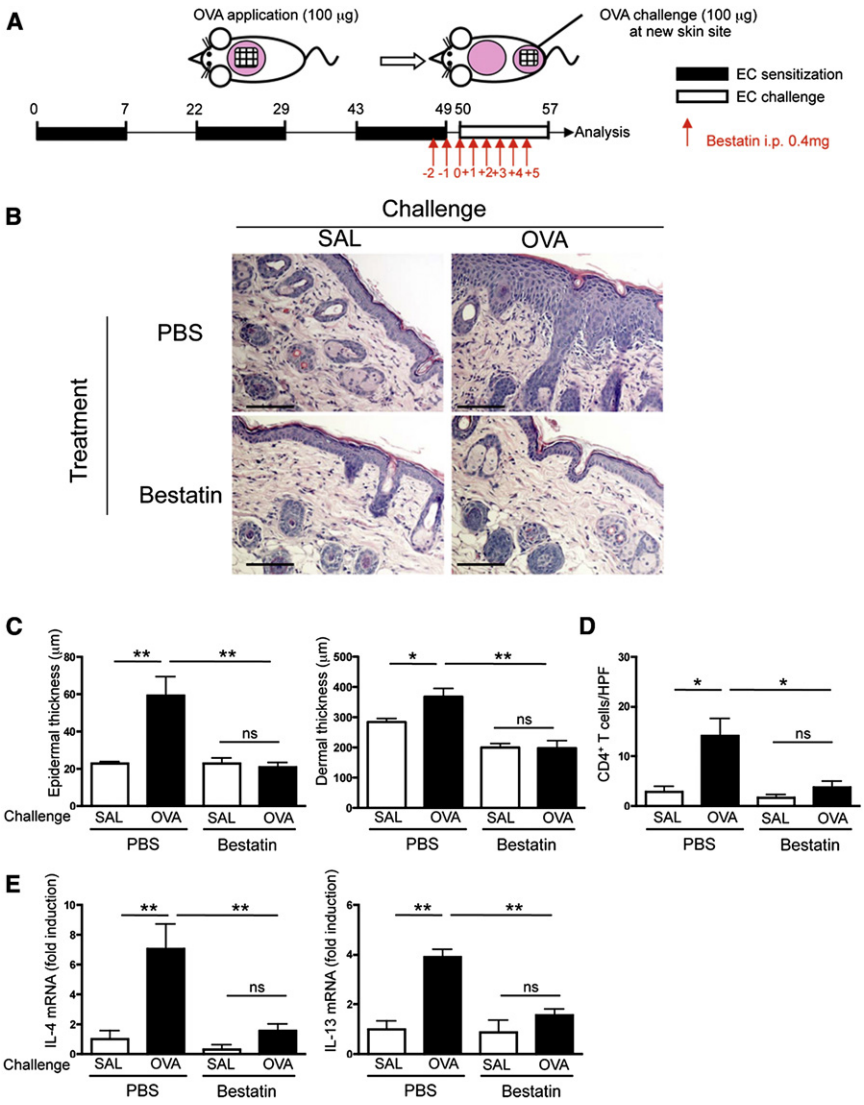

Figure 7. Pharmacologic Blockade of LTB4 Synthesis Inhibits Acute Skin Flares in Previously EC-Sensitized Mice

(A) Experimental protocol.

(B) Representative photomicrographs of H&E-stained sections from challenged skin of OVA-sensitized mice treated with bestatin or vehicle (PBS) control. Magnification, 200X. Scale bars represent 100 μm.

(C–E) Epidermal and dermal thickness (C), number of infiltrating $CD4^+$ T cells (D), and mRNA levels of IL-4 and IL-13 expressed as fold induction relative to saline-challenged untreated mice (E).

Columns and error bars represent mean and SEM, respectively. n = 3 mice per group. *p < 0.05, **p < 0.01. ns, not significant; SAL, saline.

and *Ltb4r1*$^{-/-}$ mice demonstrated that LTB4 and BLT1 are both important for neutrophil accumulation into tape-stripped mouse skin. The role of LTB4 in neutrophil recruitment to the skin is supported by the observation that epicutaneous application of LTB4 in normal, healthy volunteers results in local neutrophil infiltration (de Jong et al., 1992).

Neutrophils, but not mast cells, were the major source of the increased LTB4 in mouse skin 24 hr after mechanical injury. LTB4 failed to accumulate in mice depleted of neutrophils using Gr-1 mAb or CTX, but accumulated normally in the mechanically injured skin of mast cell-deficient *Kit*^{W}/*Kit*$^{W\text{-}v}$ mice. Although mast cells could release LTB4 early after mechanical injury, mast cell-derived LTB4 was clearly not essential for neutrophil influx into mechanically injured skin. This is consistent with our previous observation that allergic skin inflammation elicited by EC sensitization of tape-stripped skin with OVA is intact in mast cell-deficient *Kit*^{W}/*Kit*$^{W\text{-}v}$ mice (Alenius et al., 2002).

Adoptive transfer experiments demonstrated that neutrophil influx in tape-stripped mouse skin was largely dependent on BLT1 and on both paracrine and autocrine actions of neutrophil-derived LTB4. LTB4 played a role in neutrophil recruitment to the skin in response to bacterial infection and sterile wounding. In addition, neutrophil-derived LTB4 is required for the recruitment of neutrophils to the joints and for the development of inflammatory arthritis (Chen et al., 2006; Chou et al., 2010; Kim et al., 2006). These observations suggest that LTB4 generated by neutrophils is important for their recruitment to the skin in response to a variety of inflammatory stimuli, as well as for their recruitment to other tissues.

Additional factors such as inflammatory chemokines and cytokines produced by resident skin cells may contribute to the initial neutrophil influx in the tape-stripped skin, as suggested by the residual neutrophil infiltration observed in *Ltb4r1*$^{-/-}$ mice. Furthermore, production of IL-1β and IL-6 by skin-infiltrating WT neutrophils may explain the finding that *Ltb4r1*$^{-/-}$ neutrophils could be detected in tape-stripped skin when cotransferred with WT neutrophils, but not when transferred alone. Both the rapid induction (within 6 hr) of CXCL2, IL-1β, and IL-6 mRNA and neutrophil influx into tape-stripped skin were blunted in *Myd88*$^{-/-}$ mice. This observation suggests that signaling via Toll-like receptors, possibly by endogenous alarmins (Guiducci et al., 2010), and/or signaling via IL-1 receptor (IL-1R) family members released from damaged keratinocytes plays an important role in the initial neutrophil influx into injured skin.

A central finding of the present study is that $CD4^+$ effector T cells from OVA-sensitized WT mice fail to transfer allergic inflammation to *Ltb4r1*$^{-/-}$ recipients. Taken together with the observation that *Ltb4r1*$^{-/-}$ $CD4^+$ T cells fail to transfer allergic inflammation, this result indicates that the expression of BLT1 on both non-T cells and T cells is important for allergic skin inflammation. Cotransfer of WT neutrophils, but not *Ltb4r1*$^{-/-}$ neutrophils, rescued the ability of $CD4^+$ effector T cells to

accumulate in antigen-challenged skin of *Ltb4r1*$^{-/-}$ recipients and cause allergic skin inflammation. Intradermal administration of WT and *Ltb4r1*$^{-/-}$, but not *Lta4h*$^{-/-}$, neutrophils rescued the ability of WT CD4$^+$ effector T cells to transfer allergic skin inflammation to *Ltb4r1*$^{-/-}$ recipients. This finding demonstrates that neutrophil-derived LTB4 is critical for the ability of CD4$^+$ effector T cells to cause allergic skin inflammation. These observations demonstrate that LTB4 production by neutrophils is critical both for the BLT1-dependent accumulation of neutrophils and the BLT1-dependent recruitment of antigen-specific CD4$^+$ effector T cells to cutaneous sites of antigen introduction.

The LTB4-synthesis inhibitor bestatin blocked neutrophil influx into tape-stripped skin and inhibited the development of allergic inflammation at cutaneous sites of antigen challenge in mice. This result suggests that blockade of LTB4-BLT1 interaction may be a useful strategy in preventing acute flares that are precipitated in quiescent skin in patients with AD by scratching and introduction of previously encountered antigen.

LTB4 concentrations were elevated in OVA-sensitized mouse skin sites, as in AD skin lesions, and mice EC-sensitized with OVA exhibited increased scratching. However, unlike AD skin lesions, which contain few, if any, neutrophils (Leiferman, 1994), EC-sensitized mouse skin sites exhibit neutrophil infiltration (Spergel et al., 1998). Neutrophils, like eosinophils, may not persist intact in chronic AD lesions. It thus remains possible that scratching established skin lesions induces a transient, acute neutrophil influx, which triggers LTB4-dependent recruitment of BLT1$^+$ effector T cells, resulting in the exacerbation of allergic skin inflammation.

Scratching is a hallmark of AD and causes further disruption of an already abnormal skin barrier, allowing cutaneous introduction of antigen. Our finding that the LTB4-BLT1 axis is implicated in two sequential steps in allergic inflammation at sites of cutaneous antigen exposure, namely recruitment of neutrophils and neutrophil-dependent recruitment of effector T cells, makes the LTB4-BLT1 axis a potential therapeutic target in AD.

EXPERIMENTAL PROCEDURES

Human Skin Biopsy

Two healthy nonallergic adult subjects were asked, after informed consent was obtained, to scratch the inner side of their forearm a total of 12 times over a period of 24 hr (every hour, eight times on day 0 and four times on day 1), each time using 20 strokes of their index fingernail. One hour after the last scratching, a 4 mm punch biopsy was obtained from the scratched site and from the corresponding unscratched site of the contralateral forearm. Histologic examination was performed on hematoxylin and eosin (H&E)-stained sections by an experienced dermatopathologist who was blinded to the source of the material.

Mice and Sensitization

Ltb4r1$^{-/-}$ and *Ltb4r1*$^{-/-}$OT-II mice on C57BL/6 background were previously described (Tager et al., 2003). *Lta4h*$^{-/-}$ mice, a kind gift of B.H. Koller (Byrum et al., 1999), were bred on C57BL/6 background for ten generations. WT C57BL/6 mice were obtained from Charles River Laboratories. *J-Kit*^{W}/*Kit*$^{W\text{-}v}$ (WBB6F1) mice and congenic WBB6F1 WT mice, OT-II, and CD45.1$^+$ mice on C57BL/6 background were obtained from the Jackson Laboratory. LysEGFP reporter mice on a C57BL/6 genetic background were previously described (Faust et al., 2000).

The animals were kept in a specific pathogen-free environment. All procedures performed on the animals were in accordance with the Institutional Animal Care and Use Committee of the Boston Children's Hospital or the University of California, Los Angeles (UCLA) Chancellor's Animal Research Committee.

For mechanical injury, the shaved back skin or the ear skin was tape-stripped six times with Tegaderm (Westnet, Canton, MA, USA). For neutrophil depletion, WT mice were treated with CTX or PBS control as described previously (Zuluaga et al., 2006). In selected experiments, WT mice were treated by i.p. injection with 100 μg Gr-1 mAb (RB6-8C5, eBioscience) or isotype control at days −3 and −1 of tape stripping. Neutrophil depletion was confirmed by flow cytometry at day 0 before tape stripping. Mice were treated by i.p. injection at 0.4 mg/day with bestatin (Cayman Chemical) or vehicle control, daily for 2 days before tape stripping.

EC sensitization of 6-to-8-week-old female mice was performed as described previously (Spergel et al., 1998). In brief, the skin of anesthetized mice was shaved and tape-stripped six times by transparent IV dressing (Tegaderm; Owens & Minor, Franklin, MA, USA). One hundred micrograms of chicken egg OVA (grade V; Sigma-Aldrich, St. Louis) in 100 μl of normal saline or placebo (100 μl of normal saline) was placed on a patch of sterile gauze (1 × 1 cm), which was secured to the skin with a transparent bio-occlusive dressing. Each mouse had a total of three 1-week exposures to the patch separated from each other by 2-week intervals. In the acute skin-challenge model, mice were EC-sensitized with OVA for 7 weeks, then challenged on days 0 and 3 after sensitization (days 50 and 53 from the start) by means of application of OVA or saline to shaved, tape-stripped, and previously unsensitized skin and were studied on day 7 of challenge (day 57 from the start). Mice were treated by i.p. injection at 0.4 mg/day with bestatin or vehicle control daily for 7 days, beginning 2 days before the 7-day challenge, as shown in Figure 7A.

Antibodies and Flow cCytometry Analysis of Ear Skin

Ear skin was used for flow cytometry analysis because of a technical difficulty of preparing cell suspension from the dorsal skin. Cells from ears were isolated as described previously (He et al., 2008). In brief, ears were separated in two halves and floated on trypsin-EDTA (Cellgro) for 30 min at 37°C for allowing dissociation of the epidermis. Both epidermal and dermal sheets were incubated for a further 1–2 hr at 37°C. The resulting cell suspensions were stained with the appropriate antibodies and analyzed by flow cytometry.

For surface staining, the following antibodies were used: fluorochrome-labeled anti-CD11b, Gr-1, CD4, CD44, Vα2, Vβ5, CD45.1, and CD45.2 (all from eBioscience). Cells were analyzed on FACSCanto, and the data was analyzed with FlowJo software.

Measurement of Skin LTB4 Concentrations

LTB4 concentrations in human and mouse skin were determined using solid-phase extraction followed by enzyme immunoassay kit measurement according to the manufacturer's instructions (Cayman Chemical).

Neutrophil Preparation from Bone Marrow

Mouse neutrophils were prepared from the femur and tibia of WT and *Ltb4r1*$^{-/-}$ mice using discontinuous Percoll density centrifugation as previously described (Jia et al., 2007). Preparations were >90% pure, as determined by flow cytometry analysis with double staining using Gr-1 and CD11b mAbs.

Adoptive Transfer of Neutrophils

CD45.1$^+$ WT recipient mice were treated with CTX as described above, and ear skin was tape-stripped 20 hr before neutrophil transfer. A total of 1 × 10^7 bone marrow neutrophils were isolated from CD45.2$^+$ WT, *Ltb4r1*$^{-/-}$, and *Lta4h*$^{-/-}$ mice and i.v. injected into CTX-treated WT mice. The percentage of adoptively transferred neutrophils recruited to the ear skin was analyzed 4 hr and 24 hr after transfer using a FACSCanto (BD Biosciences).

In cotransfer experiment, 5 × 10^6 neutrophils from WT and *Ltb4r1*$^{-/-}$ mice were labeled with 1 μM CellTracker Green CMFDA and 15 μM CellTracker Orange CMTMR (Invitrogen) at 37°C for 10 min. *Ltb4r1*$^{-/-}$ or WT neutrophils loaded with CMTMR dye (red) were mixed with 5 × 10^6 WT neutrophils loaded with CMFDA dye (green) at an input ratio of ~1:1 and adoptively transferred by i.v. injection into CTX-treated WT recipients, whose ears were tape-stripped 20 hr earlier. Four hours later, the ratio of green:red fluorescent cells in the ears was determined and adjusted to the input ratio, and the relative skin-homing index was calculated.

Adoptive Transfer of Allergic Skin Inflammation

Splenocytes from EC-sensitized WT or *Ltb4r1*$^{-/-}$ mice were cultured with OVA for 5 days, then CD4$^+$ T cells were purified by autoMACS and injected i.v. into naive recipients (5 × 10^6 CD4$^+$ T cells per mouse), which were challenged the same day by application of 100 μg OVA to shaved and tape-stripped dorsal skin. Skin challenge was repeated on day 3, and the skin site was examined on day 7. In selected experiments, 5 × 10^6 splenic CD4$^+$ T cells from WT mice EC-sensitized with OVA were injected i.v. into naive *Ltb4r1*$^{-/-}$ recipients on day 0, and WT, *Ltb4r1*$^{-/-}$, or *Lta4h*$^{-/-}$ neutrophils were administered i.v. (1 × 10^7) or i.d. (1 × 10^6) on day 0 and day 3 of 1-week EC challenge.

Skin Homing of CD4$^+$ T Cells

CD4$^+$ T cells were prepared from the spleens of OT-II mice on WT or *Ltb4r1*$^{-/-}$ background and activated with irradiated syngeneic splenocytes (3,000 rads) (1:10 ratio) as APCs in the presence of OVA (100 μg/ml) and IL-2 (10 ng/ml) for 5 days. A total of 5 × 10^6 activated transgenic CD4$^+$ T cells from WT or *Ltb4r1*$^{-/-}$ background were injected i.v. into naive recipients, followed by application of OVA and CT or CT alone to tape-stripped ear skin as previously described (He et al., 2008). In selected experiments, recipients received WT or *Ltb4r1*$^{-/-}$ neutrophils i.v. (1 × 10^7) or i.d. (1 × 10^6) on day 0 and again on day 3 of 1-week EC challenge. At the end of 1-week EC challenge, a single-cell suspension was prepared from ear skin as described above, stained with the appropriate antibodies, and analyzed by flow cytometry.

Quantitative PCR Analysis of Cytokines and Histological Analysis

Total RNA was extracted from homogenized skin tissue or from cultured cells with an RNAqueous extraction kit (Ambion), following the manufacturer's instructions. Complementary DNA (cDNA) was generated with the iScript cDNA synthesis kit (Bio-Rad Laboratories). Quantitative real-time PCR was done using the Taqman Gene Expression Assay, a universal PCR master mix, and the ABI Prism 7300 sequence-detection system with commercial primers and probes, all from Applied Biosystems. Fold induction of target gene expression was calculated using the comparative method for relative quantitation by normalization to the internal control β2-microglobulin, as described previously (He et al., 2007). For histological analysis, skin specimens were fixed in 10% buffered formalin and embedded in paraffin. Multiple 4 μm sections of skin were stained with H&E. Neutrophils were counted in ten randomly chosen areas 25 × 25 μm in size. CD4 staining of skin sections was performed as previously described (Spergel et al., 1998). Epidermal and dermal thickening, eosinophils, and CD4$^+$ T cells were counted blindly in 10–15 high-power fields at a magnification of 400X.

Immunoglobulin Determinations

Mice were bled, and serum concentrations of OVA-specific IgE, IgG1, and IgG2a Abs were measured using modified OVA-specific ELISAs as previously described (He et al., 2008).

Response of Splenocytes to OVA

Single-cell suspensions from spleen of EC-sensitized mice were cultured in complete RPMI 1640 (Invitrogen) supplemented with 10% fetal calf serum, 0.05 mM 2-mercaptoethanol, 100 U/ml penicillin, and 100 μg/ml streptomycin at 3 × 10^6/ml in 24-well plate following stimulation with OVA (100 μg/ml) for 4 days. Supernatants were collected for cytokine measurements with ELISA.

Statistical Analysis

Two-tailed Student's t test or one-way ANOVA was used for determining statistical differences between groups. A p value smaller than 0.05 was considered statistically significant.

SUPPLEMENTAL INFORMATION

Supplemental Information includes five figures and Supplemental Experimental Procedures and can be found with this article online at http://dx.doi.org/10.1016/j.immuni.2012.06.018.

ACKNOWLEDGMENTS

This work was supported by the Atopic Dermatitis Research Network National Institutes of Health/National Institute of Allergy and Infectious Diseases contract HHSN272201000020C (R.S.G.); U.S. Public Health Service grants AR-047417 (R.S.G.), AI 065858-03 (D.M.L.) HL-085100, AI-076471, and HL-092020, GM-076084; a Research Scholar Grant from the American Cancer Society (H.R.L.); and AI-050892 and AI-040618 (A.D.L.), R01 AI078910 (L.S.M.), T32 AR058921 (J.S.C.), R24 CA92865 (UCLA Small Animal Imaging Resource Program); and the Boston Children's Hospital Faculty Career Development Fellowship (M.K.O.). D.M.L. is currently an employee of Novartis Pharma AG and owns stock and/or options >$10,000 and has ownership and consulting income >$10,000 from Synovex. M.C. is currently an employee of Pfizer. We thank M.J. Massaad and H.C. Oettgen for reading the manuscript. We also thank J. Beaupre for her technical assistance.

Received: December 8, 2011
Accepted: June 27, 2012
Published online: October 11, 2012

REFERENCES

Alenius, H., Laouini, D., Woodward, A., Mizoguchi, E., Bhan, A.K., Castigli, E., Oettgen, H.C., and Geha, R.S. (2002). Mast cells regulate IFN-gamma expression in the skin and circulating IgE levels in allergen-induced skin inflammation. J. Allergy Clin. Immunol. *109*, 106–113.

Byrum, R.S., Goulet, J.L., Snouwaert, J.N., Griffiths, R.J., and Koller, B.H. (1999). Determination of the contribution of cysteinyl leukotrienes and leukotriene B4 in acute inflammatory responses using 5-lipoxygenase- and leukotriene A4 hydrolase-deficient mice. J. Immunol. *163*, 6810–6819.

Chen, M., Lam, B.K., Kanaoka, Y., Nigrovic, P.A., Audoly, L.P., Austen, K.F., and Lee, D.M. (2006). Neutrophil-derived leukotriene B4 is required for inflammatory arthritis. J. Exp. Med. *203*, 837–842.

Chou, R.C., Kim, N.D., Sadik, C.D., Seung, E., Lan, Y., Byrne, M.H., Haribabu, B., Iwakura, Y., and Luster, A.D. (2010). Lipid-cytokine-chemokine cascade drives neutrophil recruitment in a murine model of inflammatory arthritis. Immunity *33*, 266–278.

de Jong, E.M., van Erp, P.E., van Vlijmen, I.M., and van de Kerkhof, P.C. (1992). The inter-relation between inflammation and epidermal proliferation in normal skin following epicutaneous application of leukotriene-B4—an immunohistochemical study. Clin. Exp. Dermatol. *17*, 413–420.

Faust, N., Varas, F., Kelly, L.M., Heck, S., and Graf, T. (2000). Insertion of enhanced green fluorescent protein into the lysozyme gene creates mice with green fluorescent granulocytes and macrophages. Blood *96*, 719–726.

Fleischer, A.B., Jr., and Boguniewicz, M. (2010). An approach to pruritus in atopic dermatitis: a critical systematic review of the tacrolimus ointment literature. J. Drugs Dermatol. *9*, 488–498.

Fogh, K., Herlin, T., and Kragballe, K. (1989). Eicosanoids in skin of patients with atopic dermatitis: prostaglandin E2 and leukotriene B4 are present in biologically active concentrations. J. Allergy Clin. Immunol. *83*, 450–455.

Friedrich, E.B., Tager, A.M., Liu, E., Pettersson, A., Owman, C., Munn, L., Luster, A.D., and Gerszten, R.E. (2003). Mechanisms of leukotriene B4—triggered monocyte adhesion. Arterioscler. Thromb. Vasc. Biol. *23*, 1761–1767.

Funk, C.D. (2001). Prostaglandins and leukotrienes: advances in eicosanoid biology. Science *294*, 1871–1875.

Goodarzi, K., Goodarzi, M., Tager, A.M., Luster, A.D., and von Andrian, U.H. (2003). Leukotriene B4 and BLT1 control cytotoxic effector T cell recruitment to inflamed tissues. Nat. Immunol. *4*, 965–973.

Gregorio, J., Meller, S., Conrad, C., Di Nardo, A., Homey, B., Lauerma, A., Arai, N., Gallo, R.L., Digiovanni, J., and Gilliet, M. (2010). Plasmacytoid dendritic cells sense skin injury and promote wound healing through type I interferons. J. Exp. Med. *207*, 2921–2930.

Guiducci, C., Tripodo, C., Gong, M., Sangaletti, S., Colombo, M.P., Coffman, R.L., and Barrat, F.J. (2010). Autoimmune skin inflammation is dependent on

plasmacytoid dendritic cell activation by nucleic acids via TLR7 and TLR9. J. Exp. Med. *207*, 2931–2942.

He, R., Oyoshi, M.K., Jin, H., and Geha, R.S. (2007). Epicutaneous antigen exposure induces a Th17 response that drives airway inflammation after inhalation challenge. Proc. Natl. Acad. Sci. USA *104*, 15817–15822.

He, R., Oyoshi, M.K., Garibyan, L., Kumar, L., Ziegler, S.F., and Geha, R.S. (2008). TSLP acts on infiltrating effector T cells to drive allergic skin inflammation. Proc. Natl. Acad. Sci. USA *105*, 11875–11880.

Hilger, R.A., Neuber, K., and König, W. (1991). Conversion of leukotriene A4 by neutrophils and platelets from patients with atopic dermatitis. Immunology *74*, 689–695.

Huang, W.W., Garcia-Zepeda, E.A., Sauty, A., Oettgen, H.C., Rothenberg, M.E., and Luster, A.D. (1998). Molecular and biological characterization of the murine leukotriene B4 receptor expressed on eosinophils. J. Exp. Med. *188*, 1063–1074.

Jia, Y., Subramanian, K.K., Erneux, C., Pouillon, V., Hattori, H., Jo, H., You, J., Zhu, D., Schurmans, S., and Luo, H.R. (2007). Inositol 1,3,4,5-tetrakisphosphate negatively regulates phosphatidylinositol-3,4,5- trisphosphate signaling in neutrophils. Immunity *27*, 453–467.

Kim, N.D., Chou, R.C., Seung, E., Tager, A.M., and Luster, A.D. (2006). A unique requirement for the leukotriene B4 receptor BLT1 for neutrophil recruitment in inflammatory arthritis. J. Exp. Med. *203*, 829–835.

Kim, D.K., Kim, H.J., Sung, K.S., Kim, H., Cho, S.A., Kim, K.M., Lee, C.H., and Kim, J.J. (2007). 12(S)-HPETE induces itch-associated scratchings in mice. Eur. J. Pharmacol. *554*, 30–33.

Koro, O., Furutani, K., Hide, M., Yamada, S., and Yamamoto, S. (1999). Chemical mediators in atopic dermatitis: involvement of leukotriene B4 released by a type I allergic reaction in the pathogenesis of atopic dermatitis. J. Allergy Clin. Immunol. *103*, 663–670.

Leiferman, K.M. (1994). Eosinophils in atopic dermatitis. J. Allergy Clin. Immunol. *94*, 1310–1317.

Miyahara, N., Takeda, K., Miyahara, S., Taube, C., Joetham, A., Koya, T., Matsubara, S., Dakhama, A., Tager, A.M., Luster, A.D., and Gelfand, E.W. (2005). Leukotriene B4 receptor-1 is essential for allergen-mediated recruitment of CD8+ T cells and airway hyperresponsiveness. J. Immunol. *174*, 4979–4984.

Nosbaum, A., Hennino, A., Berard, F., and Nicolas, J.F. (2010). Patch testing in atopic dermatitis patients. Eur. J. Dermatol. *20*, 563–566.

Ohnishi, H., Miyahara, N., and Gelfand, E.W. (2008). The role of leukotriene B(4) in allergic diseases. Allergol. Int. *57*, 291–298.

Okano-Mitani, H., Ikai, K., and Imamura, S. (1996). Leukotriene A4 hydrolase in peripheral leukocytes of patients with atopic dermatitis. Arch. Dermatol. Res. *288*, 168–172.

Patcha, V., Wigren, J., Winberg, M.E., Rasmusson, B., Li, J., and Särndahl, E. (2004). Differential inside-out activation of beta2-integrins by leukotriene B4 and fMLP in human neutrophils. Exp. Cell Res. *300*, 308–319.

Peters-Golden, M., and Henderson, W.R., Jr. (2007). Leukotrienes. N. Engl. J. Med. *357*, 1841–1854.

Ruzicka, T., Simmet, T., Peskar, B.A., and Ring, J. (1986). Skin levels of arachidonic acid-derived inflammatory mediators and histamine in atopic dermatitis and psoriasis. J. Invest. Dermatol. *86*, 105–108.

Sicherer, S.H., and Leung, D.Y. (2007). Advances in allergic skin disease, anaphylaxis, and hypersensitivity reactions to foods, drugs, and insects. J. Allergy Clin. Immunol. *119*, 1462–1469.

Spergel, J.M., Mizoguchi, E., Brewer, J.P., Martin, T.R., Bhan, A.K., and Geha, R.S. (1998). Epicutaneous sensitization with protein antigen induces localized allergic dermatitis and hyperresponsiveness to methacholine after single exposure to aerosolized antigen in mice. J. Clin. Invest. *101*, 1614–1622.

Spergel, J.M., Mizoguchi, E., Oettgen, H., Bhan, A.K., and Geha, R.S. (1999). Roles of TH1 and TH2 cytokines in a murine model of allergic dermatitis. J. Clin. Invest. *103*, 1103–1111.

Tager, A.M., Dufour, J.H., Goodarzi, K., Bercury, S.D., von Andrian, U.H., and Luster, A.D. (2000). BLTR mediates leukotriene B(4)-induced chemotaxis and adhesion and plays a dominant role in eosinophil accumulation in a murine model of peritonitis. J. Exp. Med. *192*, 439–446.

Tager, A.M., Bromley, S.K., Medoff, B.D., Islam, S.A., Bercury, S.D., Friedrich, E.B., Carafone, A.D., Gerszten, R.E., and Luster, A.D. (2003). Leukotriene B4 receptor BLT1 mediates early effector T cell recruitment. Nat. Immunol. *4*, 982–990.

Terawaki, K., Yokomizo, T., Nagase, T., Toda, A., Taniguchi, M., Hashizume, K., Yagi, T., and Shimizu, T. (2005). Absence of leukotriene B4 receptor 1 confers resistance to airway hyperresponsiveness and Th2-type immune responses. J. Immunol. *175*, 4217–4225.

Thorsen, S., Fogh, K., Broby-Johansen, U., and Søndergaard, J. (1990). Leukotriene B4 in atopic dermatitis: increased skin levels and altered sensitivity of peripheral blood T-cells. Allergy *45*, 457–463.

Wenzel, S.E., Trudeau, J.B., Kaminsky, D.A., Cohn, J., Martin, R.J., and Westcott, J.Y. (1995). Effect of 5-lipoxygenase inhibition on bronchoconstriction and airway inflammation in nocturnal asthma. Am. J. Respir. Crit. Care Med. *152*, 897–905.

Werner, S., Krieg, T., and Smola, H. (2007). Keratinocyte-fibroblast interactions in wound healing. J. Invest. Dermatol. *127*, 998–1008.

Werz, O. (2002). 5-lipoxygenase: cellular biology and molecular pharmacology. Curr. Drug Targets Inflamm. Allergy *1*, 23–44.

Zuluaga, A.F., Salazar, B.E., Rodriguez, C.A., Zapata, A.X., Agudelo, M., and Vesga, O. (2006). Neutropenia induced in outbred mice by a simplified low-dose cyclophosphamide regimen: characterization and applicability to diverse experimental models of infectious diseases. BMC Infect. Dis. *6*, 55.