

COX-2 inhibition enhances the T_H2 immune response to epicutaneous sensitization

Dhafer Laouini, PhD, Abdala ElKhal, PhD, Ali Yalcindag, MD, Seiji Kawamoto, MD, PhD, Hans Oettgen, MD, PhD, and Raif S. Geha, MD *Boston, Mass*

Background: Mechanical injury to the skin by scratching is an important feature of atopic dermatitis (AD).

Objective: To investigate the role of COX-2 in allergic skin inflammation elicited by epicutaneous (EC) sensitization via introduction of ovalbumin through shaved tape-stripped skin. **Methods:** COX-2 mRNA was measured by quantitative PCR, and COX-2 protein was measured by Western blotting. We investigated the effect of administration of the COX-2 selective inhibitor NS-398 during EC sensitization with ovalbumin in a mouse model of AD characterized by eosinophil skin infiltration, elevated total and antigen specific IgE, and a systemic T_H2 response to antigen. We further examined the response of COX-2-deficient mice to EC immunization with ovalbumin.

Results: Tape stripping caused a transient increase in skin COX-2 mRNA. In contrast, COX-2 mRNA was not increased after ovalbumin sensitization. Infiltration by eosinophils and expression of IL-4 mRNA in ovalbumin-sensitized skin sites, ovalbumin specific IgE and IgG₁ antibody responses, and IL-4 secretion by splenocytes after ovalbumin stimulation were all significantly increased in EC mice that received NS-398. In contrast, ovalbumin specific IgG_{2a} antibody response and IFN- γ secretion by splenocytes after ovalbumin stimulation were significantly decreased in these mice. COX-2-deficient mice also exhibited an enhanced systemic T_H2 response to EC sensitization.

Conclusion: These results demonstrate that COX-2 limits the T_H2 response to EC sensitization and suggest that COX inhibitors may worsen allergic skin inflammation in patients with AD. (*J Allergy Clin Immunol* 2005;116:390-6.)

Key words: Atopic dermatitis, allergic skin inflammation, NS-398, COX-2, T_H1, T_H2

Prostaglandins are formed by the oxidative cyclization of the central carbons within 20 carbon polyunsaturated fatty acids. 5-COX is the key enzyme involved in the

Abbreviations used

AD:	Atopic dermatitis
BAL:	Bronchoalveolar lavage
CysLT:	Cysteinyl leukotriene
DC:	Dendritic cell
EC:	Epicutaneous
HPF:	High-power field
PG:	Prostaglandin
PPAR:	Peroxisome proliferator-activated receptor
WT:	Wild type

conversion of arachidonic acid to prostaglandin (PG) G₂ and PGH₂. PGH₂ is subsequently converted to a variety of eicosanoids that include PGE₂, PGD₂, PGF_{2 α} , PGI₂, and thromboxane A₂.¹ The spectrum of prostaglandins produced depends on the downstream enzymatic machinery expressed in a particular cell type. Prostaglandins have both autocrine and paracrine effects. These are mediated by an array of receptors, which are differentially expressed by various cell types. Two classes of prostaglandin receptors exist: the membrane G-coupled receptor class, ie, E-prostanoid 1-4 receptors for PGE₂; and the nuclear peroxisome proliferator-activated receptor (PPAR) class, ie, PPAR α , PPAR γ , and PPAR δ , which acts as a transcription factor on ligand binding.² Nonsteroidal anti-inflammatory drugs inhibit COX, leading to a marked decrease in prostaglandin synthesis and inflammation.³

Two COX isoforms, COX-1 and COX-2, have been identified and are encoded by distinct genes.⁴ COX-1 is expressed in nearly all tissues under basal conditions, suggesting that its major function is to generate prostaglandin precursors for homeostatic regulation.⁵ COX-2 is mainly an inducible enzyme. Inflammatory cytokines, which include IL-1 and TNF- α , and growth factors, which include TGF- α , platelet-derived growth factor, epidermal growth factor, and fibroblast growth factor, all have been shown to induce COX-2 expression.⁶⁻⁹

Prostaglandins have profound effects on the immune response. A large body of data suggests that addition of PGE₂ *in vitro* inhibits IL-12 production and promotes IL-10 production by antigen-presenting cells, inhibits the production of T_H1 cytokines, and promotes T_H2 cell differentiation.^{10,11} Furthermore, PGE₂ was shown to enhance IL-4-driven isotype switching to IgE.¹² Topical application of PGE₂ suppresses the cutaneous immune

From the Division of Immunology, Children's Hospital; and the Department of Pediatrics, Harvard Medical School.

Dr Laouini and Dr ElKhal contributed equally to the article.

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Reprint requests: Raif S. Geha, MD, Enders 8, Division of Immunology, Children's Hospital, 300 Longwood Avenue, Boston, MA 02115. E-mail: raif.geha@childrens.harvard.edu.

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response.¹³ The PPAR γ agonist 15-deoxy- δ (12,14)-prostaglandin J(2) also inhibits IL-12 production by macrophages¹⁴ and ameliorates experimental autoimmune encephalomyelitis.¹⁵ In a model of allergic airway inflammation, COX inhibition by nonsteroidal anti-inflammatory drug increased IL-5 and IL-13 production in bronchoalveolar lavage (BAL) fluid and airway hyperresponsiveness/AHR.¹⁶ Furthermore, lung inflammatory indices, which include BAL cells, proteins, and IgE as well as lung inflammation as determined by histopathology, were significantly increased in the absence of either COX-1 or COX-2.¹⁷

COX-1 is basally expressed at low levels in skin.¹⁸ Skin injury by UV light has been shown to induce COX-2 expression,¹⁹ whereas mechanical injury increases PGE₂ in the skin.²⁰ This may be mediated by IL-1 and TNF- α released from keratinocytes, fibroblasts, and mast cells.²¹⁻²³ Atopic dermatitis (AD) is an inflammatory skin disease that frequently occurs in subjects with personal or family history of atopic disease.²⁴ Mechanical injury to the skin by scratching is an important feature of AD. We have developed a mouse model of allergic skin inflammation elicited by epicutaneous (EC) sensitization with ovalbumin. This model displays many of the features of human AD, including a dermatitis characterized by dermal infiltration of T cells and eosinophils and increased local expression of T_H2 cytokines and by a systemic allergen specific T_H2 response characterized by IgG₁ and IgE antibodies and IL-4 secretion by splenocytes after *in vitro* stimulation with ovalbumin.²⁵ We used this model to assess the role of COX-2 in allergic skin inflammation.

METHODS

Mice

BALB/c mice were obtained from the Jackson Laboratory (Bar Harbor, Me). COX-2^{+/-} mice on C57BL6x129/SvImJ background and genetically matched controls were obtained from Taconic (Germantown, NY). Homozygous COX-2-deficient mice were obtained by genotyping the offspring of COX-2^{+/-} parents, and, as previously described, were not fertile.²⁶ All mice were kept in a pathogen-free environment. All procedures performed on the mice were in accordance with the Animal Care and Use Committee of the Children's Hospital.

EC sensitization

EC sensitization of female mice 4 to 6 weeks old was performed as described previously.²⁵ Briefly, the skin of anesthetized mice was shaved and tape-stripped 6 times. Ovalbumin (grade V; Sigma Chemical Co, St Louis, Mo) 100 μ g in 100 μ L normal saline, or placebo (100 μ L normal saline), was placed on a patch of sterile gauze (1 cm $\times$ 1 cm), which was secured to the skin with a transparent bio-occlusive dressing (Tegaderm, Owens & Minor Inc, Franklin, Mass). Each mouse had a total of three 1-week exposures to the patch separated by 2-week intervals. On day 49, the mice were killed and their tissues examined.

Treatment with COX-2 inhibitor

Mice were given 1 mg/kg of the selective COX-2 inhibitor NS-398 (Biomol Research Laboratories, Inc, Plymouth Meeting, Pa) intra-

peritoneally daily for the duration of the sensitization period. NS-398 (25 mg/mL in dimethyl sulfoxide) was diluted in a 5% NaHCO₃ solution before injection.

Histological analysis

Specimens were fixed in 10% buffered formalin and embedded in paraffin. Multiple 4- μ m sections were stained with hematoxylin and eosin. Individual cell types were counted blinded in 15 to 20 high-power fields (HPFs) at 1000 $\times$.

Quantitative RT-PCR for COX enzyme mRNA expression

Five hundred milligrams of skin was homogenized by using a Polytron RT-3000 (Kinematica AG, Brinkmann Instruments Inc) in lysis buffer solution provided in the RNeasy extraction kit (Ambion Inc, Austin, Tex). RT was performed by using transcript first-strand cDNA synthesis kit (Roche Diagnostic, Foster City, Calif). PCR reactions were run on an ABI Prism 7700 (Applied Biosystems, Foster City, Calif) sequence detection system platform. Taqman primers with 6-carboxyfluorescein-labeled probe were obtained from Applied Biosystems. The housekeeping gene β 2-microglobulin was used as a control. The relative gene expression among the different samples was determined by using the method described by Pfaffl.²⁷

Determination of COX-2 protein expression and PGE₂ levels in skin

Five hundred milligrams of skin was homogenized in 1 mL 0.1 mol/L PBS solution (pH = 7.4) containing 1 mmol/L EDTA, 0.1 mmol/L indomethacin, and a cocktail of protease inhibitors. Fifteen microliters of this solution was used for Western blotting for COX-2, with a rabbit anti-COX-2 antiserum (Abcam Inc, Cambridge, Mass) followed by horseradish peroxidase-conjugated donkey antirabbit antibody (Amersham Bioscience, Temecula, Calif). The blots were reprobed with mAb to actin (Chemicon International, Piscataway, NJ) followed by horseradish peroxidase-conjugated sheep antimouse antibody (Amersham Bioscience) for loading control. The rest of the material was extracted as described,²⁸ and the extract used to determine PGE₂ concentration by ELISA (Cayman Chemicals, Ann Arbor, Mich).

Competitive RT-PCR evaluation of cytokine mRNA in skin

Competitive RT-PCR evaluation of cytokine mRNA in skin was performed as described previously.²⁹ Skin biopsies were immediately frozen in dry ice. The samples were homogenized in Trizol (GIBCO BRL, Carlsbad, Calif) by using a Polytron RT-3000. RNA extraction was performed following the manufacturer's instructions. cDNA was synthesized from 10 μ g total RNA in a 40- μ L reaction mix by using Superscript II (GIBCO BRL). The primers used to amplify cDNA for β 2-microglobulin, IL-4, and IFN- γ and DNA amplification were as described previously.²⁹ To quantify cytokine mRNA, a fixed amount of reverse-transcribed cellular mRNA was coamplified in the presence of serial dilutions of a multispecific internal plasmid control (pMUS3), which contains nucleotide sequences of multiple cytokines.³⁰ Results were expressed as a ratio of cytokine cDNA to β 2-microglobulin cDNA. We have recently found that the results of competitive RT-PCR for determination of cytokine mRNA in skin compare favorably with those of quantitative RT-PCR. In 2 experiments in BALB/c mice, each using 6 mice with EC with ovalbumin and 6 mice with EC with saline, we found that the mean increase in skin IL-4 mRNA expression after ovalbumin sensitization was 4.9-fold using competitive PCR compared with 4.3-fold using quantitative RT-PCR.

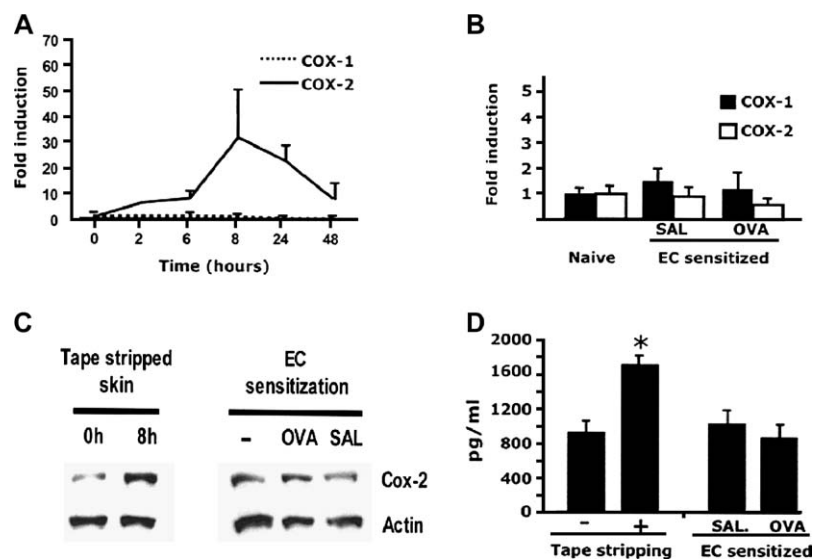


FIG 1. COX-2 and COX-1 mRNA expression in the skin of BALB/C mice after tape stripping ($n = 2$ per group; **A**) and after EC sensitization with ovalbumin (OVA) and saline (SAL) sensitization ($n = 5$ per group; **B**), expressed as fold induction over levels in unmanipulated skin. **C**, COX-2 protein expression in skin after tape stripping (left panel) and EC sensitization (right panel). Results are representative of 3 experiments. **D**, PGE₂ levels in skin ($n = 4$ per group). Columns and bars represent means and SEMs. * $P < .05$.

Serum antibody determinations

IgG₁, IgG_{2a}, and IgE antiovalbumin antibodies were determined by ELISA following the procedures we previously described.²⁵

IL-4 and IFN- γ synthesis by spleen cells

Single cell suspensions of spleen cells were prepared and cultured at 2×10^6 /mL in 24-well plates in the presence of ovalbumin (50 μ g/mL) as previously described.³¹ Supernatants were collected after 96 hours. IL-4 and IFN- γ were determined by ELISA (Pharmingen, San Diego, Calif).

Statistical analysis

The nonparametric Mann-Whitney test was used to compare the different mice groups.

RESULTS

Tape stripping induces expression of COX-2 mRNA in normal mouse skin

We used quantitative RT-PCR to examine the effect of tape stripping on COX-2 mRNA expression in mouse skin. Low levels of COX-2 mRNA were detectable in uninjured skin. After tape stripping 6 times, COX-2 mRNA expression increased, with peak levels 8 hours poststripping, and returning to normal 48 hours later (Fig 1, A). In contrast, there was no detectable increase in the levels of COX-1 mRNA levels in the skin after tape stripping. COX-2 and COX-1 mRNA levels in ovalbumin-sensitized skin sites did not significantly differ from those in saline-sensitized or unmanipulated skin sites (Fig 1, B). Western blotting analysis demonstrated that COX-2 protein expression in the skin increased 8 hours after tape stripping (Fig 1, C). In contrast, there was no detectable increase in COX-2 protein expression in ovalbumin-

sensitized skin sites compared with saline-sensitized skin sites or with unmanipulated skin (Fig 1, C). The increased COX-2 mRNA expression observed 8 hours after stripping was associated with significantly increased level of the COX metabolite PGE₂ (Fig 1, D). There was no increase in PGE₂ levels in either ovalbumin-sensitized or saline-sensitized skin sites.

Eosinophil infiltration is increased in ovalbumin-sensitized skin sites of mice treated with COX-2 inhibitor

There was no difference in the numbers of eosinophils in saline sensitized sites of untreated mice and mice treated with NS-398 (Fig 2, A). Ovalbumin sensitization caused a significant increase in the number of eosinophils in the skin of control untreated BALB/c mice, consistent with previous observations.²⁵ There were significantly more eosinophils in ovalbumin-sensitized skin of mice treated with NS-398 than in ovalbumin-sensitized skin of untreated controls (Fig 2, A). Ovalbumin sensitization caused an increase in skin mononuclear cells that was modestly but significantly higher in mice treated with NS-398 compared with untreated controls (Fig 2, B).

IL-4 expression is increased in EC skin sites of mice treated with COX-2 inhibitor

We used competitive PCR to measure cytokine mRNA in skin. Low and comparable levels of IL-4 and IFN- γ mRNA were detected in saline sensitized skin from untreated mice and mice treated with NS-398 (Fig 3). Consistent with previous results,²⁵ expression of IL-4 mRNA, but not IFN- γ mRNA, markedly increased in ovalbumin-sensitized skin sites of untreated control BALB/c mice. IL-4 mRNA was significantly increased

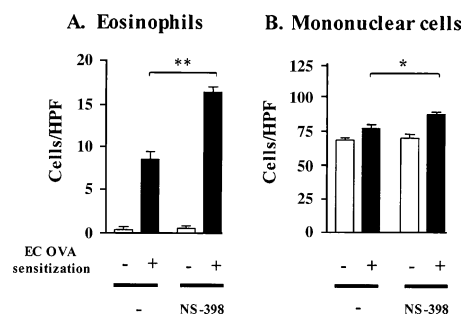


FIG 2. Effect of the COX-2 inhibitor NS-398 on the number of infiltrating eosinophils (**A**) and mononuclear cells (**B**) in ovalbumin (OVA)-sensitized and saline-sensitized skin sites of untreated mice and in mice treated with NS-398. The columns and error bars represent means $\pm$ SEMs/HPF of cells calculated by examining 15 to 20 HPFs per mouse ($n = 6$). * $P < .05$. ** $P < .01$.

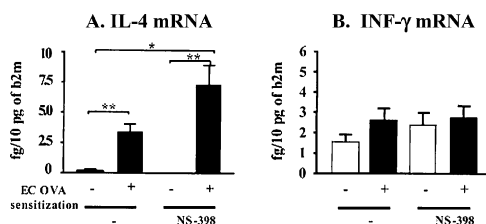


FIG 3. Effect of the COX-2 inhibitor NS-398 on IL-4 (**A**) and IFN- γ (**B**) mRNA expression in saline and ovalbumin (OVA)-sensitized skin. Levels were normalized to β 2-microglobulin. Pooled results of experiments using 6 mice per group. Bars represent means $\pm$ SEMs. * $P < .05$. ** $P < .01$.

in ovalbumin-sensitized skin of mice treated with NS-398 compared with untreated ovalbumin-sensitized controls. These results suggest that COX-2 products normally downregulate the T_H2 cytokine profile of infiltrating T cells.

Treatment with COX-2 inhibitor enhances antigen specific IgE and IgG₁ antibody responses to EC sensitization with ovalbumin

The T_H2 cytokine IL-4 plays an important role in isotype switching to IgE and IgG₁, whereas the T_H1 cytokine IFN- γ plays an important role in isotype switching to IgG_{2a}.³² To investigate whether COX-2 inhibition enhanced the systemic T_H2 response to EC sensitization with ovalbumin, we measured total and ovalbumin specific IgE and IgG₁ in serum. Fig 4, A, shows that ovalbumin specific IgG₁ and IgE levels were significantly higher in mice treated with NS-398. Treatment with NS-398 had no effect on the IgG_{2a} antibody response. These results suggest that COX products normally downregulate the systemic IgE and IgG₁ antibody response to EC-introduced antigen.

COX-2 inhibition causes increased systemic T_H2 response and decreased systemic T_H1 response to EC sensitization

We have previously shown that splenocytes from BALB/c mice with EC with ovalbumin secrete IL-4, and

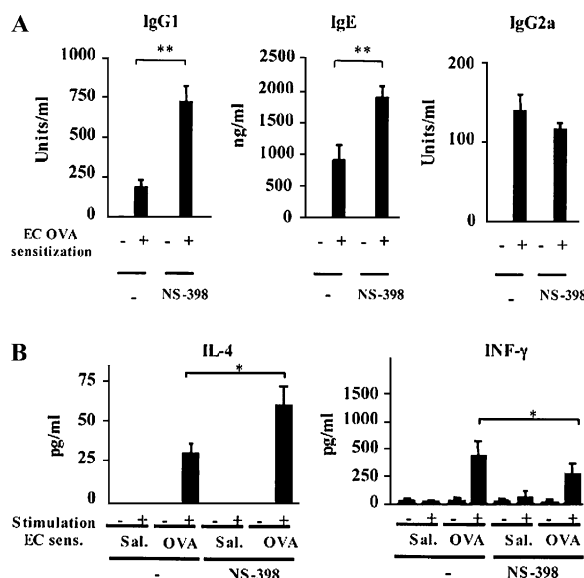


FIG 4. Effect of the COX-2 inhibitor NS-398 on (**A**) serum levels of ovalbumin (OVA) specific IgG, IgE, and IgG_{2a} and (**B**) cytokine production by spleen cells from EC mice ($n = 6$ for each group). Columns and error bars represent means $\pm$ SEMs. * $P < .05$. ** $P < .01$. SAL., Saline; Sens., sensitization.

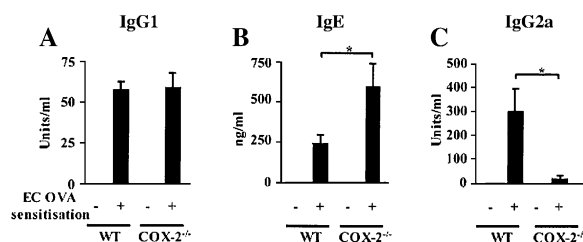


FIG 5. Serum levels of ovalbumin (OVA) specific IgG₁ (**A**), IgE (**B**), and IgG_{2a} (**C**) in EC mice, COX-2^{-/-} mice, and WT controls ($n = 6$ for each group). Columns and error bars represent means $\pm$ SEMs. * $P < .05$.

IFN- γ after ovalbumin stimulation *in vitro*.³¹ Fig 4, B, shows that splenocytes from EC mice treated with NS-398 secreted significantly higher amounts of IL-4, and significantly less IFN- γ , than splenocytes of unsensitized, untreated controls. These results suggest that COX products normally limit the systemic T_H2 response to EC-introduced antigen and promote the systemic T_H1 response.

Increased systemic T_H2 response and decreased systemic T_H1 response in COX-2-deficient mice

NS-398 may have effects other than COX-2 enzyme inhibition. To ascertain that the effect of NS-398 on the T_H response to EC sensitization was a result of COX-2 inhibition, we examined the response of COX-2^{-/-} mice to EC immunization. Fig 5 shows that ovalbumin specific IgE levels were significantly higher and ovalbumin specific IgG_{2a} levels were significantly lower in

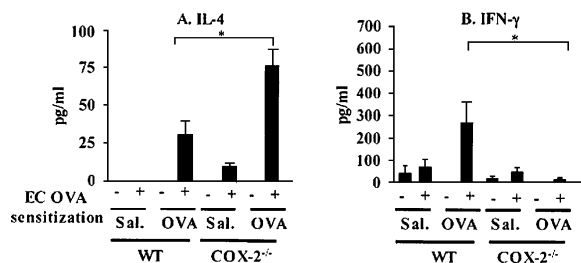


FIG 6. Cytokine production by spleen cells from EC mice, COX-2^{-/-} mice, and WT controls (n = 6 for each group). Columns and error bars represent means $\pm$ SEMs. *P < .05. SAL., Saline.

COX-2-deficient mice than in wild-type (WT) controls. COX-2 deficiency had no detectable effect on the IgG₁ antibody response. Fig 6 shows that splenocytes from EC-immunized COX-2^{-/-} mice secreted significantly more IL-4 and significantly less IFN-γ than splenocytes of genetically matched WT controls.

DISCUSSION

The results of this study suggest that COX-2 products limit the systemic T_H2 response and enhance the systemic T_H1 response to epicutaneously introduced antigen and promote skin infiltration with eosinophils in a mouse model of allergic dermatitis.

Both COX-1 and COX-2 were expressed in unmanipulated mouse skin (Fig 1, A), consistent with previous reports on mouse and human skin.^{18,33,34} Mechanical injury by tape stripping transiently upregulated COX-2 mRNA but not COX-1 mRNA expression in mouse skin. This finding is consistent with the observation that PGE₂ levels increase in human skin after tape stripping,²⁰ which we confirmed in this mouse study (Fig 1, C). Keratinocytes, fibroblasts, mast cells, endothelial cells, and tissue macrophages have all been reported to express COX-2 after activation.²¹⁻²³ IL-1β and TNF-α are known inducers of COX-2 mRNA expression.^{6,7} A correlation has been observed between levels of PGE₂ and IL-1α in tape-stripped skin.²⁰

The COX-2 selective inhibitor NS-398 enhanced dermal infiltration with eosinophils in our model (Fig 2, A). Eosinophil infiltration of the skin in our model is dependent on their expression of the eotaxin receptor CCR3,³¹ and that eotaxin expression is dependent on IL-4.²⁹ Expression of mRNA for the T_H2 cytokine IL-4, but not for the T_H1 cytokine IFN-γ, in ovalbumin-sensitized skin sites was significantly enhanced in NS-398-treated mice (Fig 3). This may have contributed to increased eosinophil skin infiltration. The T_H2 cytokine IL-5 is also important for eosinophil infiltration of the skin in our model. Although we did not measure IL-5 expression, it is likely that it was also enhanced in NS-398-treated mice and contributed to their exaggerated skin eosinophilia, because IL-5 and IL-4 expression by T_H2 cells is usually concordant.

The increased infiltration by mononuclear cells observed in ovalbumin-sensitized skin of NS-398-treated mice (Fig 2, B) may reflect the stronger T_H2 response exhibited by these mice. Tape stripping induces the expression of the T_H2 selective chemokines thymus and activation-regulated chemokine and cutaneous T cell-attracting chemokine, which attract T_H2 cells in the skin (unpublished data). Secretion of the T_H2 cytokines IL-4 and IL-13 by infiltrating T_H2 cells further upregulates the expression of T_H2 selective chemokines by skin cells³⁵ and enhances T_H2 cell infiltration. Increased secretion of T_H2 cytokines in the skin of NS-398-treated mice may contribute to the enhanced infiltration of T cells in the skin of these mice.

Treatment of mice with the COX-2 inhibitor NS-398 promoted the T_H2 systemic response to EC sensitization. This was evidenced by enhanced serum IgG₁ and IgE antibody levels to ovalbumin mice (Fig 4, A) and increased IL-4 secretion by splenocytes in response to stimulation with ovalbumin (Fig 4, B). In contrast, IFN-γ secretion was significantly decreased (Fig 4, B). Studies with COX-2^{-/-} mice strongly supported the conclusion that the enhancing effect of NS-398 on the T_H2 response to EC immunization was a result of inhibition of COX-2 activity. After EC immunization, these mice mounted a significantly higher ovalbumin specific IgE antibody response and their splenocytes secreted significantly more IL-4 and significantly less IFN-γ than splenocytes from WT controls (Figs 5 and 6).

Taken together, our results suggest that COX-2 products normally limit the development of T_H2 cytokines and promote the development of T_H1 cytokines in response to EC sensitization with antigen. The fact that there was no detectable increase in COX-2 or COX-1 mRNA expression, COX-2 protein expression, or PGE₂ levels in ovalbumin-sensitized skin compared with either saline sensitized or unmanipulated skin (Fig 1, B-D) suggests that the effects of COX-2 inhibition may be exerted early in the sensitization phase of our model, which is dependent on tape stripping, because we are unable to sensitize the mice without it. However, we cannot rule out an effect on later events via the inhibition of baseline COX-2 activity in the skin.

Our findings of increased IL-4 response to EC sensitization with inhibition or lack of COX-2 is in agreement with previous findings that administration of the COX inhibitor indomethacin 2 days before and during intraperitoneal immunization enhances mRNA expression and secretion of the T_H2 cytokines IL-5 and IL-13 in the lung after allergen challenge.¹⁶ Similarly, allergic lung inflammation as evidenced by eosinophils in BAL fluid and lung histopathology and T_H2 cytokine secretion are enhanced in intraperitoneally immunized mice deficient in COX-1 or COX-2.^{17,36} In these studies, COX inhibition did not result in a significant increase in the IgE antibody response to intraperitoneal immunization, and T_H cytokine secretion by splenic T cells was not examined.

There is a plethora of evidence that the COX-2 product PGE₂, which is present in skin of patients with AD,³⁷

inhibits the development of T_H1 cells and promotes the development of T_H2 cells *in vitro*,^{10,38} although discrepant results have also been reported.^{39,40} This inhibitory effect is exerted in a large part at the level of the dendritic cells, because PGE_2 is a potent inhibitor of IL-12 production by these cells¹⁰ and an inhibitor of IL-12 β 1 receptor and IL-12 β 2 receptor expression.⁴¹ On the basis of these *in vitro* results, one would expect that decreased PGE_2 generation in the skin subsequent to inhibition or lack of COX-2 may promote the T_H1 response and inhibit the T_H2 response to EC sensitization. In fact, the reverse was observed. However, PGE_2 may not be the most abundant or most relevant prostaglandin generated in injured skin. Langerhans cells generate high amounts of PGD_2 but very little amounts of other prostaglandins.⁴² The prostanoid PGI_2 limits lung allergic inflammation,⁴³ and mice deficient in the PGI_2 receptor mount an exaggerated T_H2 response.^{43,44} Further studies are needed to examine the nature of prostaglandins that accumulate in the skin after mechanical injury and the roles of individual prostaglandins in modulating the T_H response to EC sensitization.

Inhibition or lack of COX-2 activity may result in enhanced leukotriene synthesis because of both increased availability of arachidonic acid substrate and release from the inhibitory effect of prostaglandins on 5-lipoxygenase-activating protein expression.⁴⁵ Increased amounts of leukotriene C_4 in the skin may promote CC chemokine ligand 19-dependent mobilization of antigen bearing dendritic cells (DC) to lymph nodes,⁴⁶ resulting in the exaggeration of what is already a T_H2 -skewed response to EC sensitization. Cysteinyl leukotrienes (cysLTs) may promote the induction of T_H2 responses by DCs, as suggested by the observation that intranasal administration of DCs pulsed with antigen and cysLTs enhance allergic inflammation compared with DCs pulsed with antigen alone.⁴⁷ CysLTs also promote eosinophil locomotion and hence infiltration at skin sites of allergic sensitization.⁴⁸

EC sensitization of mice is relevant to human sensitization because it mimics allergen sensitization via abraded skin in patients with AD. Although there are no data on COX-2 expression in human AD, COX products are increased in the skin of patients with AD,³⁷ and COX-2 gene expression is induced by IL-13, which is expressed in AD lesions.⁴⁹ Our results clearly show that COX-2 inhibition may exacerbate AD by promoting the systemic and cutaneous T_H2 response and are best avoided in this disease.

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