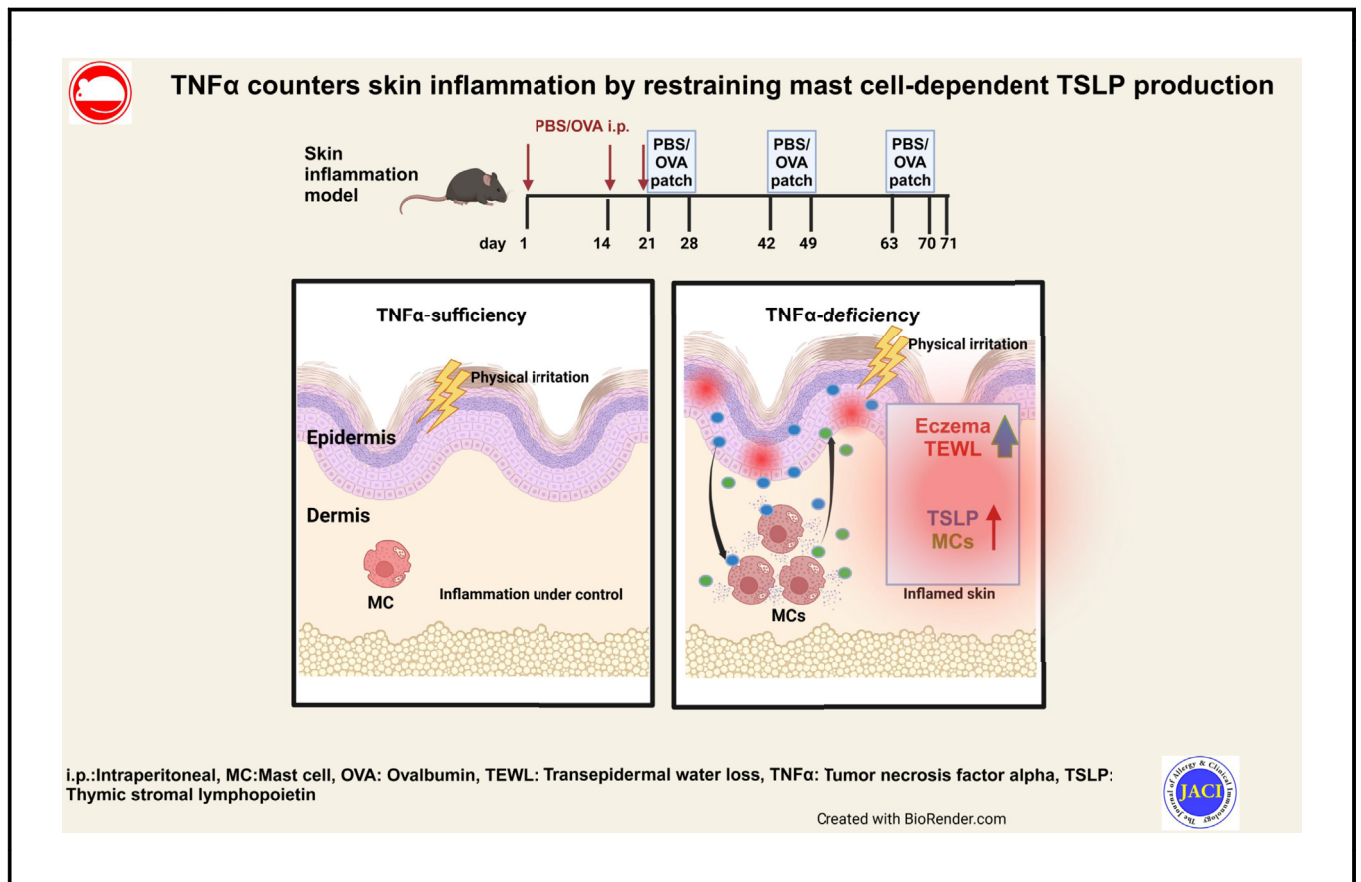


# TNF- $\alpha$ counters skin inflammation by restraining mast cell-dependent thymic stromal lymphopoietin production



Davender Redhu, PhD, Vandana Kumari, PhD, Kristin Franke, PhD, Karin Hartmann, MD, Margitta Worm, MD, Magda Babina, PhD

## GRAPHICAL ABSTRACT



**Capsule summary:** TNF- $\alpha$  deficiency promotes skin inflammation driven by mast cells and thymic stromal lymphopoietin.

# TNF- $\alpha$ counters skin inflammation by restraining mast cell-dependent thymic stromal lymphopoietin production



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**Background:** TNF- $\alpha$  is an important proinflammatory cytokine, but its neutralization in the management of inflammatory skin disorders like psoriasis may trigger eczematous skin lesions as an adverse reaction.

**Objectives:** This study aimed to elucidate whether TNF- $\alpha$  may protect from skin inflammation and to identify in detail the underlying mechanisms.

**Methods:** Wild-type, TNF- $\alpha$ -deficient, thymic stromal lymphopoietin (TSLP) receptor-deficient, mast cell (MC)-deficient, TNF- $\alpha$ -TSLP receptor double-deficient, and TNF- $\alpha$ -MC double-deficient mice were subjected to a skin inflammation model and inspected by physical, clinical, histologic, immunohistochemical, and bioanalytic techniques.

**Results:** TNF- $\alpha$  deficiency promoted skin inflammation. This was accompanied by MC hyperplasia and potent TSLP production in lesional skin and serum of TNF- $\alpha$ -deficient mice. Specifically, MCs were found to be responsible for inducing high levels of TSLP in the epidermis, compromising barrier function and initiating inflammation. In contrast, the production of immunoglobulins, including IgE, was reduced in mice lacking TNF- $\alpha$ .

**Conclusions:** TNF- $\alpha$  restrains MC-dependent TSLP production and the onset of eczema. (*J Allergy Clin Immunol* 2025;156:150-8.)

**Key words:** Tumor necrosis factor alpha, eczema, mast cells, thymic stromal lymphopoietin

## Abbreviations used

AD:	Atopic dermatitis
KC:	Keratinocyte
MC:	Mast cell
MRGPRX2:	Mas-related G protein-coupled receptor X2
OVA:	Ovalbumin
PAR-2:	Protease-activated receptor 2
PBS:	Phosphate-buffered saline
qPCR:	Real-time quantitative PCR
SCF:	Stem cell factor
SEM:	Standard error of mean
TEWL:	Transepidermal water loss
TSLP:	Thymic stromal lymphopoietin
TSLPR:	TSLP receptor
WT:	Wild type

TNF- $\alpha$  is a key proinflammatory mediator in the body, thereby promoting a wide array of inflammatory diseases.<sup>1,2</sup> Consequently, TNF- $\alpha$  is successfully targeted in autoimmune and inflammatory disorders like rheumatoid arthritis and psoriasis.<sup>3,4</sup> However, TNF- $\alpha$ -targeted therapies can induce inflammatory conditions like atopic dermatitis (AD)-like eczema as drug-induced adverse reactions (so-called  $\gamma$ -type reactions) associated with cytokine or immune imbalance,<sup>5</sup> challenging the traditional understanding of TNF- $\alpha$ 's function.<sup>6</sup> The mechanisms underlying this intriguing phenomenon remain elusive, including the inflammatory cells initiating the onset of lesions.

In most subtypes of eczema, T cells are considered the primary drivers of the condition, while involvement of mast cells (MCs) is less clear.<sup>7-12</sup> MCs produce large amounts of mediators that elicit inflammation, barrier dysfunction, and pruritus.<sup>10-15</sup> Emerging evidence also suggests that MCs engage in close interactions with sensory neurons, thereby forming central circuits in the instigation and maintenance of inflammatory processes.<sup>10,13,15,16</sup>

We used a mouse strain, C57BL/6, that is resistant to easily develop experimental AD and found that TNF- $\alpha$  deficiency promotes AD-like skin inflammation after repeated cutaneous allergen exposures. Using TNF- $\alpha$ , thymic stromal lymphopoietin receptor (TSLPR), and MCs as well as TNF- $\alpha$ -TSLPR and TNF- $\alpha$ -MC double-deficient mice, we uncovered a complex interplay between TNF- $\alpha$ , MCs, and thymic stromal lymphopoietin (TSLP) in skin inflammation. In this circuit, the lack of TNF- $\alpha$  prompts MC hyperplasia, leading to TSLP stimulation in the epidermis, provoking a self-amplifying loop whereby MCs and TSLP engage in a vicious circle. Interestingly, in accordance with TSLP-driven eczema's being uncoupled from the adaptive immune system,<sup>7-9</sup> the uncovered disorder in TNF- $\alpha$ -devoid surroundings depends on MCs and TSLP but progresses regardless of the presence of allergen.

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## METHODS

### Mice

TNF- $\alpha^{-/-}$ , TSLPR $^{-/-}$ , and C57Bl/6J-Mcpt5-Crex $B6;129S7$ -Dicer $^{tm1Smr}/J$  (MC-deficient) mice were generated as described previously.<sup>17-19</sup> TNF- $\alpha^{-/-}$  mice on B6/J background were crossed to TSLPR $^{-/-}$  mice to generate TNF- $\alpha^{-/-}$ TSLPR $^{-/-}$  double-knockout mice and C57Bl/6J-Mcpt5-Crex $B6;129S7$ -Dicer $^{tm1Smr}/J$  mice were bred with B6;129S-Tnf $^{tm1Gkl}/J$  to obtain TNF- $\alpha$ -MC double-deficient mice. Wild-type (WT) animals were purchased from Janvier (Saint-Berthevin, France). All animals were bred and maintained under pathogen-free conditions in the Charité animal facility. Animal experiments were performed in accordance with approved protocols by the local state office of health and social affairs (LaGeSo G0296/06, G0076/10, and G0262/15). Further details regarding mice strains and breeding are provided in the Methods section in this article's Online Repository available at [www.jacionline.org](http://www.jacionline.org).

### Skin inflammation model

Ten-week-old female WT, TNF- $\alpha$ -deficient, TSLPR-deficient, TNF- $\alpha$ -TSLPR double-deficient, MC-deficient, and TNF- $\alpha$ -MC double-deficient mice were sensitized with 3 intraperitoneal injections of 10  $\mu$ g ovalbumin (OVA) adsorbed to 1.5 mg Al(OH)<sub>3</sub> (alum) on days 1, 14, and 21. On day 21, the belly was shaved and tape stripped, and 100  $\mu$ g OVA allergen or phosphate-buffered saline (PBS) was applied epicutaneously using the patch test method for 1 week.<sup>20</sup> Each mouse underwent three 1-week allergen exposures at the same skin site with 2-week intervals in between. The experimental schemes are graphically depicted in the figures.

Antibodies were intradermally applied to the WT and TNF- $\alpha$ -deficient mice, 1 day before the patch, half a day before patch renewal, and in the middle of the patch-free week, as depicted in the figures. The antibody application doses for anti-TSLP or mIgG<sub>2a</sub> (both from R&D Systems, Minneapolis, Minn) were 20  $\mu$ g per mouse.

On day 71, mice were anesthetized and humanely killed, and blood and skin samples were collected for further analysis. Photographs of the patch area were taken for symptom score assessment, and skin biopsy samples were used for immunohistochemistry, frozen slowly in liquid nitrogen, or fixed in formalin for paraffin embedding. The remaining skin was frozen for mRNA isolation and stored at  $-80^{\circ}\text{C}$  until further processing.

### Assessment of skin inflammation

Clinical severity was assessed using a skin score assessing papulation, erythema, excoriation/crusting, dryness, and lesion extent, which were evaluated independently in a blinded and randomized fashion. Severity for each parameter was graded as follows: 0 for no symptoms, 1 for mild symptoms, 2 for intermediate symptoms, and 3 for severe symptoms. The maximum skin score was considered to be 15 and no eczema 0.

### Skin barrier assessment

Transepidermal water loss (TEWL) was measured by using the Tewameter TM 300 device (CK Electronic, Cologne, Germany).

### Histology

Skin samples underwent histologic examination after sectioning at a thickness of 5  $\mu$ m and staining with hematoxylin for 1 minute. Epidermal and dermal thicknesses were evaluated using AxioVision measuring tools on the AxioPlan light microscope (Zeiss, Berlin, Germany) at 100 $\times$  magnification. Ten measurements were conducted for each mouse, with the results quantified in micrometers, followed by calculation of mean and standard error of the mean (SEM).

### Immunohistochemistry

Skin biopsy samples were fixed in 4% paraformaldehyde at 4 $^{\circ}\text{C}$  for 1 hour and embedded in paraffin, then cut in cross section at a thickness of 5  $\mu$ m. To stain MCs, paraffin was removed from the sections using xylol, ethanol, and distilled water. After rehydration in Tris-buffered saline, staining was performed with 0.1% toluidine blue in 0.5 N HCl for 1 hour. For T-cell staining, skin sections after rehydration were blocked with 1% goat serum (Dako, Jena, Germany) for 20 minutes and incubated with H<sub>2</sub>O<sub>2</sub> for 10 minutes. Skin sections were washed 3 times with 0.05% Tween 20 in PBS, followed by avidin/biotin blocking (Dako). After washing, sections were stained with anti-CD4 (1:30) and anti-CD8 (1:30), both from BD Pharmingen (Franklin Lakes, NJ), for 1 hour. Finally samples were incubated with biotin-conjugated goat anti-rat immunoglobulin-specific polyclonal secondary antibody (1:200, Dako), followed by staining using an AEC substrate kit (Dako) and counterstained with Papainicolaou stain (1:2).

Positively stained cells (MCs and T cells) were counted with an AxioPlan light microscope (Zeiss) at 100 $\times$  magnification and expressed as cells per 1 mm<sup>2</sup> (mean  $\pm$  SEM).

Detailed descriptions of ELISA, RNA isolation, and real-time quantitative PCR (qPCR) are listed in the Methods section in the Online Repository.

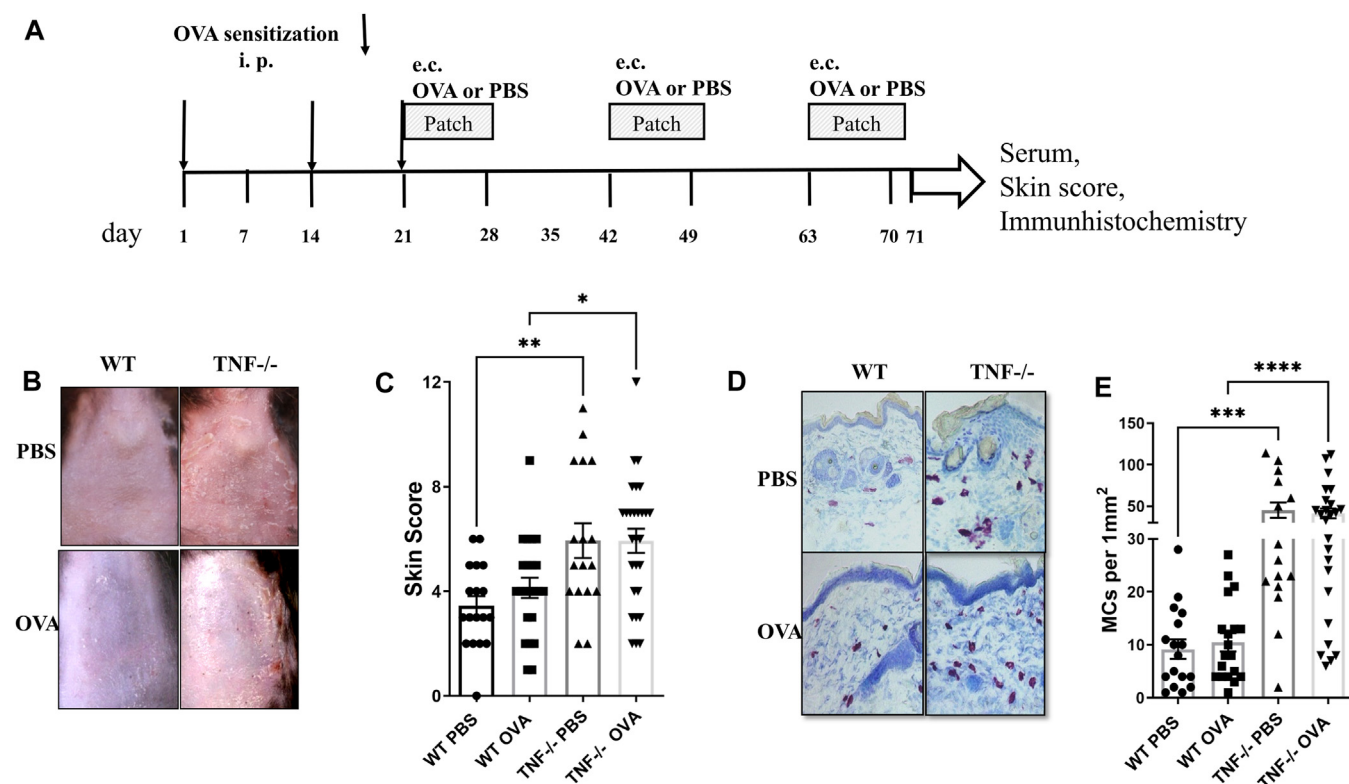
### Statistical analysis

Statistical analysis was performed by 1-way ANOVA followed by Dunnett multiple comparison test for parametric data, or linear regression, or the Kruskal-Wallis test with Dunn multiple comparisons test for nonparametric data. For comparisons between 2 groups, the 2-tailed unpaired *t* test was used. GraphPad Prism v9 (GraphPad Software, La Jolla, Calif) was used for data analysis. *P* < .05 was considered statistically significant.

## RESULTS

### TNF- $\alpha$ deficiency promotes skin inflammation

In the steady state, the skin of WT and TNF- $\alpha$ -deficient mice was comparable in epidermal and dermal thickness, number of T cells, MCs, and keratinocytes (KCs), suggesting no major role of TNF- $\alpha$  in the development and maintenance of the physiologic skin structure. Conversely, when mice were subjected to an experimental skin disease model (Fig 1, A), TNF- $\alpha$ -deficient mice developed eczematous skin lesions, while their WT counterparts were largely protected (Fig 1, B and C). Surprisingly, there was no difference between OVA- and PBS-treated skin in eczema manifestation. These findings indicate a beneficial effect of TNF- $\alpha$  in preventing the onset of an innate-type eczema, which is driven by repetitive physical irritation (as a result of shaving,



**FIG 1.** TNF- $\alpha$  deficiency predisposes skin inflammation and MC hyperplasia. **(A)** Scheme of eczema model. **(B)** Representative example of eczematous skin in WT and TNF- $\alpha$  knockout mice treated epicutaneously (e.c.) with PBS or OVA. **(C)** Depiction of skin score consisting of on erythema, extension, dryness, excoriation, and crusting (with each parameter scored 0-3, then summed). **(D)** Representative acidic toluidine blue staining of skin sections (original magnification 40 $\times$ ). **(E)** Aggregated MC numbers across mice. Each dot represents single animal from total of 5 separate experiments (\* $P < .05$ , \*\* $P < .01$ , \*\*\* $P < .001$ , \*\*\*\* $P < .0001$ ).

tape stripping, and patching) and the mouse responses to the patch (biting, scratching) independent of an allergen.<sup>21</sup>

To better understand the mechanism by which TNF- $\alpha$  deficiency promotes eczema development we analyzed the epidermal thickness as well as the numbers of CD4<sup>+</sup>, CD8<sup>+</sup>, and MCs in the lesional skin. The epidermis was of comparable thickness across all mouse groups (see Fig E1, A, in the Online Repository available at [www.jacionline.org](http://www.jacionline.org)). The number of CD4<sup>+</sup> T cells was increased in the presence of allergen, yet this augmentation was not influenced by the genotype. Conversely, CD8<sup>+</sup> T cells were only enhanced in TNF- $\alpha$ -deficient and OVA-treated mice (Fig E1, B and C). Notably, there was a significant rise in the abundance of MCs in TNF- $\alpha$ -deficient skin regardless of treatment, aligning closely with the development of dermatitis (Fig 1, D and E).

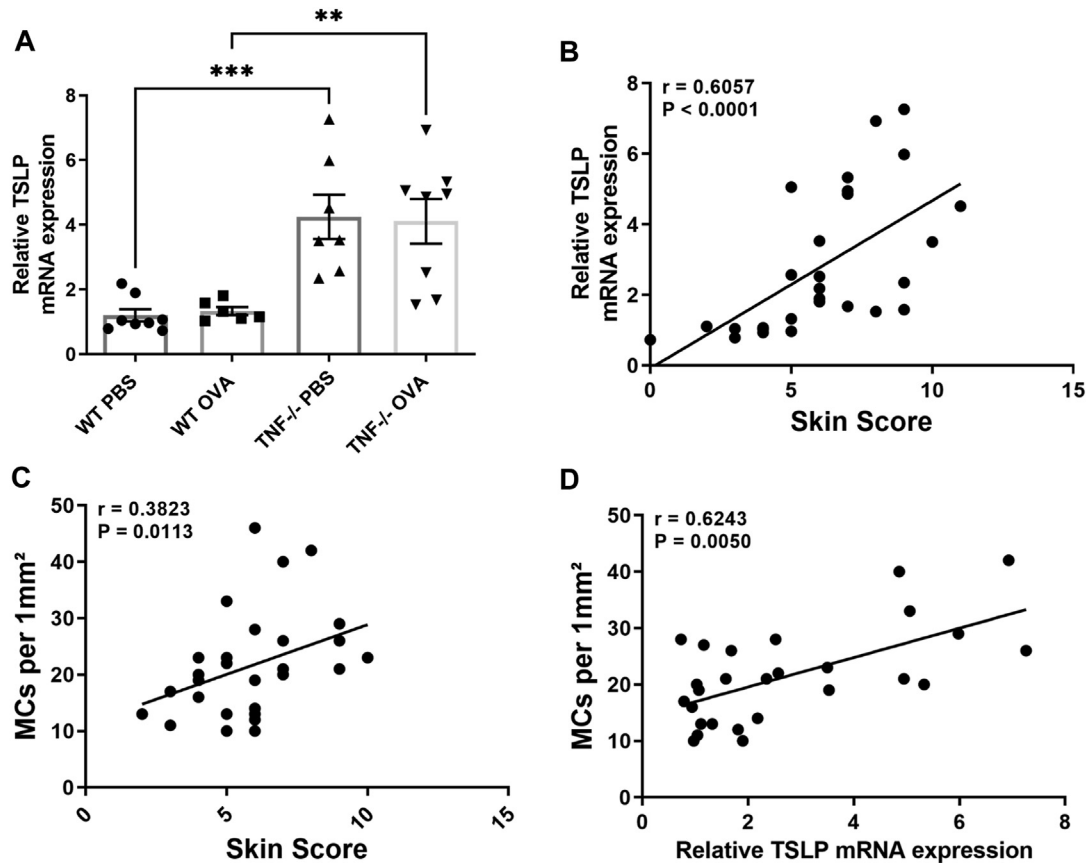
Assessment of the immunoglobulin response including OVA IgE, total IgE, OVA IgG<sub>1</sub>, total IgG, OVA IgG<sub>2a</sub>, and total IgA in sera revealed impaired production of OVA IgE, total IgE, and OVA IgG<sub>1</sub> in the TNF- $\alpha$ -deficient mice (see Fig E2, A-C, in the Online Repository available at [www.jacionline.org](http://www.jacionline.org)), while levels of total IgG, OVA IgG<sub>2a</sub>, and total IgA were comparable between the genotypes (Fig E2, D-F). Thus, while the absence of TNF- $\alpha$  enables eczema manifestation on repetitive barrier disruption, these responses are accompanied by impaired production of immunoglobulins.

### TSLP is enhanced in eczematous skin and correlates with eczema severity

To better understand the inflammatory microenvironment in TNF- $\alpha$ -deficient skin, we studied the expression of several cytokines. Most entities were moderately altered and/or did not display an association with eczema severity (see Fig E3 in the Online Repository available at [www.jacionline.org](http://www.jacionline.org)). For example, IL-1 $\beta$  and IL-6 were only increased in TNF- $\alpha$ -deficient, OVA-treated animals, while IL-10 was uniquely upregulated in the TNF- $\alpha$ -deficient, PBS-treated group. An upward trend in both TNF- $\alpha$ -null groups was detected for stem cell factor (SCF) and IL-13 (without reaching statistical significance in the latter). The most prominently upregulated cytokine was TSLP, however, which was strongly elevated in TNF- $\alpha$ -/- skin independent of whether an allergen was applied (OVA) or the skin was only subjected to repetitive physical irritation (PBS) (Fig 2, A). Moreover, TSLP expression correlated with eczema severity (Fig 2, B). MC hyperplasia was likewise associated with the degree of eczema (Fig 2, C), and MC numbers and TSLP expression correlated as well (Fig 2, D). Conversely, eczema severity did not align with the abundance of other leukocytes like T cells (data not shown).

Given that TSLP is an established trigger and executor of an innate-type eczema,<sup>22</sup> our results suggested that TNF- $\alpha$  functions to restrain dermatitis by attenuating TSLP production. Therefore,





**FIG 2.** TNF- $\alpha$  restrains TSLP expression in inflamed murine skin *in vivo*. **(A)** Relative TSLP mRNA expression in skin of WT and TNF- $\alpha^{-/-}$  mice quantified by qPCR. **(B)** Correlation of TSLP mRNA expression and skin score of WT and TNF- $\alpha^{-/-}$  mice. **(C)** Correlation of MC number and skin score, **(D)** Correlation of MC number and TSLP mRNA expression in lesional skin. Each dot represents single mouse; means  $\pm$  SEMs are from 3 independent experiments (\*\* $P < .01$ , \*\*\* $P < .001$ ).

TNF- $\alpha$  deficiency in intact murine skin promotes eczema, with TSLP emerging as a strong candidate to explain this unanticipated response.

### TSLP is the driver of skin inflammation in TNF- $\alpha$ -deficient skin

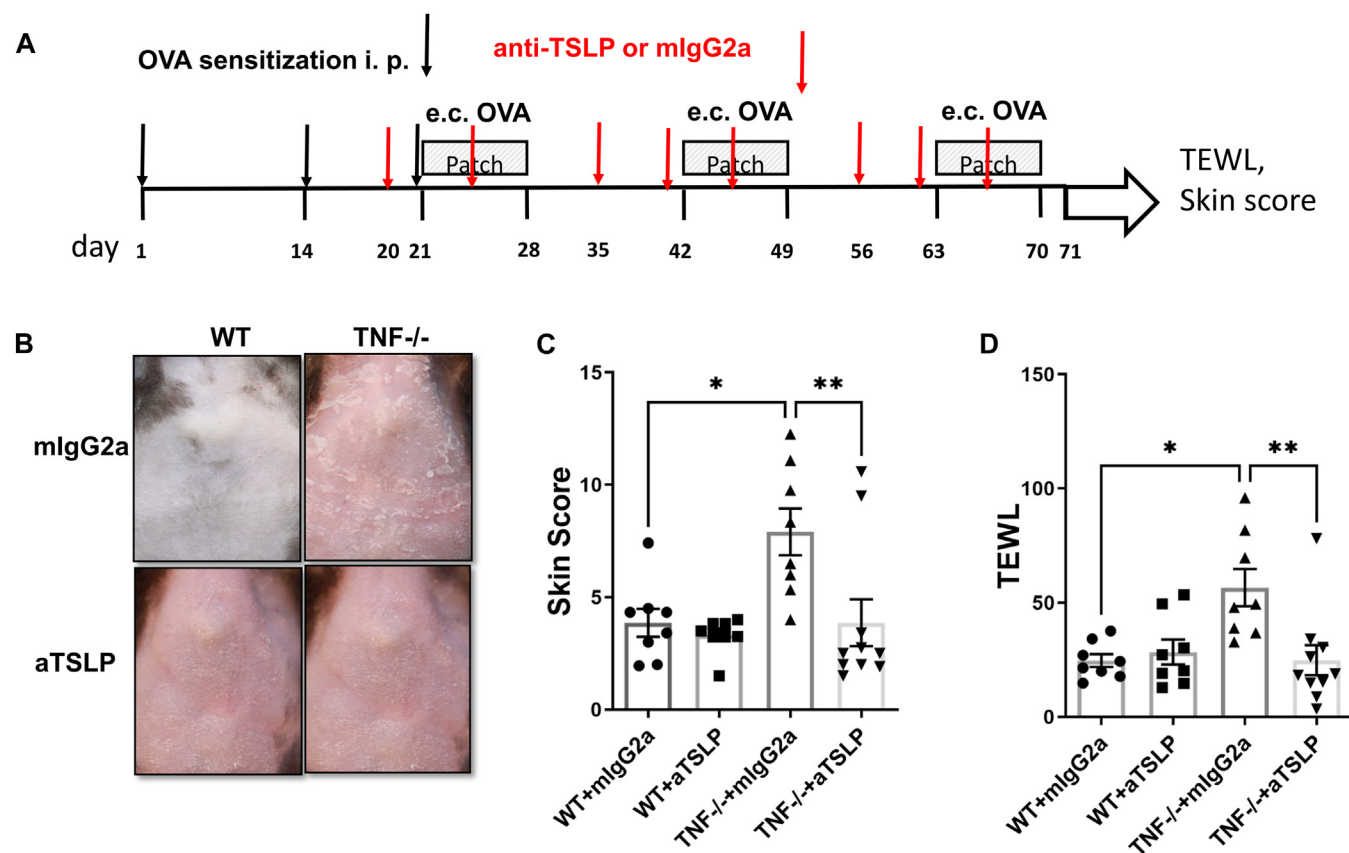
To prove the causal involvement of TSLP in this model, we utilized a neutralizing antibody to disrupt TSLP function in TNF- $\alpha$ -deficient (compared to TNF- $\alpha$ -sufficient) conditions (Fig 3, A). Blocking TSLP indeed decreased the severity observed in TNF- $\alpha^{-/-}$  mice to the level of WT mice, as indicated by severity (skin score) and TEWL (Fig 3, B-D). Interestingly, although not reaching statistical significance, MC numbers also trended to decrease with anti-TSLP treatment in both genotypes (see Fig E4 in the Online Repository available at [www.jacionline.org](http://www.jacionline.org)). Subsequently, we generated TNF- $\alpha$ -TSLPR double-knockout mice to validate the key involvement of the TSLP/TSLPR axis in the absence of TNF- $\alpha$ . The double-knockout mice replicated the results observed with the anti-TSLP antibody, leading to a reduction in skin score and TEWL to match levels obtained in WT mice (Fig 4, A-C). We also observed an increase in circulating TSLP levels in TNF-deficient mice that was maintained in the TNF- $\alpha^{-/-}$ /TSLPR $^{-/-}$  mice (Fig 4, D). A trend was evident in the TSLPR $^{-/-}$  mice (although not statistically

significant), indicating a compensatory mechanism to the lacking TSLPR signaling. On the basis of our collective findings, we conclude that TSLP is the key driver of skin inflammation in the absence of TNF- $\alpha$ .

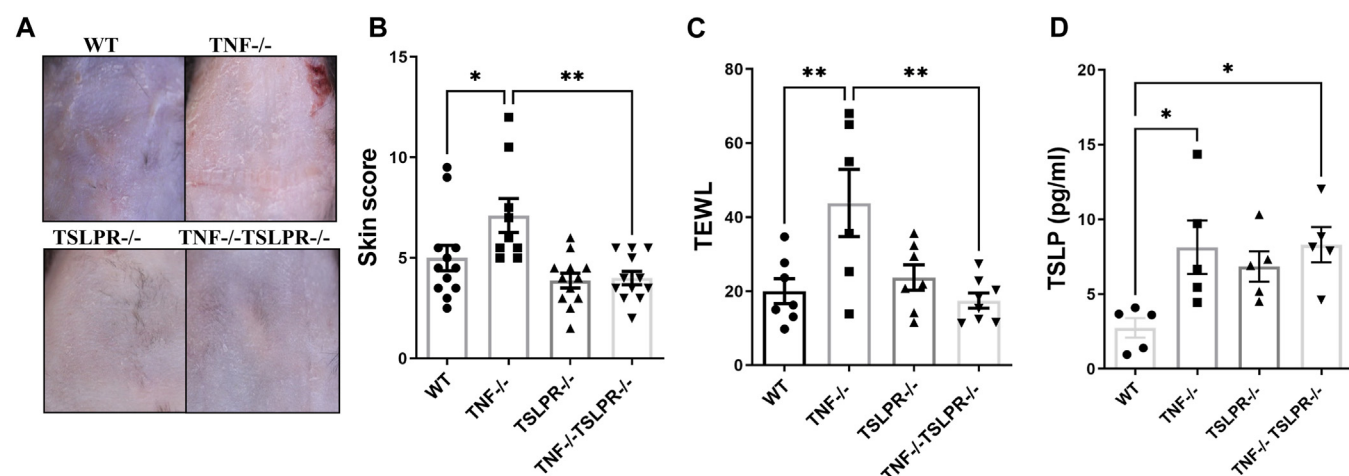
### MC deficiency ameliorates eczema promoted by the absence of TNF- $\alpha$

Considering the correlation between MCs and eczema severity on the one hand and TSLP levels on the other (Fig 2, C and D), we hypothesized that MCs may act as intermediaries in promoting TSLP activity. While MCs can produce some TSLP themselves,<sup>23</sup> the most significant producers of the cytokine are epithelial cells, including skin KCs.<sup>24,25</sup> In fact, we have recently documented that MCs can stimulate KCs to generate TSLP through a tryptase/protease-activated receptor 2 (PAR-2)-dependent mechanism.<sup>26</sup>

To delineate the role of MCs in eczema development under TNF- $\alpha$  deficiency, we crossed B6;129S-Tnf<sup>tm1Gkl</sup>/J (TNF- $\alpha$  deficient) with C57Bl/6J-Mcpt5-CrexB6;129S7-Dicer<sup>tm1Smr</sup>/J (MC deficient) mice to generate animals devoid of MCs and TNF- $\alpha$  (Fig 5, A). Using these MC-TNF- $\alpha$  double-deficient mice, we finally determined that MCs are indeed crucial to eczema development, as revealed by skin score and TEWL normalization (Fig 5, B-E). No significant differences in TSLP serum levels



**FIG 3.** Eczema in TNF- $\alpha$ -deficient skin is driven by TSLP. **(A)** Scheme of eczema model using anti-TSLP antibody. **(B)** Representative example of eczematous skin in WT and TNF- $\alpha$ -knockout mice treated with anti-TSLP antibody or isotype control. **(C)** Skin score and **(D)** TEWL in WT and TNF- $\alpha^{-/-}$  mice. Each dot corresponds to 1 mouse; means  $\pm$  SEMs are from 3 independent experiments (\* $P$  < .05, \*\* $P$  < .01).

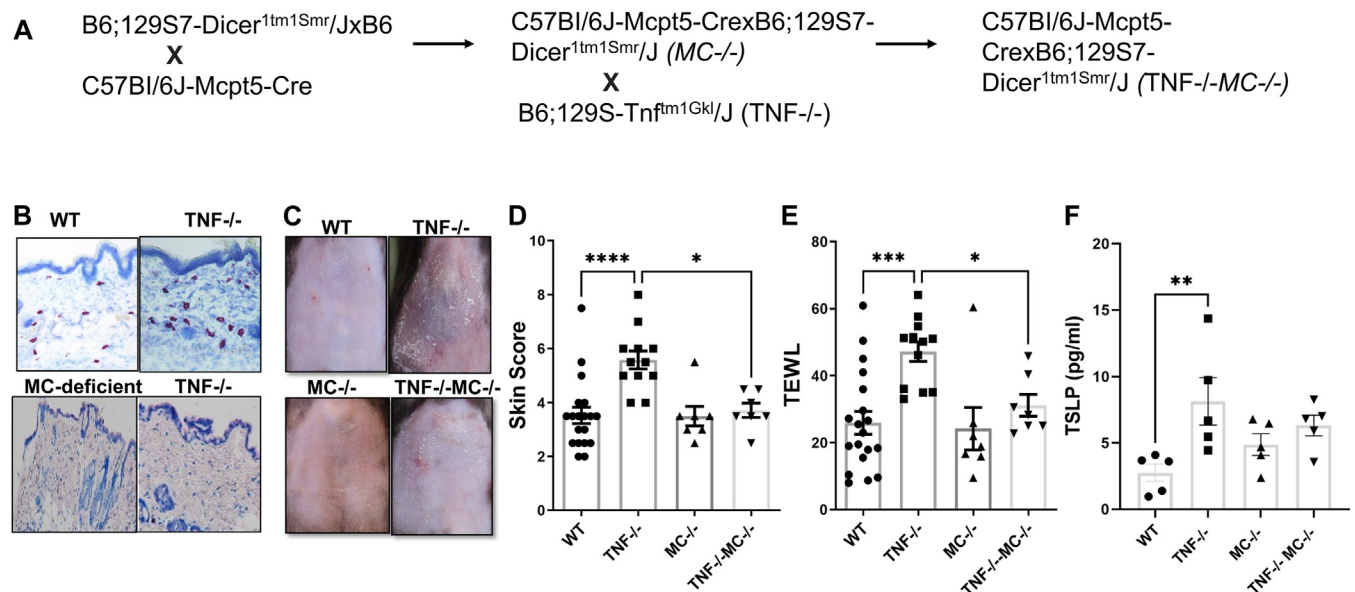


**FIG 4.** TSLPR/TNF- $\alpha$  double-knockout mice are protected from eczema development compared to mice deficient in TNF- $\alpha$  only. **(A)** Representative example of eczematous skin. **(B)** Skin score, **(C)** TEWL, and **(D)** TSLP serum levels in WT, TNF- $\alpha^{-/-}$ , TSLPR- $\alpha^{-/-}$ , and TNF- $\alpha^{-/-}$ TSLPR- $\alpha^{-/-}$  mice treated with epicutaneous (e.c.) OVA. Each dot corresponds to 1 mouse; means  $\pm$  SEMs are from 3 to 5 experiments (\* $P$  < .05, \*\* $P$  < .01).

were found between the two MC-deficient groups—that is, TNF- $\alpha^{-/-}$ MC- $\alpha^{-/-}$  mice could not mount the same systemic TSLP response as TNF- $\alpha^{-/-}$  single-deficient mice, and TNF deficiency could thus not support TSLP in the absence of MCs (Fig 5, F).

## DISCUSSION

The proinflammatory role of TNF- $\alpha$  in chronic inflammatory disorders is well established.<sup>27,28</sup> Conversely, we and others have reported on the onset of eczematous skin lesions in



**FIG 5.** MCs play key role in progression of eczema. **(A)** Scheme of generation of MC-deficient and TNF- $\alpha$ <sup>-/-</sup>MC<sup>-/-</sup> double-deficient mice. **(B)** Representative histologic staining images of MCs and **(C)** representative example of eczematous skin in 4 genotypes. **(D)** Skin score, **(E)** TEWL, and **(F)** serum TSLP levels in WT, TNF- $\alpha$ -knockout, MC-deficient, and TNF- $\alpha$ <sup>-/-</sup>MC<sup>-/-</sup> double-deficient mice. Each dot represents 1 mouse; means  $\pm$  SEMs are from 3 to 5 experiments is given as bar (\* $P$  < .05, \*\*\* $P$  < .001, \*\*\*\* $P$  < .0001).

individuals undergoing systemic TNF- $\alpha$ -neutralizing therapy; consequently, eczema has been categorized as an adverse effect associated with anti-TNF- $\alpha$  treatment.<sup>5,6,29-31</sup> We now unravel how TNF- $\alpha$  deficiency can predispose to eczema in an *in vivo* model, recapitulating the events of human disease. An important finding was that the skin inflammation promoted by TNF- $\alpha$  deficiency occurs irrespective of allergen presence, indicating it is primarily caused by the innate immune system. Indeed, we accordingly noted comparable infiltration of CD4<sup>+</sup> T cells into the skin of WT and TNF- $\alpha$ -deficient mice. Although the numbers of CD8<sup>+</sup> T cells were slightly increased in TNF- $\alpha$ -knockout mice, this phenomenon was only observed after topical OVA sensitization and was therefore not a prerequisite for lesion development. We observed a significant decrease in humoral responses in the absence of TNF- $\alpha$ , which also affected levels of total and allergen-specific IgE. These observations align with prior research that has demonstrated a central function of TNF- $\alpha$  in humoral immunity through its facilitation of primary B-cell follicles and germinal centers.<sup>17,32</sup>

Given that TNF- $\alpha$  exhibited protective effects in the skin microenvironment, the absence of this cytokine appears to increase the susceptibility to eczema that does not rely on B cells, fitting most other models.<sup>10,33,34</sup> Indeed, there has been a recent shift toward IgE-independent mechanisms to explain MC activation in the context of different diseases, including eczema; in particular, the significance of Mas-related G protein-coupled receptor X2 (MRGPRX2)/Mrgprb2 as a potent mechanism for MC activation has been recognized.<sup>10,23,35-37</sup>

Considering the upregulation of TSLP in affected skin and its correlation with the severity of eczema, an investigation was conducted to ascertain if TSLP is the driver of eczema in the absence of TNF- $\alpha$ . Indeed, interference with the TSLP/TSLPR axis neutralized the eczema-promoting effect of TNF- $\alpha$  deficiency. Both eczema score and TEWL nearly normalized by

anti-TSLP antibody or TSLPR deficiency in TNF- $\alpha$ -null mice. These findings are consistent with reports that have established a crucial role of TSLP as the initiator of eczema, whereby the TSLP-dependent subtype chiefly relies on the innate immune system and is equally observed in RAG<sup>-/-</sup> mice lacking B- or T-cell responses.<sup>22,34,35,38,39</sup>

It may seem puzzling that a lack of TNF- $\alpha$  leads to an increase in TSLP production considering TNF- $\alpha$ 's established role as a stimulator of TSLP *in vitro*. Nonetheless, our own prior research has demonstrated that IL-1 has a more significant role in triggering TSLP in the skin milieu, with TNF- $\alpha$  not contributing to its generation in acutely irritated skin.<sup>21</sup> Nonetheless, this observation likewise fails to explain the heightened TSLP levels in TNF- $\alpha$ -deficient mice, suggesting the presence of an intermediary link between TNF- $\alpha$  deficiency and TSLP induction. Considering that only mice lacking TNF- $\alpha$  displayed MC hyperplasia along with increased TSLP expression, we speculated that MCs could serve as this intermediary factor.

Essential insights into the cascade that stimulates TSLP production in the skin have been gained over the last few years. In this context, physical skin irritation was specifically identified as triggering the release of IL-1, which in turn promotes the production of TSLP.<sup>21,26,40</sup> Moreover, MC activation through MRGPRX2/b2 (induced by neuropeptides, for example) led to the secretion of tryptase, which consecutively activates PAR-2 on KCs.<sup>40</sup> IL-1 and PAR-2 stimulate NF- $\kappa$ B (nuclear factor kappa-light-chain enhancer of activated B cells) and NFAT (nuclear factor of activated T cells) on adjacent motifs, respectively, thereby synergizing on the TSLP promoter.<sup>40</sup> Meixiong et al<sup>41</sup> found that MRGPRX2/b2 plays a more significant role in inducing tryptase compared to Fc $\epsilon$ RI, triggering histamine-independent itch in an eczema model, aligning with reports on tryptase or Mrgprb2 deficiency providing a shield against eczema.<sup>34,42</sup> Recently, MCs have been demonstrated to directly

instruct KCs to produce TSLP by a tryptase–PAR-2 mechanism *in vitro* and *in vivo*.<sup>26</sup> Using a TSLP-dependent eczema model, recent research found that the Mrgprb2-tryptase pathway was responsible for triggering epidermal TSLP expression, leading to the onset of eczema.<sup>34</sup> Our present findings support the notion that persistent or repetitive activation of the interconnected pathways outlined above can lead to TSLP production and therefore TSLP-driven eczema.

TNF- $\alpha$ , primarily produced by KCs in response to skin barrier disruption and inflammatory stimuli, plays a critical regulatory role in skin immunity,<sup>43–46</sup> but how does its absence contribute to MC hyperplasia and consequent TSLP hyperexpression? Several lines of evidence demonstrate that this is likely accomplished at several levels. In fact, TNF- $\alpha$  can directly suppress MC activity, particularly degranulation via the MRGPRX2 pathway, potentially through upregulation of ICOSL (inducible T-cell costimulator ligand),<sup>47,48</sup> and this can contribute to the increased MC activity under TNF- $\alpha$  deficiency.

Indirectly, TNF- $\alpha$  attenuates several MC supportive factors such as SCF, the major survival factor of MCs, and substance P, a ligand of MRGPRX2 and key driver of MC degranulation.<sup>49</sup> We could replicate TNF- $\alpha$ 's negative impact on SCF; TNF- $\alpha$ -deficient mice showed higher expression in the skin. Moreover, TSLP itself can promote skin MC survival.<sup>50</sup> Its increase under TNF- $\alpha$  deficiency further promotes MC hyperplasia, and we found a downward trend in the MC compartment after application of anti-TSLP, supporting a feedforward loop. TSLP can also influence MC activity, including degranulation,<sup>51–54</sup> and MCs were identified as the cells expressing the highest levels of TSLPR across the body.<sup>55,56</sup> As discussed above, tryptase released from MCs is a potent inducer of TSLP in KC, while histamine indirectly amplifies the inflammatory milieu via IL-6, IL-8, and SCF induction.<sup>26</sup> This cascade can establish a feedforward loop where TSLP further supports MC abundance and activity, exacerbating inflammation. Collectively, direct and indirect regulatory effects are expected to combine with the end result that TNF- $\alpha$  interferes with MC abundance and function *in vivo*.

This offers a molecular rationale as to why anti-TNF- $\alpha$  medications can induce dermatitis as an adverse effect in certain individuals.<sup>5</sup> Although psoriasis and AD share numerous similarities,<sup>56</sup> the pivotal role of TNF- $\alpha$  in driving the pathology of psoriasis is widely recognized, explaining the effectiveness of TNF- $\alpha$ -targeted treatments.<sup>3</sup> Conversely, TNF- $\alpha$  may turn out a crucial distinguishing factor between AD and psoriasis, initiating the latter while offering protection against the former.

A limitation of this study is that our data solely originates from murine models. Furthermore, we utilized BL6 mice, recognized for their tendency to exhibit heightened innate and MC-dependent immune reactions.<sup>20,57–63</sup> The mice additionally demonstrated reduced T<sub>H</sub>2 responses coupled with diminished IgE responses; it is precisely this model that seems to best correspond to patients who develop eczematous lesions after anti-TNF- $\alpha$  therapy. The strain dependence is also emphasized by the following: OVA patching induces significant eczema in BALB/c mice, while C57BL/6 mice show minimal responses in the same model.<sup>64</sup> C57BL/6 mice are largely protected, but in combination with a second component, like TNF- $\alpha$  deficiency, C57BL/6 mice become prone to develop eczema, much like BALB/c mice, which is driven by TSLP.<sup>14,65</sup> Our results thus highlight the significance of the genetic background and its linked immunologic profile, which may translate to variations among patient subgroups.

Overall, our study shows that skin homeostasis and the innate immune response can be altered on systemic cytokine knockout (or cytokine-targeted treatment in humans), which, on triggers like skin irritation, may result in the onset of unexpected inflammatory processes or the so-called paradox reaction in humans. Eczema developing in the absence of TNF- $\alpha$  potentially reflects a subcategory that may be amenable to TSLP-directed treatment or modalities targeting MCs and their products. This awareness can foster the implementation of tailored treatment approaches that are patient-centric and personalized.

## DISCLOSURE STATEMENT

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**Clinical implication: Targeting MCs or their products may represent novel approaches for personalized eczema therapy.**

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