

Toll-like receptor 2 is important for the T_H1 response to cutaneous sensitization

Haoli Jin, MD, PhD,^{a,*} Lalit Kumar, PhD,^{a,*} Clinton Mathias, PhD,^a David Zurakowski, PhD,^b Hans Oettgen, MD, PhD,^a Leonid Gorelik, PhD,^c and Raif Geha, MD^a *Boston and Cambridge, Mass*

Background: Atopic dermatitis and allergic contact dermatitis are skin disorders triggered by epicutaneous sensitization with protein antigens and contact sensitization with haptens, respectively. Skin is colonized with bacteria, which are a source of Toll-like receptor (TLR) 2 ligands.

Objective: We sought to examine the role of TLR2 in murine models of atopic dermatitis and allergic contact dermatitis.

Methods: TLR2^{-/-} mice and wild-type littermates were epicutaneously sensitized with ovalbumin (OVA) or contact sensitized with oxazolone (OX). Skin histology was assessed by means of hematoxylin and eosin staining and immunohistochemistry. Ear swelling was measured with a micrometer. Cytokine mRNA expression was examined by means of quantitative RT-PCR. Antibody levels and splenocyte secretion of cytokines in response to OVA stimulation were measured by means of ELISA. Dendritic cells were examined for their ability to polarize T-cell receptor/OVA transgenic naive T cells to T_H1 and T_H2.

Results: In response to OVA sensitization, TLR2^{-/-} mice experienced skin infiltration with eosinophils and CD4⁺ cells, as well as upregulation of T_H2 cytokine mRNAs that was comparable with that seen in wild-type littermates. In contrast, epidermal thickening, IFN- γ expression in the skin, IFN- γ production by splenocytes, and IgG2a anti-OVA antibody levels were impaired in TLR2^{-/-} mice. After OX ear challenge, contact sensitized TLR2^{-/-} mice exhibited defective ear swelling with impaired cellular infiltration, decreased epidermal thickening and local IFN- γ expression, and impaired OX-specific IgG2a responses. Dendritic cells from TLR2^{-/-} mice

induced significantly lower production of IFN- γ but normal IL-4 and IL-13 production in naive T cells.

Conclusions: These results indicate that TLR2 promotes the IFN- γ response to cutaneously introduced antigens. (*J Allergy Clin Immunol* 2009;123:875-82.)

Key words: Toll-like receptor 2, atopic dermatitis, contact hypersensitivity, oxazolone

Toll-like receptors (TLRs) are a group of pathogen-associated molecular pattern receptors important for innate and adaptive immunity.^{1,2} TLR2 can heterodimerize with either TLR1 or TLR6. TLR1/2 ligands include triacyl lipopeptides of bacteria and the synthetic lipopeptide Pam3Cys. TLR2/6 ligands include bacterial diacyl lipopeptides and the synthetic lipopeptide Pam2Cys, lipoteichoic acids and peptidoglycan from *Staphylococcus aureus* and other gram-positive bacteria, macrophage-activating lipopeptide from *Mycoplasma* species, and fungal zymosan.³ TLR2 also recognizes fungal lipomannan, mutin type transmembrane glycoprotein of trypanosomes, and viral proteins, such as the hemagglutinin of measles, independently of TLR1 and TLR6.^{3,4} TLR2 is also thought to bind endogenous ligands, such as hyaluronan fragments, biglycan, eosinophil-derived neurotoxin, and serum amyloid A.⁵⁻⁸ TLR2 is expressed on immune cells, including T cells, B cells, mast cells, eosinophils, macrophages, and dendritic cells (DCs), and nonimmune cells, including keratinocytes, epithelial cells, fibroblasts, and smooth muscle cells.^{2,9} TLR2 expression is upregulated by LPS; inflammatory cytokines, such as IL-1, TNF- α , and IFN- γ ; and bacterial and viral infection. TLR2 signals through the adaptor MyD88.¹

DCs play a critical role in the polarization of naive T cells into T_H1 and T_H2 cells. Activation of TLR2 in DCs promotes IFN- γ production and T_H1 responses.^{10,11} In addition, TLR2 can directly stimulate T cells to produce more IFN- γ ,¹² and TLR2^{-/-} mice exhibit deficient T_H1-dependent humoral immune response to the gram-positive extracellular bacterium *Streptococcus pneumoniae*.¹³ However, under different conditions, TLR2 agonists have been reported to favor a T_H2 immune response *in vitro* and *in vivo*.^{7,14}

There are conflicting data regarding the effect of TLR2 engagement on the allergic response. A number of studies suggest that TLR2 ligation inhibits the allergic response. Coadministration of the probiotic bacterium *Lactobacillus plantarum* augmented T_H1 responses through TLR2 and led to protection against Der p 1–induced allergic responses.¹⁵ The TLR2 agonists Pam3CSK and lipoprotein reduced airway inflammation, airway hyperresponsiveness (AHR), and serum levels of IgE after allergen inhalation challenge.¹⁶ *Mycoplasma pneumoniae* infection preceding allergen challenge reduces airway epithelial mucin

From ^athe Division of Immunology, Children's Hospital, and the Department of Pediatrics, Harvard Medical School, Boston; ^bthe Department of Anesthesia and Surgery, Children's Hospital, Boston; and ^cBiogen Idec, Inc, Cambridge.

*These authors contributed equally to this work.

Supported by the Atopic Dermatitis and Vaccinia Immunization Network; National Institutes of Health/National Institute of Allergy and Infectious Diseases contract NO1 (AI 40030); and a US Public Health Service grant AR-047417 to R.G. H.J. has received a postdoctoral fellowship from the American Heart Association.

Disclosure of potential conflict of interest: H. Jin has received research support from the American Heart Association. H. Oettgen has served as a consultant and scientific advisor for Schering-Plough and as a consultant for Genentech and has received research support from Novartis. L. Gorelik is employed by Biogen Idec, Inc. The rest of the authors have declared that they have no conflict of interest.

Received for publication August 20, 2008; revised January 22, 2009; accepted for publication February 4, 2009.

Reprint requests: Raif Geha, MD, Division of Immunology, Room 10210, Karp Family Research Building, Children's Hospital, One Blackfan Circle, Boston, MA 02115.

E-mail: raif.geha@childrens.harvard.edu.

0091-6749/\$36.00

© 2009 American Academy of Allergy, Asthma & Immunology

doi:10.1016/j.jaci.2009.02.007

Abbreviations used

ACD:	Allergic contact dermatitis
AD:	Atopic dermatitis
AHR:	Airway hyperresponsiveness
DC:	Dendritic cell
NF- κ B:	Nuclear factor κ B
OVA:	Ovalbumin
OX:	Oxazolone
TLR:	Toll-like receptor
WT:	Wild-type

expression in mice, partly through the TLR2-IFN- γ pathway.¹⁷ Finally, the TLR2/6 agonist macrophage-activating lipopeptide, in combination with IFN- γ , strongly reduced AHR, eosinophilia, and T_H2 cytokine levels in bronchoalveolar fluid.¹⁸ In contrast to the above studies, intranasal immunization of mice with ovalbumin (OVA) in combination with the TLR2 ligand peptidoglycan has been reported to augment lung inflammation and AHR compared with intranasal OVA immunization alone.¹⁹

TLR2 is expressed in keratinocytes.^{20,21} The skin of patients with atopic dermatitis (AD) is heavily colonized with *S aureus*, a rich source of TLR2 ligands.²² This adversely affects eczema severity.²³ Furthermore, AD skin lesions might contain endogenous TLR2 ligands, such as eosinophil-derived neurotoxin, the level of which is increased in the blood of patients with AD.^{24,25} TLR2 function, but not expression, has been reported to be impaired in monocytes and keratinocytes from patients with AD.²⁶ There are conflicting data whether the TLR2 polymorphism R753Q, which impairs TLR2 function, is associated with AD.^{27,28}

Acute AD skin lesions are dominated by local expression of T_H2 cytokines, whereas chronic AD skin lesions and allergic contact dermatitis (ACD) lesions are characterized by mixed expression of both T_H2 and T_H1 cytokines.^{29,30} IFN- γ has been implicated in keratinocyte apoptosis in patients with AD²⁸ and in epidermal thickening in a mouse model of allergic skin inflammation.³¹ We tested the hypothesis that TLR2 ligands could play an important role in AD by promoting a T_H1 response to antigens introduced through the skin. We demonstrate that TLR2 promotes epidermal thickening and the IFN- γ response to cutaneously introduced antigen in a mouse model of AD elicited by epicutaneous sensitization with OVA, as well as in a mouse model of ACD elicited by contact sensitization with the hapten oxazolone (OX).

METHODS**Mice**

TLR2^{-/-} mice on a C57BL/6 background were kindly provided by Biogen Idec, Inc (Cambridge, Mass); C57BL/6 wild-type (WT) mice were purchased from Charles River Laboratories (Wilmington, Mass). All mice were kept in a pathogen-free environment and fed an OVA-free diet. All procedures performed on the mice were in accordance with the Animal Care and Use Committee of the Children's Hospital Boston.

Epicutaneous sensitization with OVA

Four- to 6-week-old female mice were epicutaneously sensitized, as described previously.³² Briefly, the dorsal skin of anesthetized mice was shaved and tape stripped 6 times. One hundred micrograms of OVA (Grade V; Sigma Chemical Co, St Louis, Mo) in 100 μ L of normal saline or placebo (100 μ L of normal saline) was placed on a patch of sterile gauze (1 $\times$ 1 cm),

which was secured to dorsal skin with a transparent bio-occlusive dressing (Tegaderm; WestNet, Inc, Arvada, Colo). Each mouse had a total of three 1-week exposures to the patch separated by 2-week intervals. Mice were euthanized immediately at the end of the third cycle of sensitization (day 49).

Hapten-induced contact hypersensitivity

Mice were sensitized by means of the application of 100 μ L of 2% OX (Sigma) in ethanol to previously shaved abdominal skin. Five days later, 10 μ L of 1% OX was applied to the dorsal and ventral surfaces of the right ear. Ethanol was applied to the left ear. Ear thickness was measured after 24, 48, and 72 hours by using a modified spring-loaded micrometer (Mitutoyo, Aurora, Ill).

Histologic and immunohistochemical analysis of mouse skin

Dorsal or ear skin specimens were fixed in 10% buffered formalin and embedded in paraffin. Multiple 4-mm sections of skin were stained with hematoxylin and eosin by Histo-Scientific Research Laboratories (Mount Jackson, Va). CD4 staining of skin sections was performed as previously described.³² Eosinophils and CD4⁺ cells were counted blind in 10 to 15 high-power fields at a magnification of $\times$ 400. The epidermal thickness of 6 different randomly chosen sites was measured in each skin section from each mouse.

Quantitative RT-PCR for TLR-2, cytokines, and chemokines

Specimens of skin were homogenized with a Polytron RT-3000 (Kinematic AG, Lucerne, Switzerland) in lysis buffer solution provided in the RNeasy extraction kit (Ambion, Inc, Austin, Tex). Reverse transcription was performed with the iScript cDNA synthesis kit (Bio-Rad Laboratories, Hercules, Calif). PCR reactions were run on an ABI Prism 7300 (Applied Biosystems, Foster City, Calif) sequence detection system platform. Taqman primers and probes were obtained from Applied Biosystems. The housekeeping gene β_2 -microglobulin was used as a control. Relative gene expression was determined by using the method described by Pfaffl.³³

Cell culture, proliferation assay, and *in vitro* cytokine production

Single-cell suspensions of spleen cells were cultured in complete RPMI 1640 (JRH Biosciences, Inc, Lenexa, Kan) supplemented with 10% FCS, 1 mmol/L sodium pyruvate, 2 mmol/L L-glutamine, 0.05 mmol/L 2-mercaptoethanol, 100 U/mL penicillin, and 1 mg/mL streptomycin at 2×10^5 /mL in 96-well plates or 2×10^6 /mL in 24-well plates in the presence of OVA (50 μ g/mL). Proliferation was measured in triplicate wells of 96-well plates by using tritiated thymidine incorporation after 72 hours of culture. Cytokine secretion in supernatants from 24-well plates after 96 hours of culture was determined by means of ELISA according to the manufacture's instructions (BD Pharmingen, San Jose, Calif).

Serum antibody determination

The BD Pharmingen protocol for sandwich ELISA was used to quantify serum antibodies from epicutaneously OVA- or OX-sensitized mice. OX-specific antibodies were measured as previously described.³⁰

***In vitro* assessment of antigen-presenting cell function of DCs**

CD11c⁺ DCs were sorted from spleens by using Miltenyi mouse CD11c beads (Miltenyi Biotec, Bergisch Gladbach, Germany) and cocultured with WT, OVA-specific, T-cell receptor transgenic OTII CD4⁺ T cells at a 1:10 ratio in the presence of graded concentrations (0, 10, 100, and 1000 ng/mL) of OVA₃₂₃₋₃₃₉ peptide. After 96 hours, T-cell proliferation and cytokine production were measured as described above.

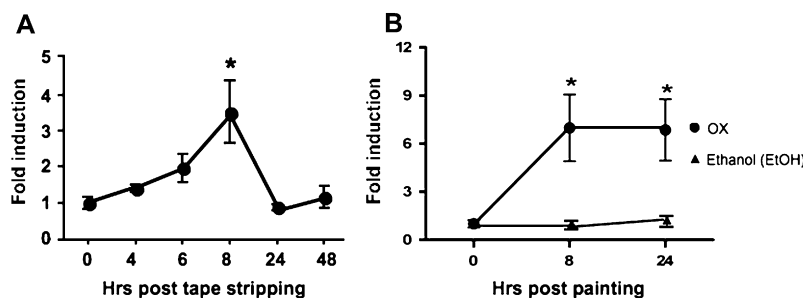


FIG 1. TLR2 mRNA expression after skin tape stripping and painting with OX. Quantitative PCR analysis of TLR2 mRNA expression in mouse skin after tape stripping ($n = 5$; **A**) or application of 2% OX in ethanol to shaved skin ($n = 4$; **B**) is shown. Results were normalized for glyceraldehyde-3-phosphate dehydrogenase expression and expressed as fold induction compared with time 0. Values are presented as the mean $\pm$ SD. * $P < .05$.

Statistical analysis

Quantitative PCR expression of TLR2 mRNA in mouse skin after tape stripping was assessed by means of 1-way ANOVA, with the F test used to determine change in fold induction relative to time 0 (baseline). Comparison of TLR2 induction between OX- and ethanol-treated skin was evaluated by means of 2-way ANOVA, with group and time as fixed factors in the model and the interaction of group-by-time included. Two-way ANOVA was used to determine changes in epidermal thickening, skin-infiltrating CD4⁺ T cells, eosinophils, and mRNA levels of cytokines. Generalized linear models with 2 factors were used to analyze changes in ear swelling over time and to compare OX-specific levels of serum IgG1 and IgG2a. Differences in proliferation and secretion of cytokines were analyzed by using ANOVA. Statistical analysis was performed with the SPSS software package (SPSS, Inc, Chicago, Ill). Two-tailed P values of less than .05 were considered statistically significant for all comparisons.

RESULTS

TLR2 expression in skin is upregulated by tape stripping and OX painting

Mechanical injury induced by scratching is a hallmark of human AD. Tape stripping of mouse back skin was used to mimic mechanical injury inflicted by scratching. Fig 1, A, shows that tape stripping upregulated TLR2 mRNA expression in mouse skin, with a peak increase of 3.2 ± 1.5 -fold over baseline ($P < .05$) 8 hours after stripping. A similar increase (2.8-fold) was revealed by means of microarray analysis of skin 8 hours after stripping (data not shown).

We also examined whether application of the hapten OX on shaved skin of the belly upregulates TLR2 mRNA expression. Fig 1, B, shows that application of OX in ethanol upregulated TLR2 mRNA expression in mouse skin, with a peak increase of 7.0 ± 4.1 -fold 8 hours after stripping. This increase was sustained at 24 hours. There was no increase in skin TLR2 mRNA expression after application of ethanol solvent (data not shown). These results show that mechanical skin injury, which is necessary for epicutaneous sensitization with protein antigen, and hapten application to the skin upregulate TLR2 expression in the skin.

Impaired epidermal thickening and upregulation of IFN- γ expression in epicutaneously sensitized skin of TLR2^{-/-} mice

To determine the role of TLR2 in allergic skin inflammation elicited by epicutaneous sensitization with protein antigen, we examined the response of TLR2^{-/-} mice to repeated epicutaneous

application of OVA on tape-stripped skin. As shown previously,³⁴ epicutaneous sensitization of C57BL/6 WT mice with OVA led to epidermal thickening with foci of spongiosis (Fig 2, A) and to marked infiltration of the dermis with CD4⁺ T cells and modest infiltration with eosinophils (Fig 2, B). OVA-sensitized TLR2^{-/-} mice exhibited impaired epidermal thickening compared with that seen in WT mice (Fig 2, A). There was a 2.6 ± 0.4 -fold increase in epidermal thickness in OVA-sensitized skin of WT mice compared with a 1.1 ± 0.3 -fold increase in TLR2^{-/-} mice ($n = 3$ per group, $P < .05$). Spongiosis was not detected in TLR2^{-/-} mice. WT and TLR2^{-/-} mice had comparable levels of skin infiltration with CD4⁺ cells and eosinophils (Fig 2, B). Expression of mRNA for the chemokines CCL17/thymus and activation-regulated chemokine, a ligand for CCR4 expressed on skin-homing T cells, and CCL11/eotaxin-1 mRNA, a ligand for CCR3 expressed on eosinophils, was comparably upregulated in OVA-sensitized skin from WT and TLR2^{-/-} mice (see Fig E1 in this article's Online Repository at www.jacionline.org).

Epicutaneous sensitization with OVA caused upregulation of mRNA for the T_H2 cytokines IL-4, IL-5, and IL-13 and the T_H1 cytokine IFN- γ in the skin of C57BL/6 WT mice (Fig 2, C). There was no significant difference in the upregulation of IL-4, IL-5, and IL-13 between OVA-sensitized skin of TLR2^{-/-} mice and their WT littermates. In contrast, IFN- γ mRNA was not upregulated in OVA-sensitized skin of TLR2^{-/-} mice. These results demonstrate that TLR2 is important for the local T_H1 response but is dispensable for the local T_H2 response to epicutaneous sensitization with protein antigen.

Defective systemic T_H1 response to epicutaneous sensitization with OVA in TLR2-deficient mice

Epicutaneous sensitization with OVA results in a mixed systemic T_H2 and T_H1 OVA-specific response.³² Splenocytes from OVA-sensitized TLR2^{-/-} mice and WT mice secreted comparable amounts of the T_H2 cytokines IL-4 and IL-13 in response to *in vitro* stimulation with OVA (Fig 3, A). In contrast, splenocytes from TLR2^{-/-} mice secreted significantly lower amounts of IFN- γ in response to OVA stimulation than splenocytes from WT control animals. TLR2^{-/-} mice mounted normal OVA-specific IgG1 and IgE antibody responses to epicutaneous sensitization (Fig 3, B). However, their OVA-specific IgG2a response was severely diminished compared with that seen in WT control animals. These results suggest that TLR2 is required for the development of a systemic T_H1 response to epicutaneous sensitization.

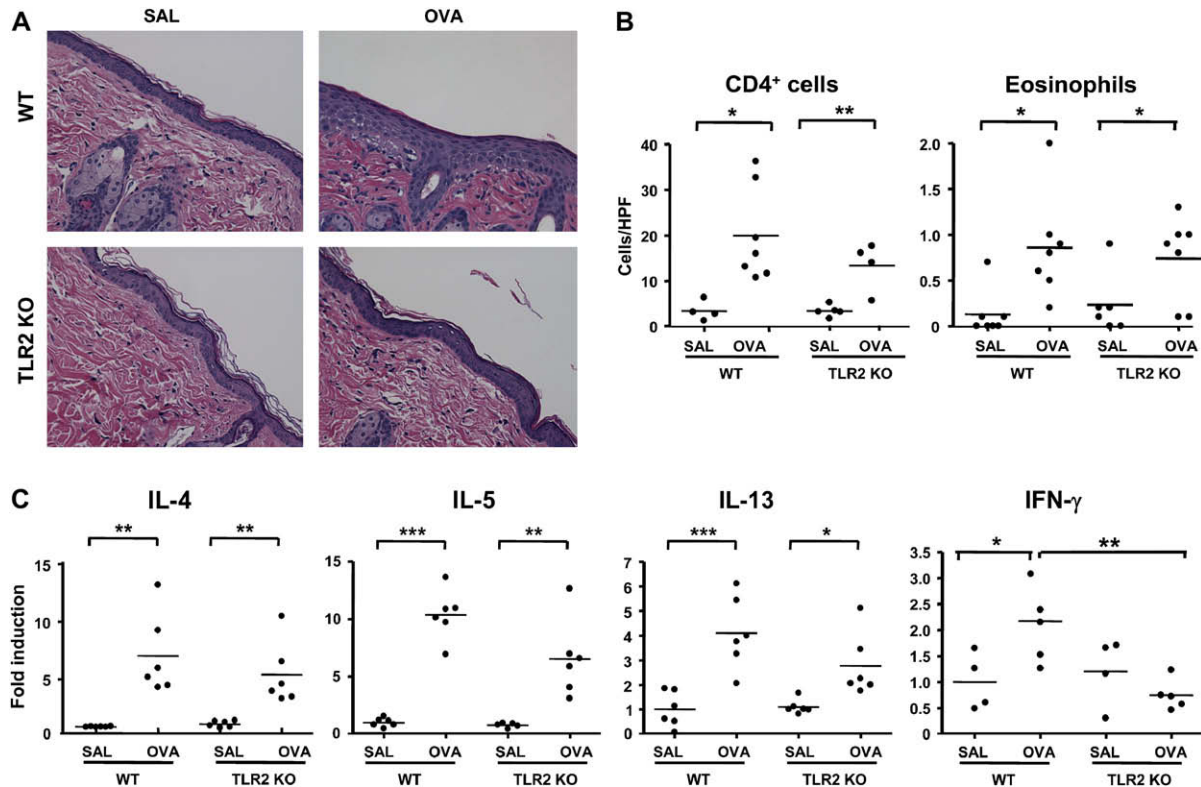


FIG 2. Impaired epidermal thickening and IFN- γ mRNA expression but normal cellular infiltration and T_H2 cytokine production in OVA-sensitized skin of TLR2^{-/-} mice. **A**, Representative skin histology (original magnification $\times 200$). **B**, Skin-infiltrating CD4⁺ T cells and eosinophils. **C**, Quantitative PCR analysis of mRNA levels of IL-4, IL-5, and IL-13 and IFN- γ . n = 6-7. * P < .05, ** P < .01, and *** P < .001. SAL, Saline; KO, knockout.

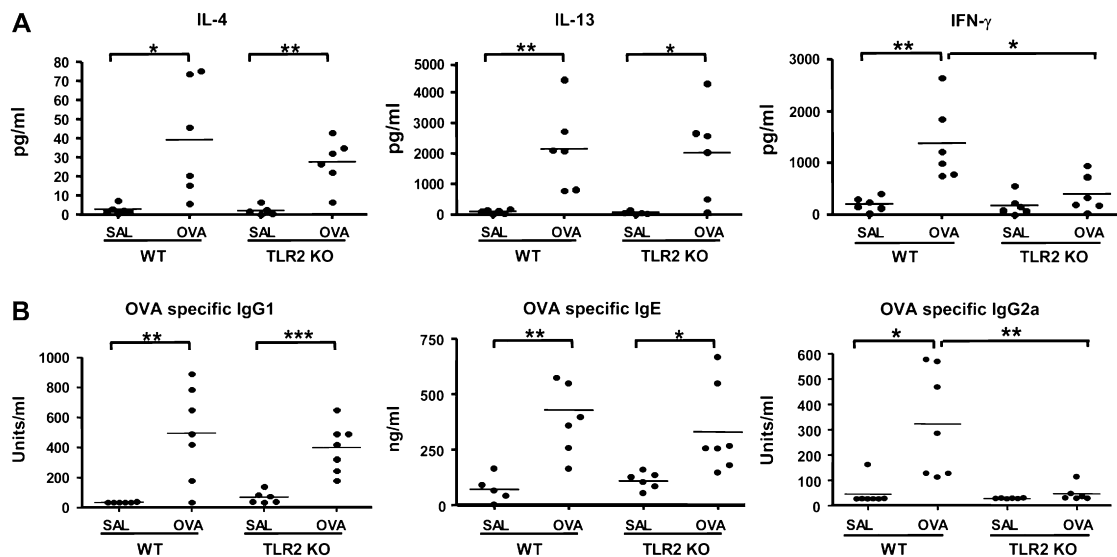


FIG 3. Impaired T_H1 systemic response to epicutaneous sensitization with OVA in TLR2^{-/-} mice. **A**, Cytokine secretion by splenocytes in response to OVA stimulation *in vitro*. **B**, Serum levels of OVA-specific immunoglobulin isotypes. n = 6-7. * P < .05, ** P < .01, and *** P < .001. SAL, Saline; KO, knockout.

Impaired contact hypersensitivity to OX in TLR2^{-/-} mice

Contact hypersensitivity to the hapten OX is a model of skin inflammation that involves local expression of both T_H1 and T_H2

cytokines. Fig 4, A, shows that OX-challenged ears of OX-sensitized WT mice exhibited swelling compared with that seen in vehicle-challenged ears. Ear swelling peaked at 24 hours, remained significant at 48 hours, and resolved by 72 hours after

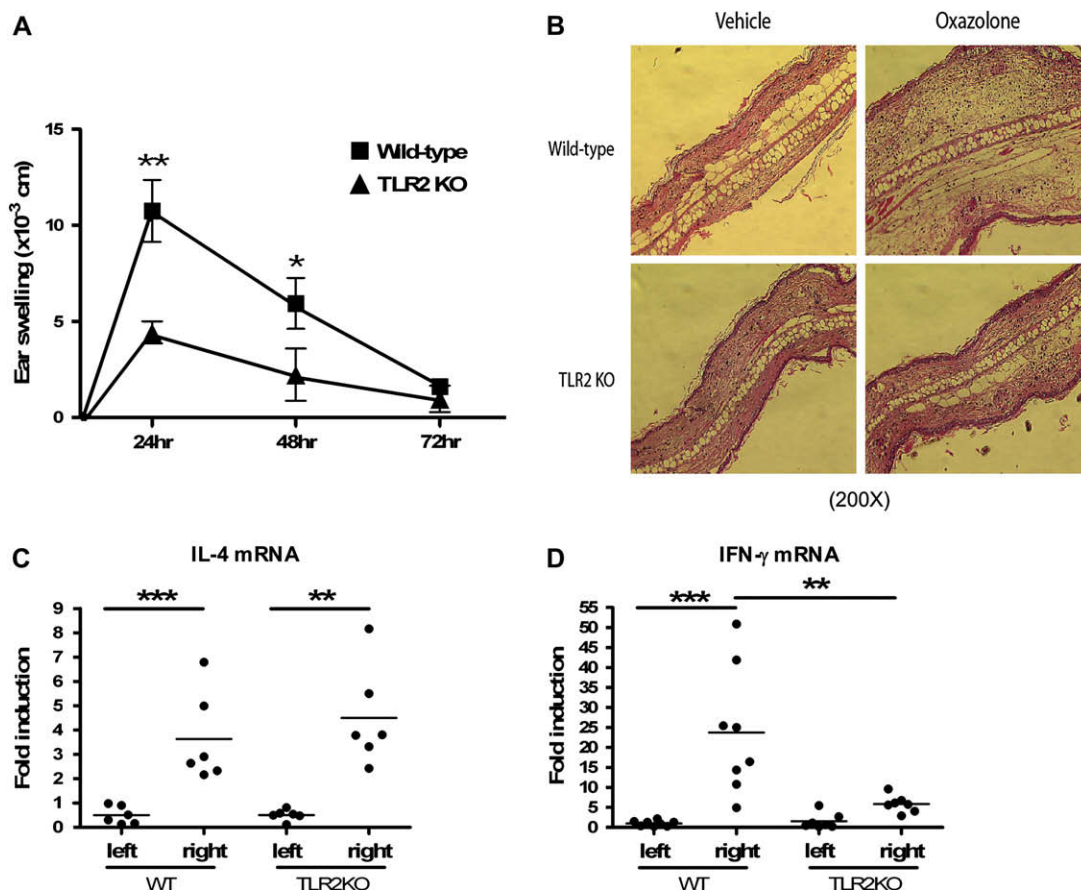


FIG 4. Impaired contact hypersensitivity in TLR2^{-/-} mice. **A**, Ear swelling in sensitized mice challenged with OX hapten. Results represent the difference in thickness between hapten- and vehicle-challenged ears ($n = 10$). **B**, Representative ear skin histology at 24 hours after challenge (original magnification $\times 200$). **C** and **D**, IL-4 (**Fig 4, C**) and IFN- γ (**Fig 4, D**) mRNA expression as fold induction in hapten-challenged over vehicle-challenged ears. * $P < .05$, ** $P < .01$, and *** $P < .0001$. KO, Knockout.

challenge. TLR2^{-/-} mice had significantly less ear swelling 24 and 48 hours after OX challenge than that seen in WT control mice. Decreased ear thickening in TLR2^{-/-} mice was confirmed by means of histologic analysis, which revealed a marked decrease in cellular infiltration and edema (**Fig 4, B**). There was significant epidermal thickening in challenged skin of WT mice but not that of TLR2^{-/-} mice (3.1 ± 0.5 -fold increase in hapten-challenged vs vehicle-challenged skin in OX-sensitized WT mice compared with a 1.1 ± 0.2 -fold increase in TLR2^{-/-} mice; $n = 6$ per group; $P < .0001$). Expression of mRNA for the cytokines IL-4 and IFN- γ was markedly upregulated in OX-challenged ears compared with that seen in vehicle-challenged ears in WT mice (**Fig 4, C** and **D**). IL-4 mRNA expression was normally upregulated in OX-challenged ears of TLR2^{-/-} mice. In contrast, upregulation of IFN- γ mRNA expression was severely diminished in the ears of these mice. These results indicate that TLR2 is important for the development of contact hypersensitivity and for local expression of IFN- γ after hapten challenge.

Impaired IgG2a response to OX in TLR2^{-/-} mice

Reduced IFN- γ upregulation in OX-challenged skin sites from TLR2^{-/-} mice could be secondary to a defect in systemic T_H1 response. Because IgG2a antibody responses are driven by IFN- γ ,³⁵

we examined the IgG2a antibody response to OX as an indirect measure of the T_H1 systemic response to OX sensitization. **Fig 5** shows that WT mice had OX-specific IgG1 and IgG2a after OX contact sensitization and challenge. TLR2^{-/-} mice had similar levels of OX-specific IgG1 as WT control mice. In contrast, they mounted significantly less OX-specific IgG2a response. These results suggest that TLR2 is important for the development of a systemic T_H1 response to contact sensitization with hapten.

Impaired ability of TLR2^{-/-} DCs to polarize the response of naive T cells to T_H1

DCs capture antigen in the skin and traffic to draining lymph node, where they prime naive T cells to become effector cells that can home to the skin.³⁶ A possible explanation for the defective T_H1 response of TLR2^{-/-} mice to epicutaneous sensitization is that their DCs are impaired in their ability to drive a T_H1 response in CD4⁺ T cells. To directly test this hypothesis, we cocultured splenic CD11c⁺ cells from WT and TLR2^{-/-} mice with T-cell receptor/OVA transgenic CD4⁺ T cells from OTII mice in the presence of the OVA₃₂₃₋₃₃₉ peptide. DCs from TLR2^{-/-} mice and WT control mice were comparable in their ability to drive proliferation and production of the T_H2 cytokines IL-4 and IL-13 by naive T cells (**Fig 6, A-C**). In

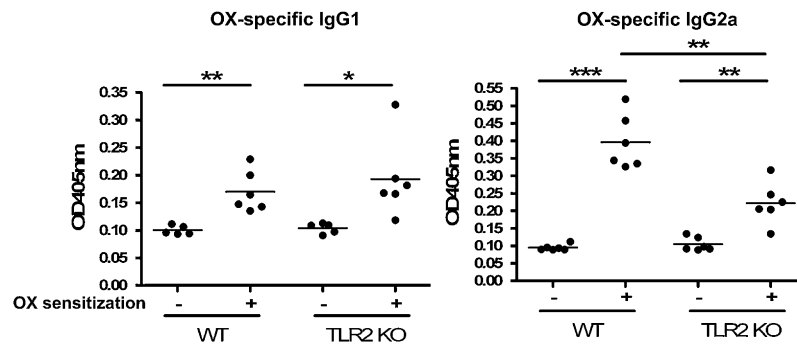


FIG 5. Impaired OX-specific IgG2a response in TLR2^{-/-} mice. Serum anti-OX IgG1 and IgG2a levels in mice sensitized and challenged with OX. Serum was taken 24 hours after the challenge. **P* < .05, ***P* < .01, and ****P* < .001. KO, Knockout.

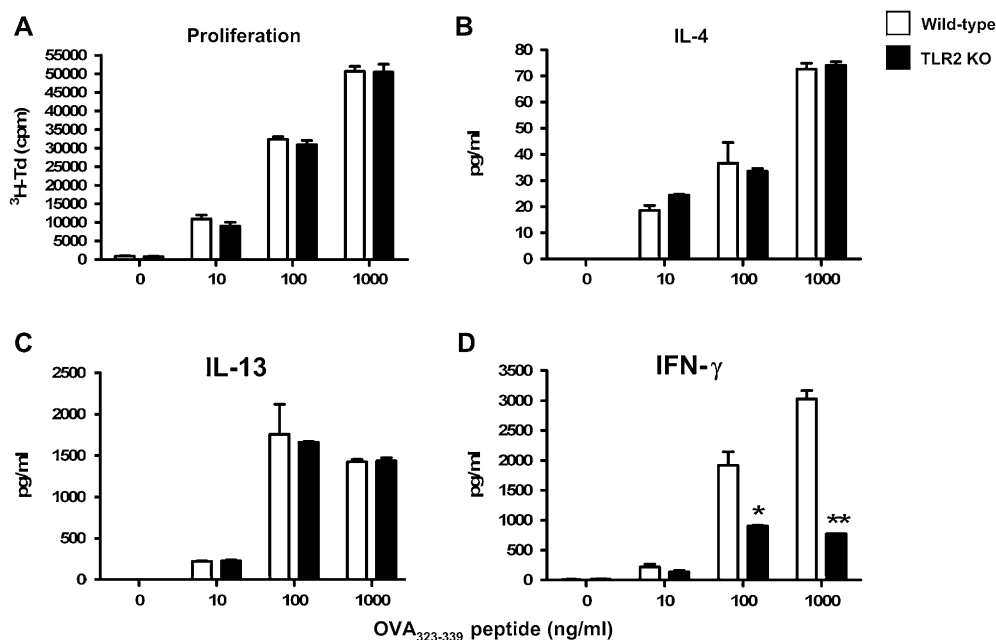


FIG 6. Impaired capacity of TLR2^{-/-} DCs to drive a T_H1 response. Proliferation (**A**) and secretion of IL-4 (**B**), IL-13 (**C**), and IFN-γ (**D**) by CD4⁺ OTII T cells stimulated with OVA₃₂₃₋₃₃₉ peptide in the presence of CD11c⁺ splenic DCs derived from WT and TLR2^{-/-} mice. Data are representative of 3 experiments. **P* < .05 and ***P* < .01. ³H-Td, Tritiated thymidine; KO, knockout.

contrast, DCs from TLR2^{-/-} mice were impaired in their ability to drive IFN-γ production (Fig 6, D). These results indicate that TLR2 expression by DCs is important for T_H1 polarization and suggest that DCs might contribute to the impaired T_H1 response to epicutaneous sensitization.

DISCUSSION

This work demonstrates that TLR2 is important for the T_H1 response to epicutaneous sensitization with protein antigen and contact sensitization with hapten.

TLR2 expression in the skin was upregulated by mechanical skin injury inflicted by means of tape stripping (Fig 1, A). Inasmuch as injury caused by tape stripping mimics injury inflicted by scratching, this observation suggests that TLR2 might be upregulated in the skin of patients with AD after scratching of dry skin. TLR2 expression was also upregulated by application of

the hapten OX (Fig 1, B). Physical injury and application of haptens upregulate nuclear factor κB (NF-κB) expression in the skin,³⁷⁻³⁹ and NF-κB is known to mediate upregulation of TLR2 expression.⁴⁰ The mechanism by which mechanical injury and hapten application upregulates TLR2 expression in skin cells could therefore involve NF-κB. Keratinocytes, DCs, mast cells, and fibroblasts all express TLR2.⁹ Immunohistologic studies are needed to determine which of these cells upregulate TLR2 in the skin after mechanical injury and application of hapten.

Infiltration with CD4⁺ cells and eosinophils and upregulation of mRNA expression for the T_H2 cytokines IL-4, IL-5, and IL-13 and the chemokines thymus and activation-regulated chemokine and eotaxin/CCL11 were all normal in OVA-sensitized skin of TLR2^{-/-} mice (Fig 2 and see Fig E1). In contrast, TLR2^{-/-} mice selectively did not show epidermal thickening and upregulation of IFN-γ mRNA expression in epicutaneously

sensitized skin sites (Fig 2, A and C). We had previously shown that IFN- γ ^{-/-} mice do not have epidermal thickening after epicutaneous sensitization with OVA but have normal infiltration of the dermis by CD4⁺ cells and eosinophils and normal local expression of T_H2 cytokines.³¹ This suggests that the decreased IFN- γ response in TLR2^{-/-} mice underlies the lack of epidermal thickening after epicutaneous sensitization. The failure of TLR2^{-/-} mice to upregulate local expression of IFN- γ mRNA was likely due to their severely impaired T_H1 systemic response to epicutaneous sensitization. This was evidenced by significantly impaired IFN- γ secretion by splenocytes from epicutaneously sensitized TLR2^{-/-} mice after OVA stimulation *in vitro* and by the failure of TLR2^{-/-} mice to mount an OVA-specific IgG2a antibody response to epicutaneous sensitization (Fig 3).

Impaired IFN- γ response and epidermal thickening of TLR2^{-/-} mice to cutaneous sensitization was also observed after contact sensitization with the hapten OX. This was evidenced by decreased swelling of hapten-challenged ears, impaired epidermal thickening, decreased cellular infiltration and edema in these ears, and selective impairment of local expression of IFN- γ mRNA but intact local expression of IL-4 mRNA (Fig 4). Because IFN- γ is known to be important for the response to OX challenge,³⁰ it is likely that decreased local expression of IFN- γ contributed to the decreased inflammation of OX-challenged ears in TLR2^{-/-} mice. TLR2^{-/-} mice were severely impaired in their capacity to mount an IgG2a response to OX but retained an intact IgG1 response (Fig 5). Because IFN- γ drives IgG2a production and IL-4 drives IgG1 production,³⁵ this result strongly suggests that the systemic T_H1 response to hapten sensitization was selectively impaired in TLR2^{-/-} mice.

Splenic DCs from TLR2^{-/-} mice were selectively impaired in their ability to drive a T_H1 response in CD4⁺ T cells. This was evidenced by a significant impairment in the ability of these DCs to drive IFN- γ production by naive OTII T cells in response to OVA peptide (Fig 6). In contrast, the ability of these DCs to support T-cell proliferation and production of T_H2 cytokines was intact. It is possible that splenic DCs in WT mice have been primed by exogenous, endogenous, or both types of TLR ligands *in vivo* to drive a T_H1 response. This is supported by the observation that the TLR2 ligands bacterial lipopeptides act as adjuvants for T_H1 responses in an antigen-presenting cell-dependent manner.¹⁰ Pathogen-associated molecular patterns from the bacterial flora of mouse skin, endogenous TLR2 ligands, or both can promote the T_H1-polarizing capacity of skin DCs that capture antigens or haptens in the skin and traffic to DLN, where they prime naive T cells. Failure of skin DCs to respond to these ligands in TLR2^{-/-} mice would impair their capacity to support a T_H1 response to cutaneous sensitization. TLR2 agonists can also directly stimulate T cells, natural killer cells, and DCs to produce IFN- γ .^{12,41,42} Loss of this effect in TLR2^{-/-} mice might contribute to their impaired T_H1 response to cutaneous sensitization.

IFN- γ is expressed in chronic AD lesions and is thought to play an important role in keratinocyte apoptosis and in perpetuating skin inflammation.^{28,43} Our results suggest that disrupting TLR2–TLR2 ligand interaction might impair the T_H1 response to antigens introduced through the skin and inhibit epidermal thickening and thus could be beneficial in treating AD. Decreased signaling through TLR2 by ligands from skin-colonizing bacteria might contribute the reported efficacy of antibiotics in ameliorating AD skin lesions.⁴⁴

We thank Drs Robert Sidbury and Birgitta Schmidt (Department of Dermatology, Harvard Medical School, Boston, Mass) for help in the analysis of skin histology.

Clinical implications: TLR2 triggering by ligands from skin bacterial flora might contribute to the T_H1 response to cutaneous sensitization in patients with AD and ACD.

REFERENCES

1. Takeda K, Kaisho T, Akira S. Toll-like receptors. *Annu Rev Immunol* 2003;21:335-76.
2. Iwasaki A, Medzhitov R. Toll-like receptor control of the adaptive immune responses. *Nat Immunol* 2004;5:987-95.
3. Akira S, Uematsu S, Takeuchi O. Pathogen recognition and innate immunity. *Cell* 2006;124:783-801.
4. Weigt H, Muhlradt PF, Emmendorffer A, Krug N, Braun A. Synthetic mycoplasma-derived lipopeptide MALP-2 induces maturation and function of dendritic cells. *Immunobiology* 2003;207:223-33.
5. Shimada M, Yanai Y, Okazaki T, Noma N, Kawashima I, Mori T, et al. Hyaluronan fragments generated by sperm-secreted hyaluronidase stimulate cytokine/chemokine production via the TLR2 and TLR4 pathway in cumulus cells of ovulated COCs, which may enhance fertilization. *Development* 2008;135:2001-11.
6. Schaefer L, Babelova A, Kiss E, Hausser HJ, Baliova M, Krzyzankova M, et al. The matrix component biglycan is proinflammatory and signals through Toll-like receptors 4 and 2 in macrophages. *J Clin Invest* 2005;115:2223-33.
7. Yang D, Chen Q, Su SB, Zhang P, Kurosaka K, Caspi RR, et al. Eosinophil-derived neurotoxin acts as an alarmin to activate the TLR2-MyD88 signal pathway in dendritic cells and enhances Th2 immune responses. *J Exp Med* 2008;205:79-90.
8. Cheng N, He R, Tian J, Ye PP, Ye RD. Cutting edge: TLR2 is a functional receptor for acute-phase serum amyloid A. *J Immunol* 2008;181:22-6.
9. Marshall JS, McCurdy JD, Olynch T. Toll-like receptor-mediated activation of mast cells: implications for allergic disease? *Int Arch Allergy Immunol* 2003;132:87-97.
10. Sieling PA, Chung W, Duong BT, Godowski PJ, Modlin RL. Toll-like receptor 2 ligands as adjuvants for human Th1 responses. *J Immunol* 2003;170:194-200.
11. Daehnel K, Gillette-Ferguson I, Hise AG, Diaconu E, Harling MJ, Heinzel FP, et al. Filaria/Wolbachia activation of dendritic cells and development of Th1-associated responses is dependent on Toll-like receptor 2 in a mouse model of ocular onchocerciasis (river blindness). *Parasite Immunol* 2007;29:455-65.
12. Imanishi T, Hara H, Suzuki S, Suzuki N, Akira S, Saito T. Cutting edge: TLR2 directly triggers Th1 effector functions. *J Immunol* 2007;178:6715-9.
13. Khan AQ, Chen Q, Wu ZQ, Paton JC, Snapper CM. Both innate immunity and type 1 humoral immunity to *Streptococcus pneumoniae* are mediated by MyD88 but differ in their relative levels of dependence on toll-like receptor 2. *Infect Immun* 2005;73:298-307.
14. Re F, Strominger JL. Toll-like receptor 2 (TLR2) and TLR4 differentially activate human dendritic cells. *J Biol Chem* 2001;276:37692-9.
15. Hisbergues M, Magi M, Rigaux P, Steuve J, Garcia L, Goudercourt D, et al. *In vivo* and *in vitro* immunomodulation of Der p 1 allergen-specific response by *Lactobacillus plantarum* bacteria. *Clin Exp Allergy* 2007;37:1286-95.
16. Patel M, Xu D, Kewin P, Choo-Kang B, McSharry C, Thomson NC, et al. TLR2 agonist ameliorates established allergic airway inflammation by promoting Th1 response and not via regulatory T cells. *J Immunol* 2005;174:7558-63.
17. Wu Q, Martin RJ, Rino JG, Jeyaseelan S, Breed R, Chu HW. A deficient TLR2 signaling promotes airway mucin production in *Mycoplasma pneumoniae*-infected allergic mice. *Am J Physiol Lung Cell Mol Physiol* 2007;292:L1064-72.
18. Weigt H, Nassenstein C, Tschernig T, Muhlradt PF, Krug N, Braun A. Efficacy of macrophage-activating lipopeptide-2 combined with interferon-gamma in a murine asthma model. *Am J Respir Crit Care Med* 2005;172:566-72.
19. Chisholm D, Libet L, Hayashi T, Horner AA. Airway peptidoglycan and immunostimulatory DNA exposures have divergent effects on the development of airway allergy hypersensitivities. *J Allergy Clin Immunol* 2004;113:448-54.
20. Baker BS, Ovigne JM, Powles AV, Corcoran S, Fry L. Normal keratinocytes express Toll-like receptors (TLRs) 1, 2 and 5: modulation of TLR expression in chronic plaque psoriasis. *Br J Dermatol* 2003;148:670-9.
21. Sumikawa Y, Asada H, Hoshino K, Azukizawa H, Katayama I, Akira S, et al. Induction of beta-defensin 3 in keratinocytes stimulated by bacterial lipopeptides through toll-like receptor 2. *Microbes Infect* 2006;8:1513-21.
22. Lin YT, Wang CT, Chiang BL. Role of bacterial pathogens in atopic dermatitis. *Clin Rev Allergy Immunol* 2007;33:167-77.
23. Abeck D, Mempel M. *Staphylococcus aureus* colonization in atopic dermatitis and its therapeutic implications. *Br J Dermatol* 1998;139(suppl 53):13-6.

24. Cheng JF, Ott NL, Peterson EA, George TJ, Hukee MJ, Gleich GJ, et al. Dermal eosinophils in atopic dermatitis undergo cytolytic degeneration. *J Allergy Clin Immunol* 1997;99:683-92.
25. Taniuchi S, Chihara J, Kojima T, Yamamoto A, Sasai M, Kobayashi Y. Serum eosinophil derived neurotoxin may reflect more strongly disease severity in childhood atopic dermatitis than eosinophil cationic protein. *J Dermatol Sci* 2001;26:79-82.
26. Hasannejad H, Takahashi R, Kimishima M, Hayakawa K, Shiohara T. Selective impairment of Toll-like receptor 2-mediated proinflammatory cytokine production by monocytes from patients with atopic dermatitis. *J Allergy Clin Immunol* 2007;120:69-75.
27. Ahmad-Nejad P, Mrabet-Dahbi S, Breuer K, Klotz M, Werfel T, Herz U, et al. The toll-like receptor 2 R753Q polymorphism defines a subgroup of patients with atopic dermatitis having severe phenotype. *J Allergy Clin Immunol* 2004;113:565-7.
28. Trautmann A, Akdis M, Kleemann D, Altnauer F, Simon HU, Graeve T, et al. T cell-mediated Fas-induced keratinocyte apoptosis plays a key pathogenetic role in eczematous dermatitis. *J Clin Invest* 2000;106:25-35.
29. Tamura T, Matsubara M, Takada C, Hasegawa K, Suzuki K, Ohmori K, et al. Effects of olopatadine hydrochloride, an antihistamine drug, on skin inflammation induced by repeated topical application of oxazolone in mice. *Br J Dermatol* 2004;151:1133-42.
30. Thomson JA, Trout AB, Kelso A. Contact sensitization to oxazolone: involvement of both interferon-gamma and interleukin-4 in oxazolone-specific Ig and T-cell responses. *Immunology* 1993;78:185-92.
31. Spergel JM, Mizoguchi E, Oettgen H, Bhan AK, Geha RS. Role of TH1 and TH2 cytokines in a murine model of allergic dermatitis. *J Clin Invest* 1999;103:1103-11.
32. Spergel JM, Mizoguchi E, Brewer JP, Martin TR, Bhan AK, Geha RS. Epicutaneous sensitization with protein antigen induces localized allergic dermatitis and hyperresponsiveness to methacholine after single exposure to aerosolized antigen in mice. *J Clin Invest* 1998;101:1614-22.
33. Pfaffl MW. A new mathematical model for relative quantification in real-time RT-PCR. *Nucleic Acids Res* 2001;29:2003-7.
34. Woodward AL, Spergel JM, Alenius H, Mizoguchi E, Bhan AK, Castigli E, et al. An obligate role for T-cell receptor alphabeta+ T cells but not T-cell receptor gammadelta+ T cells, B cells, or CD40/CD40L interactions in a mouse model of atopic dermatitis. *J Allergy Clin Immunol* 2001;107:359-66.
35. Stevens TL, Bossie A, Sanders VM, Fernandez-Botran R, Coffman RL, Mosmann TR, et al. Regulation of antibody isotype secretion by subsets of antigen-specific helper T cells. *Nature* 1988;334:255-8.
36. Robert C, Kupper TS. Review articles: mechanisms of disease: inflammatory skin diseases, T cells, and immune surveillance. *N Engl J Med* 1999;341:1817-28.
37. Karimipour DJ, Kang S, Johnson TM, Orringer JS, Hamilton T, Hammerberg C, et al. Microdermabrasion: a molecular analysis following a single treatment. *J Am Acad Dermatol* 2005;52:215-23.
38. Schmidtke A, Burian M, Altis K, Hardt K, Angioni C, Schmidt R, et al. Pharmacological and histopathological characterization of a hyperalgesia model induced by freeze lesion. *Pain* 2007;127:287-95.
39. Goebeler M, Roth J, Brocker EB, Sorg C, Schulze-Osthoff K. Activation of nuclear factor-kappa B and gene expression in human endothelial cells by the common haptens nickel and cobalt. *J Immunol* 1995;155:2459-67.
40. Johnson CM, Tapping RI. Microbial products stimulate human Toll-like receptor 2 expression through histone modification surrounding a proximal NF-kappaB-binding site. *J Biol Chem* 2007;282:31197-205.
41. Sawaki J, Tsutsui H, Hayashi N, Yasuda K, Akira S, Tanizawa T, et al. Type 1 cytokine/chemokine production by mouse NK cells following activation of their TLR/MyD88-mediated pathways. *Int Immunol* 2007;19:311-20.
42. Fricke I, Mitchell D, Mittelstadt J, Lehan N, Heine H, Goldmann T, et al. Mycobacteria induce IFN-gamma production in human dendritic cells via triggering of TLR2. *J Immunol* 2006;176:5173-82.
43. Carroll JM, Crompton T, Seery JP, Watt FM. Transgenic mice expressing IFN-gamma in the epidermis have eczema, hair hypopigmentation, and hair loss. *J Invest Dermatol* 1997;108:412-22.
44. Breuer K, Haussler S, Kapp A, Werfer T. *Staphylococcus aureus*: colonizing features and influence of an antibacterial treatment in adults with atopic dermatitis. *Br J Dermatol* 2002;147:55-61.

Have you seen the *JACI* Web site?

Enjoy these benefits and more:

- Stay current on key articles with Saved Searches and Table of Contents e-mail alerts.
- Link from cited references to abstracts and full text of other participating journals.

Visit www.jacionline.org today to see what else is new online!

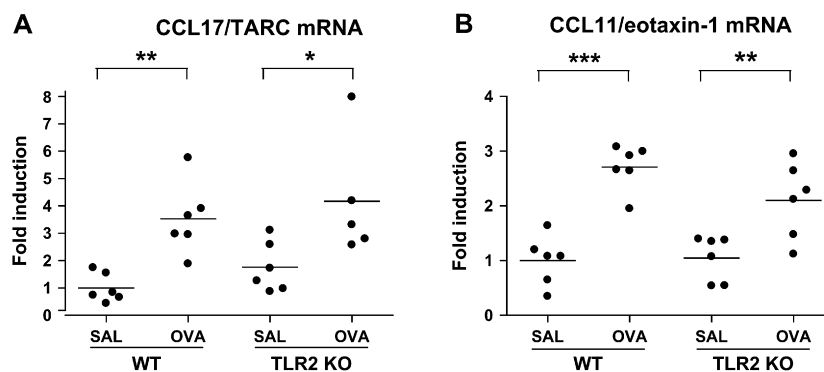


FIG E1. CCL17/thymus and activation-regulated (*TARC*) chemokine (**A**) and CCL11/eotaxin-1 (**B**) mRNA expression in OVA-sensitized skin of TLR2^{-/-} mice (n = 5-6). **P* < .05, ***P* < .01, and ****P* < .001. SAL, saline; KO, knockout.