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ORIGINAL ARTICLE

Skin and Eye Diseases

Pruritus in ordinary scabies: IL-31 from macrophages induced by overexpression of thymic stromal lymphopoietin and periostin

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Abstract

Background: Scabies is a common contagious skin disease caused by an infestation of the skin by *Sarcoptes scabiei* var. *hominis*. A hallmark symptom of scabies is severe itch.

Methods: We sought to determine the generation of a pruritogenic cytokine, interleukin (IL)-31, together with immune profiles in skin lesions of ordinary scabies through immunohistochemical and immunofluorescent studies. To elucidate the pathological mechanisms of IL-31 generation, murine peritoneal macrophages were stimulated with various T helper 2 (Th2) cytokines and proteins ex vivo.

Results: A large number of CCR4(+) Th2 cells, eosinophils, and basophils infiltrated in scabies lesions. Increased generation of IL-31, thymic stromal lymphopoietin (TSLP), and periostin was also observed. A major population of IL-31(+) cells were Arginase-1(+)/CD163(+) M2 macrophages. Murine peritoneal macrophages showed an M2 phenotype and generated IL-31 when stimulated with TSLP and periostin.

Conclusion: IL-31 appeared to be largely generated by M2 macrophages in ordinary scabies lesions. This IL-31 induction was mediated by TSLP and periostin.

KEYWORDS

IL-31, macrophage, periostin, scabies, thymic stromal lymphopoietin

1 | INTRODUCTION

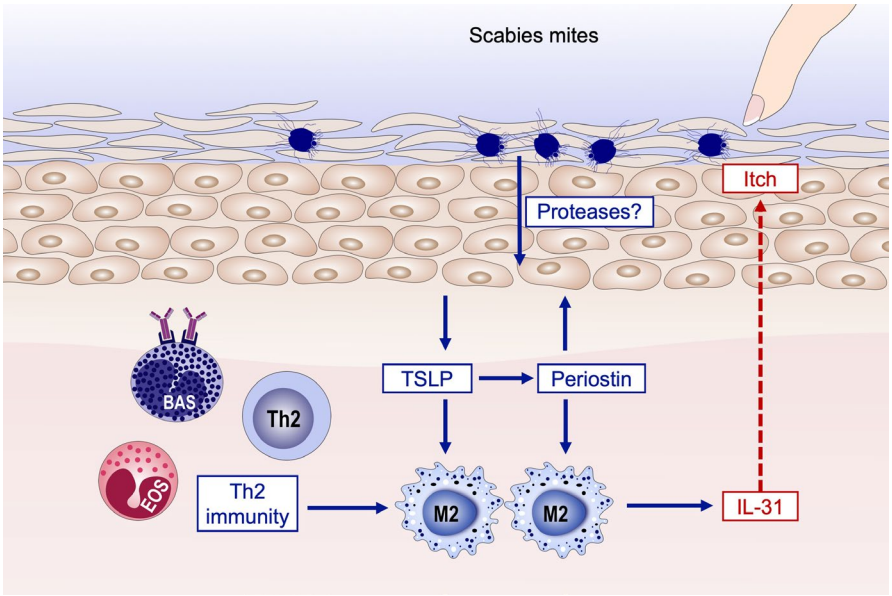
Scabies is a common contagious skin disease caused by an infestation of the skin by *Sarcoptes scabiei* var. *hominis*.[1] About 100 million individuals worldwide are reported to be infected with scabies.[2] Although scabies is more prevalent in developing countries, with prevalence rates of up to 71.4%, epidemics can occur in institutional settings such as nursing homes even in developed countries.[3] Scabies highly impacts mortality and morbidity and causes health loss.[4] Thus, scabies is a major public health problem.

Scabies has two common subtypes: ordinary scabies and crusted scabies.[1] Skin manifestations of ordinary scabies are burrows and erythematous papules/nodules, whereas psoriasis-like hyperkeratotic skin crusts with a huge number of mites are apparent in crusted scabies.[1] A hallmark symptom of scabies is severe and persistent pruritus.[5] This pruritus is thought to be provoked via type IV (delayed-type) immune responses, but precise mechanisms are not fully understood.[6]

Interleukin (IL)-31 is a pruritogenic cytokine that is produced mainly by activated T helper 2 (Th2) cells.[7] Overexpression of IL-31

Abbreviations: Ab, antibody; AD, atopic dermatitis; Ag, antigen; Arg1, arginase 1; CCR4, C-C chemokine receptor type 4; CD, cluster of differentiation; CXCR3, C-X-C chemokine receptor type 3; ELISA, enzyme-linked immunosorbent assay; IENFD, intraepidermal nerve fiber density; Ig, immunoglobulin; IL, interleukin; IL-7Rα, IL-7 receptor α; iNOS, inducible nitric oxide synthase; OSMRβ, oncostatin M receptor β; PAR-2, protease-activated receptor 2; PGP9.5, protein gene product 9.5; pM, peritoneal macrophages; TARC, thymus and activation-regulated chemokine; TSLP, thymic stromal lymphopoietin; TSLPR, thymic stromal lymphopoietin receptor.

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GRAPHICAL ABSTRACT

A large number of CCR4$^+$ cells, basophils, and eosinophils were observed in human ordinary scabies lesions, suggesting that a Th2 immune response is predominant in ordinary scabies. Increased IL-31, a pruritogenic cytokine, thymic stromal lymphopoietin (TSLP), and periostin were detected in the lesions. The majority of IL-31$^+$ cells were CD68$^+$, Arg1$^+$, and CD163$^+$ M2 macrophages and IL-31 could be induced by overexpression of TSLP and periostin. Arg1: arginase 1; CCR4: C-C chemokine receptor type 4; TSLP: thymic stromal lymphopoietin.

is detected in many pruritic diseases such as atopic dermatitis (AD)[8] and prurigo nodularis.[7] However, it is uncertain whether IL-31 is also implicated in scabies pruritus.

In this study, we sought to investigate the expression of IL-31 in scabies lesions through a histological study. We also attempted to elucidate pathological mechanisms for IL-31 generation via ex vivo stimulation studies with murine peritoneal macrophages (pM).

2 | MATERIALS AND METHODS

2.1 | Patients

Skin biopsy specimens were obtained from four patients with ordinary scabies with severe itch, four patients with tick bites without itch, and four subjects as healthy controls at Tokyo Medical and Dental University Hospital. Their diagnoses were confirmed based on clinical and histological findings. This study was approved by the ethical committee of Tokyo Medical and Dental University (#M2017-273), and all participants provided informed consent.

2.2 | Animals

Eight-week-old female C57BL/6N mice were purchased from Sankyo Lab Service. Mice were maintained under specific pathogen-free conditions in our animal facility. All animal experiments were approved by the Institutional Animal Care and Use Committee of Tokyo Medical and Dental University (#A2017236).

2.3 | Antibodies

Anti-IL-31, anti-IL-31 receptor A (IL-31RA), anti- C-C chemokine receptor type 4 (CCR4), anti-thymic stromal lymphopoietin (TSLP), anti-human TSLP receptor (TSLPR), and anti-Arginase 1 (Arg1) polyclonal antibodies (Abs) were obtained from Abcam plc. Anti-C-X-C motif chemokine receptor 3 (CXCR3), anti-CD68, anti-CD163, anti-IL-7 receptor α (IL-7Rα), anti-integrin αV, and anti-monocyte/macrophage (MOMA-2) monoclonal Abs were purchased from Abcam. Anti- oncostatin M receptor β (OSMRβ), anti-periostin, and anti-thymus, and activation-regulated chemokine (TARC) polyclonal Abs were purchased from Santa Cruz Biotechnology. Anti-IL-17A Ab was obtained from R&D Systems. Anti-human basophil antibody (BB1) was described previously.[9] Anti-protein gene product 9.5 (PGP 9.5) Ab was obtained from Enzo Life Sciences. R-Phycoerythrin-conjugated anti-MOMA-2 and allophycocyanin-conjugated anti-mouse TSLPR Abs were purchased from Bio-Rad Laboratories and BioLegend, respectively.

2.4 | Preparation of murine peritoneal macrophages

Peritoneal cells were collected from 8-week-old C57BL/6N mice, seeded at the concentration of 5×10^5/well in 6-well plates in RPMI-1640 complete medium supplemented with 10% FBS and 100 IU/mL penicillin-streptomycin and incubated for 2 hours at 37°C and 5% CO_2. Then, nonadherent cells were removed by washing with medium and the remaining adherent cells (>80% of macrophages) were incubated with or without recombinant mouse

IL-4 (10 ng/mL), IL-13 (10 ng/mL), TSLP (20 ng/mL), periostin (20 ng/mL; all were purchased from eBioscience), IFN-γ (100 U/mL; Wako Pure Chemical), or in combination thereof. After 24 hours, the cells were subjected to total RNA extraction or flow cytometric analysis. IL-31 levels in the supernatants were also determined using enzyme-linked immunosorbent assay (ELISA; Thermo Fisher Scientific KK).

2.5 | Immunohistochemistry

Formalin-fixed paraffin-embedded sections were subjected to Ag retrieval with citrate buffer for PGP 9.5, IL-31, IL-31RA, OSMR-β, CXCR3, CCR4, TARC, TSLP, and periostin or Histofine antigen retrieval buffer (pH 9; Nichirei Bioscience) for CD68 and IL-17. Sections were then incubated with methanol containing 0.3% H_2O_2 for endogenous peroxidase quenching and treated with a protein-blocking solution containing 0.25% casein (Dako) to prevent nonspecific binding of Abs. Next, sections were incubated with primary Abs at 4°C overnight followed by Histofine Simple Stain (Nichirei) and visualized with 3′-diaminobenzidine tetrahydrochloride solution (Nichirei). Sections were counterstained with Mayer's Hematoxylin. Basophils, eosinophils, and mast cells were detected using anti-BB1 Ab staining, Luna's method, and toluidine blue staining, respectively.[9,10] The total number of indicated cells was counted manually in at least three different high-power fields from each sample. The total immunostaining intensity of IL-31, IL-31RA, OSMRβ, TARC, and TSLP at the epidermis and periostin at the dermis was measured with Image J software,[11] and immunostaining intensity per unit area was calculated. Intraepidermal nerve fiber density (IENFD) was calculated by dividing the immunoreactive area of PGP 9.5, a neuron specific marker, in the epidermis by the whole epidermal area.

2.6 | Immunofluorescence staining

Formalin-fixed paraffin-embedded samples (5-μm thickness) were pretreated with Histofine antigen retrieval solution (pH 9; Nichirei) for IL-31 in combination with CD68, Arg1, or CD163 or proteinase K (Dako) for IL-31 with MOMA-2 staining. For TSLP and periostin staining in murine samples, formalin-fixed frozen sections (20-μm thickness) were used. Then, sections were incubated with the combination of indicated primary Abs followed by reaction with Alexa Fluor 488- or 568-conjugated secondary Abs (Abcam) and mounted with Fluoroshield with DAPI (GeneTex). Photomicrographs were captured with a TCS SP8 confocal microscope (Leica Microsystems).

2.7 | Real-time PCR

Total cellular RNA was extracted from cells using ISOGEN II (Nippon Gene Co.), reverse-transcribed with SuperScript™ IV VILO™ Master Mix with ezDNase enzyme (Thermo Fisher Scientific), and then, quantitative RT-PCR was performed by real-time monitoring of the increase in fluorescence of SYBR Green dye (Brilliant SYBR Green QPCR Master Mix) using the AriaMx Real-Time PCR System (both from Agilent Technologies Japan, Ltd.). The primers used for PCR were 5′-TCGGTCATCATAGCACATCTGGAG-3′ and 5′-GCACAGTCCCTTTGGAGTTAAGTC-3′ for mouse IL-31; 5′-CTCCAAGCCAAAGTCCTTAGAG-3′ and 5′-AGGAGCTGTCATTAGGGACATC-3′ for mouse Arg1; 5′-GTTCTCAGCCCAACAATACAAGA-3′ and 5′-GTGGACGGGTCGATGTCAC-3′ for mouse inducible nitric oxide synthase (iNOS); and 5′-ACCACAGTCCATGCCATCAC-3′ and 5′-TCCACCACCCTGTTGCTGTA-3′ for mouse GAPDH. mRNA expression levels were calculated by the comparative $\Delta\Delta C_T$ method relative to GAPDH.

2.8 | Flow cytometric analyses

Single-cell suspensions of cultured peritoneal macrophages (pM) were obtained using cell scrapers after fixation. They were pretreated with anti-CD16/32 antibody (BioLegend) on ice for 15 minutes, labeled with the indicated combinations of Abs using Intracellular Fix & Perm set (eBioscience), and analyzed with FACSCalibur cell analyzer (BD Biosciences).

2.9 | Statistical analysis

Student's *t* test was used to assess the statistical significance of differences between means. *P* values < 0.05 were considered to be significant.

3 | RESULTS

3.1 | Intraepidermal nerve fiber density (IENFD) and dermal mast cells

We first focused on IENFD. Increased IENFD is reported to contribute to pruritus in AD and dry skin.[12] IENFD was increased in scabies lesions compared with healthy skin (Figure 1A). Interestingly, tick bite lesions without itch had a higher IENFD ratio than scabies lesions.

Mast cells are one of the representative immune cells that are involved in itch via releasing various itch mediators such as histamine and serotonin. The number of dermal mast cells was not significantly increased in scabies lesions compared with healthy skin and tick bite lesions (Figure 1B).

3.2 | Epidermal expression of IL-31 and IL-31-positive infiltrating cells

We next examined the expression of IL-31. There was a tendency of increased epidermal IL-31 expression in scabies lesions compared with healthy skin and tick bite, although this was not statistically significant (Figure 1C). In contrast, a larger number of IL-31(+) cells infiltrated the dermis of scabies lesions compared with healthy skin and tick bites, which was statistically significant (Figure 1D).

We also investigated the expression of IL-31 receptors. IL-31 receptors form a receptor complex comprising two subunits IL-31RA and OSMRβ. These receptors are expressed by various

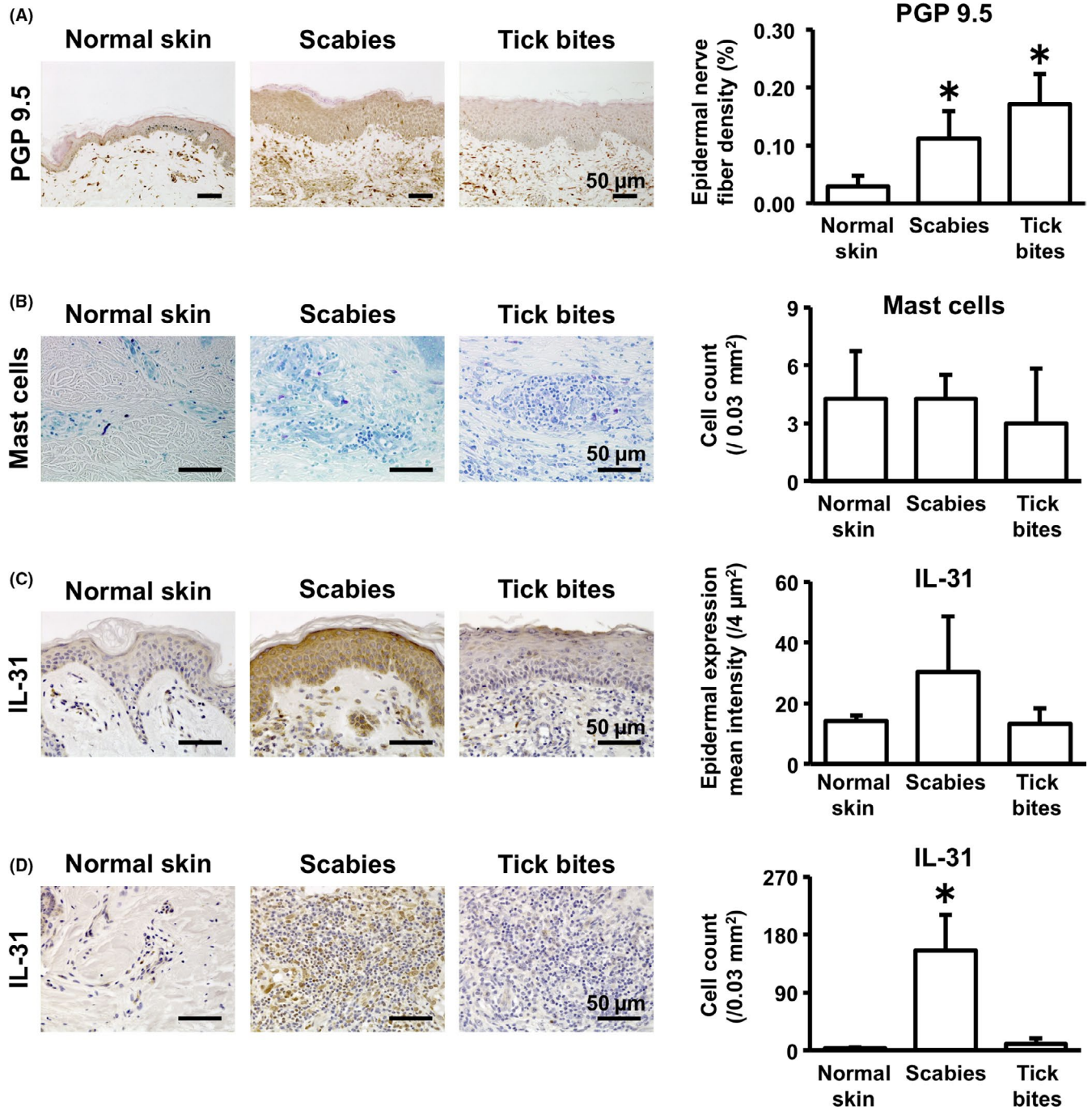
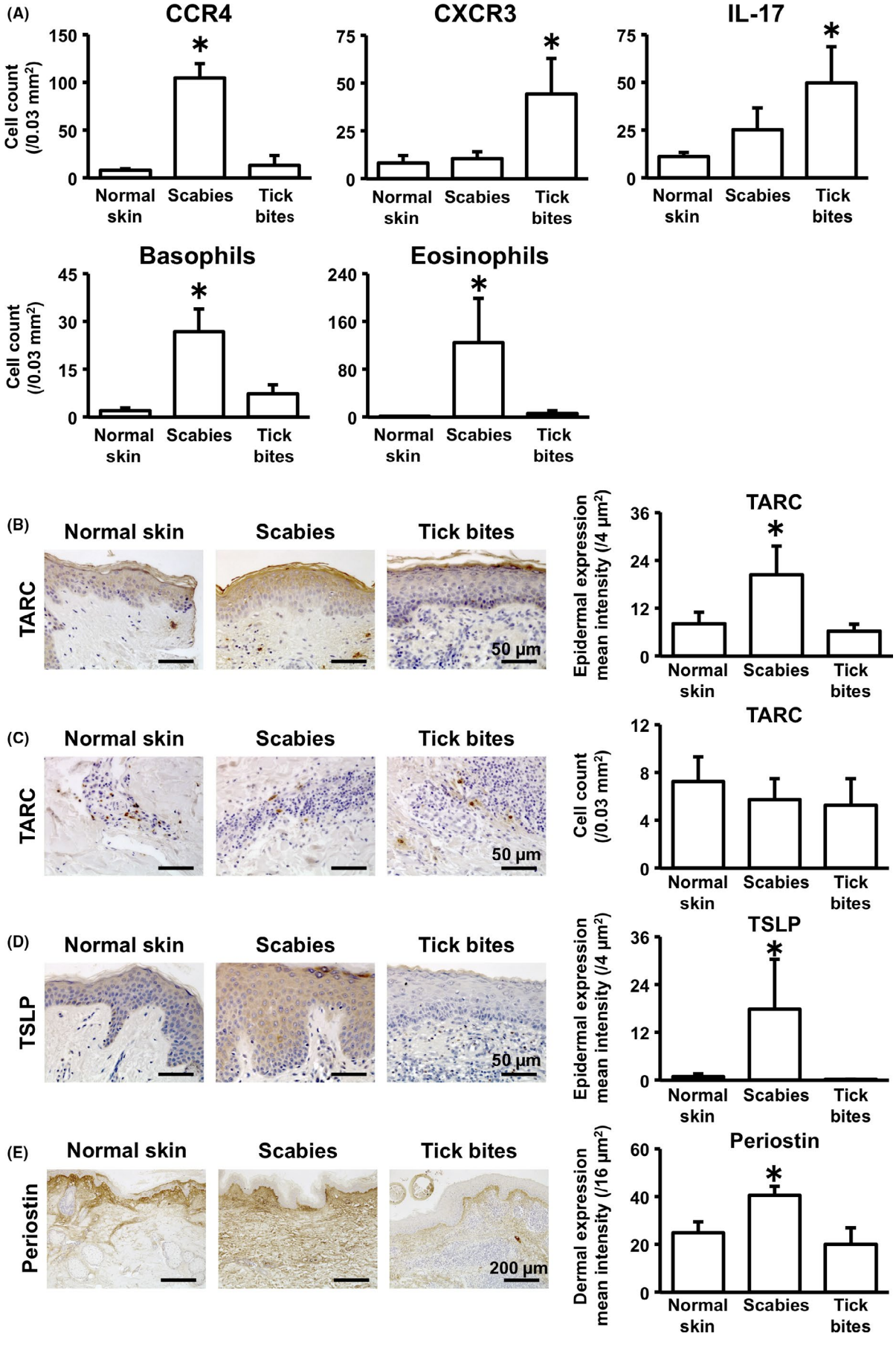


FIGURE 1 Intraepidermal nerve fiber density, dermal mast cells, and IL-31 in scabies. A, The intraepidermal nerve fiber density (IENFD), which is calculated by dividing the PGP 9.5, a neuron specific marker, immunoreactive area in the epidermis by the whole epidermal area, increased in scabies and tick bites compared with healthy control. B, Dermal mast cells did not increase in scabies (toluidine blue staining). C,D, IL-31 expression in the epidermis and dermal cells. Scale bars indicate 50 μm. Values represent mean + SD of four subjects. *$P < 0.05$ compared with normal skin

FIGURE 2 Immune profiles in scabies lesions. A, A larger number of CCR4(+) cells, basophils, and eosinophils infiltrated scabies lesions than normal skin and tick bite lesions. B,C, TARC was highly expressed by epidermal keratinocytes but not dermal infiltrative cells in scabies. D,E, Enhanced epidermal expression of thymic stromal lymphopoietin and massive dermal periostin deposition were noted in scabies. Scale bars indicate 50 μm (B-D) and 200 μm (E). Values represent mean + SD of four subjects. *$P < 0.05$ compared with normal skin


(A)
CCR4
CXCR3
IL-17
Basophils
Eosinophils
Cell count (/0.03 mm²)
Normal skin
Scabies
Tick bites
(B)
TARC
Epidermal expression mean intensity (/4 μm²)
(C)
Cell count (/0.03 mm²)
(D)
TSLP
(E)
Periostin
Dermal expression mean intensity (/16 μm²)
50 μm
200 μm

cells including epidermal keratinocytes and peripheral nerves.[8,13] Neither IL-31RA nor OSMRβ was increased in the epidermis in scabies lesions. Similarly, no differences were detected in the number of OSMRβ(+)dermal infiltrating cells (Figure S1). IL-31RA(+) cells in the dermis were hardly detected in any specimens (data not shown).

3.3 | Th2 immunity in scabies

IL-31 is produced mainly by activated Th2 cells,[14] which express CCR4.[15] The number of infiltrating CCR4(+) cells was higher in scabies lesions than healthy control and tick bites (Figure 2A). In contrast, the number of infiltrating cells expressing CXCR3 and IL-17, both of which are representative markers for Th1 cells and Th17 cells, respectively, did not increase in scabies lesions, while tick bites showed an increased number of such cells. In addition, scabies lesions featured massive infiltration of eosinophils and basophils, indicating the involvement of Th2 immunity (Figure 2A).

TARC (a ligand for CCR4), TSLP, and periostin are Th2-related proteins.[16-19] The epidermal expression of TARC was higher in scabies lesions than in healthy control and tick bites, while the number of infiltrating TARC(+) cells was comparable among groups (Figure 2B,C). TSLP promotes Th2 mediated-inflammation, leading to the production of periostin by fibroblasts, and periostin, in turn, induces keratinocyte TSLP.[16] The epidermal expression of TSLP and dermal deposition of periostin were also enhanced in scabies lesions compared with normal skin and tick bites (Figure 2D,E).

3.4 | Infiltrating macrophages express IL-31

Although the main cellular source of IL-31 is considered as activated Th2 cells,[14] monocytes/macrophages,[20,21] mast cells,[22] eosinophils,[23] and basophils[24] are also able to produce IL-31. In scabies lesions, we observed a large number of CD68(+) cells in the dermis (Figure 3A). Thus, we next tested whether monocytes/macrophages function as cellular sources of IL-31. Notably, a majority (>70%) of IL-31(+) cells were CD68(+) macrophages (Figure 3B,C). These cells expressed M2 macrophage markers,[25] Arg1 or CD163 (Figure 3D,E). In addition, IL-31(+) cells consisted of 60% of Arg1(+) cells. M1 macrophage marker iNOS(+) cells constituted only 15% of IL-31 (+) cells. It appeared that M2 macrophages developed in scabies lesions are one of the important sources of IL-31.

3.5 | Murine peritoneal macrophages express IL-31 in response to TSLP and periostin

A prior report has demonstrated that murine peritoneal macrophages (pM) are polarized cells toward M2 phenotype in vivo.[26] Consistent with this, unstimulated pM expressed Arg1 mRNA which did not further increase even under the in vitro stimulation with TSLP/periostin (Figure S2) that is known to promote M2 skewing.[27,28] In contrast, IFN-γ treatment resulted in the decreased expression of Arg1 mRNA and increased iNOS mRNA expression (Figure S2).[29] We, then, assessed IL-31 generation of pM. Notably, TSLP and/or periostin stimulated pM to express IL-31 mRNA (Figure S3). The combination of these factors further enhanced IL-31 mRNA expression and protein production (Figure 4A,B), while IL-4/-13 stimulation failed to induce IL-31 generation. IFN-γ treatment did not affect IL-31 generation. With flow cytometric analysis, we also observed that TSLP/periostin promoted generation of Arg1 and IL-31 proteins (Figure 4C,D). Apparently, pM and human macrophages expressed a TSLP receptor complex (comprising TSLPR and IL-7Rα)[30] and a periostin receptor integrin αV (Figure 5A,B).[16] Collectively, TSLP and periostin skewed macrophages toward an M2 phenotype and stimulated IL-31 production.

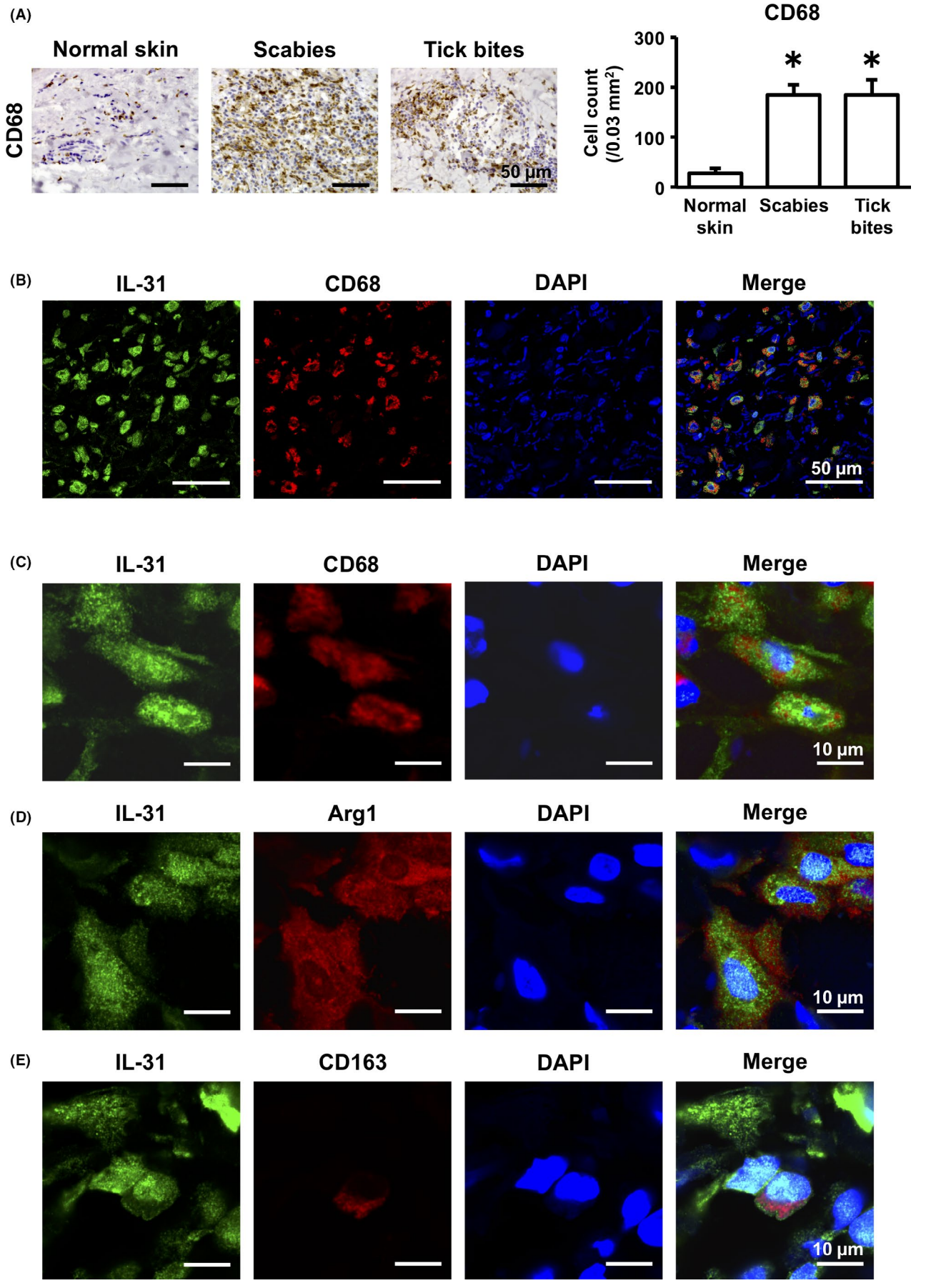
4 | DISCUSSION

The present data indicated that IL-31 seems to be one of the major pruritogenic factors in scabies lesions. Although intraepidermal sprouting of nerve fibers has been implicated in chronic pruritus,[12,31-33] IENFD was enhanced not only in scabies, but also in tick bite lesions that were not pruritic. Thus, intraepidermal nerve fiber sprouting may not be an important factor in scabies pruritus. Similarly, it seemed unlikely that mast cells largely contributed to itching, as an increase in mast cells was not observed in scabies lesions.[34]

Notably, more than half of IL-31(+) cells accumulating in the dermis were CD68(+) macrophages. A prior report also demonstrated that the major population of IL-31(+) cells in human AD lesions was CD11b(+) monocytes/macrophages and no CD3(+) cells expressed IL-31.[35] Macrophages are generally divided into two phenotypes: M1 (classically activated) and M2 (alternatively activated).[25] In scabies lesions, almost all macrophages were positive Arg1(+), indicating that they were M2 macrophages. M2 skewing is promoted by Th2-related cytokines such as IL-4 and/or IL-13.[25] Prior studies have demonstrated that Th2 cytokines are overexpressed, and mixed Th1/Th2 immune responses occur in ordinary scabies.[36-38] Consistent with this, massive infiltration of CCR4(+) Th2 cells, basophils, and eosinophils was observed in our study. Collectively, immune responses biased toward Th2 in scabies lesions promoted the development of M2 macrophages.

To understand the precise mechanisms of IL-31 production from macrophages, we performed ex vivo experiments with murine pM. TSLP and periostin, but not IL-4/13, stimulated pM to produce a significant amount of IL-31. IFN-γ-treated M1 macrophages[25] failed to

FIGURE 3 CD68(+) macrophages expressing IL-31 in scabies lesions. A, A large number of CD68 (+) cells was observed in the dermis of scabies lesions. B,C, More than 50% of IL-31 immunoreactive cells were CD68(+). D,E, IL-31(+) cells expressed Arg1 and/or CD163. Scale bars indicate 50 μm (A,B) and 10 μm (C-E). Values represent mean + SD of four subjects. *$P < 0.05$ compared with normal skin


(A)
Normal skin
Scabies
Tick bites
CD68
50 μm
CD68
Cell count (/0.03 mm²)
300
200
100
0
*
*
Normal skin
Scabies
Tick bites
(B)
IL-31
CD68
DAPI
Merge
50 μm
(C)
IL-31
CD68
DAPI
Merge
10 μm
(D)
IL-31
Arg1
DAPI
Merge
10 μm
(E)
IL-31
CD163
DAPI
Merge
10 μm

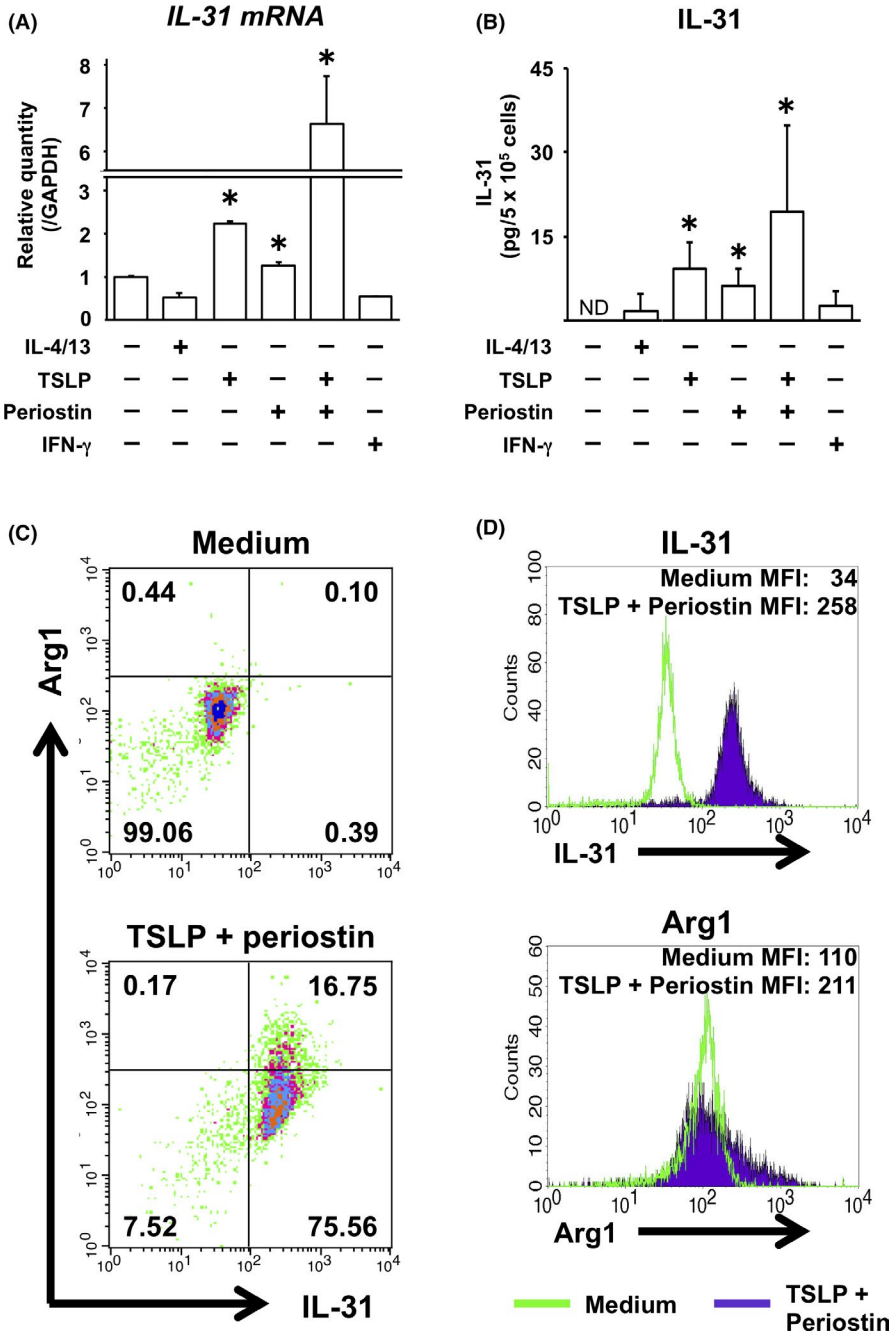
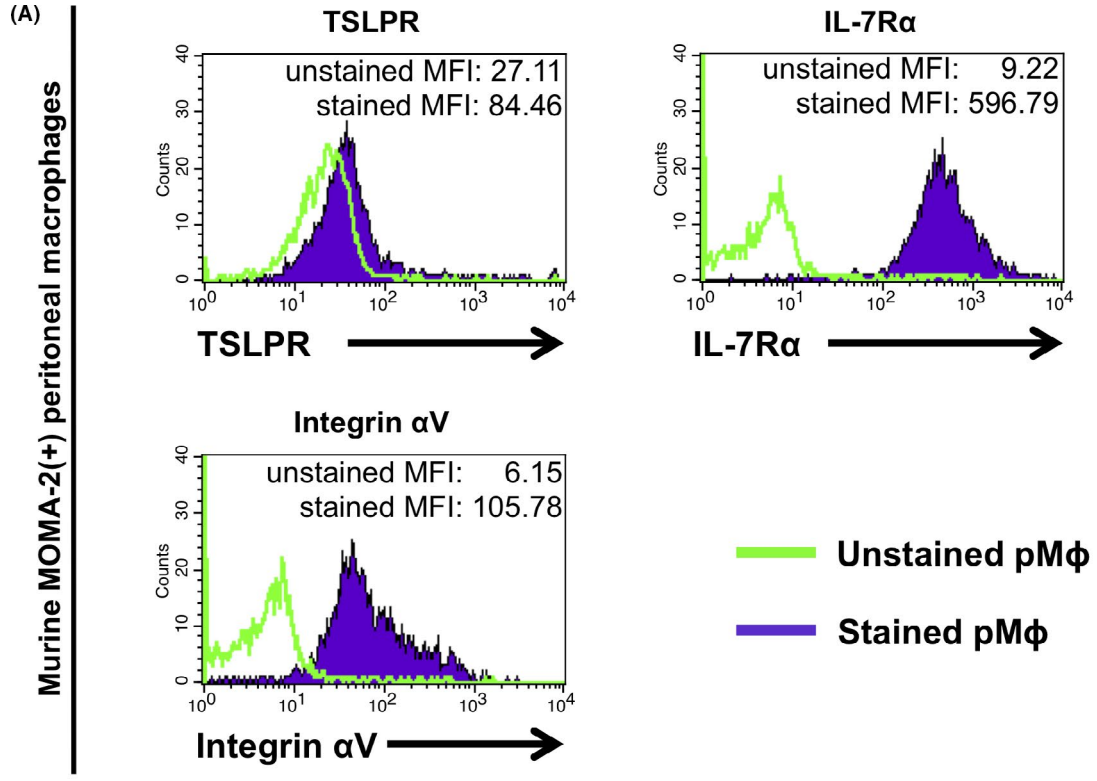


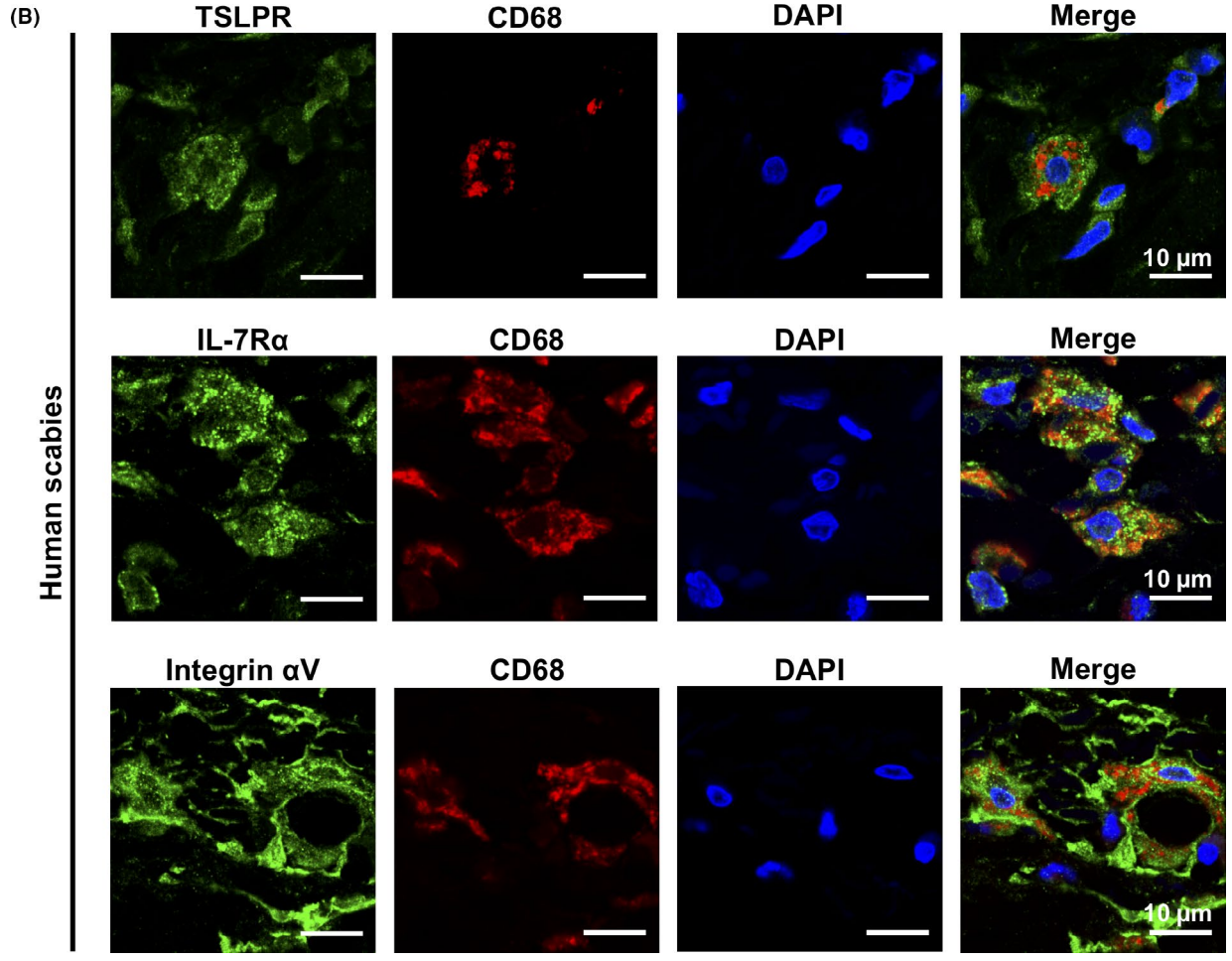
FIGURE 4 Murine peritoneal macrophages produce IL-31 in response to thymic stromal lymphopoietin (TSLP) and periostin ex vivo. Murine peritoneal macrophages (pM) were stimulated with IL-4/13, TSLP, Periostin, and/or IFN-γ for 24 h. A,B, IL-31 mRNA and protein were significantly increased when macrophages were stimulated with TSLP and periostin. C,D, Flow cytometric analysis of intracellular IL-31 and Arg1 in pM treated with TSLP plus periostin. Representative results of at least three independent experiments are shown. Values represent mean + SD of three samples. *$P < 0.05$ compared with nontreated pM. MFI, mean fluorescence intensity; ND, not detected [Color figure can be viewed at wileyonlinelibrary.com]

generate IL-31. A prior report with human monocytes/macrophages also showed that periostin induced IL-31 production.[39] These data suggested that TSLP and periostin expressed in human scabies lesions were essentially involved in IL-31 generation from M2 macrophages.

Mechanisms for TSLP/periostin overexpression in scabies are uncertain. However, it can be assumed that proteases from scabies mites[40] stimulate epidermal keratinocytes to express TSLP through its receptor, protease-activated receptor type 2 (PAR-2),[41] as observed in TSLP expression induced by house dust mite allergens in human skin.[42] TSLP activates CCR4(+) Th2 cells and basophils[43,44]; these cells are capable of producing Th2-related cytokines IL-4/13 that promote periostin secretion from fibroblasts.[16] Thus, the protease-TSLP-periostin axis might be an essential pathway for IL-31 generation from M2 macrophages.

FIGURE 5 Expression of thymic stromal lymphopoietin (TSLP) and periostin receptors on macrophages. A, Flow cytometric analysis of murine peritoneal macrophages for TSLP receptor complex comprising TSLPR and IL-7Rα, and periostin receptor integrin αV. Representative results of at least three independent experiments are shown. B, Immunohistochemical analysis of human scabies lesions. Infiltrating CD68(+) macrophages express TSLPR, IL-7Rα, and integrin αV. MFI, mean fluorescence intensity


(A)
Murine MOMA-2(+) peritoneal macrophages
TSLPR
unstained MFI: 27.11
stained MFI: 84.46
Counts
TSLPR
IL-7Rα
unstained MFI: 9.22
stained MFI: 596.79
IL-7Rα
Integrin αV
unstained MFI: 6.15
stained MFI: 105.78
Integrin αV
Unstained pMϕ
Stained pMϕ


(B)
Human scabies
TSLPR
CD68
DAPI
Merge
10 μm
IL-7Rα
CD68
DAPI
Merge
10 μm
Integrin αV
CD68
DAPI
Merge
10 μm

Besides IL-31, one may need to consider the direct contribution of TSLP and proteases to scabies itch. In mice, TSLP can stimulate peripheral nerve fibers and elicit scratching behavior.[41] This may also be the case in humans, as TSLP receptors are expressed by human dorsal root ganglia.[41] Proteases are capable of stimulating TSLP generation from keratinocytes.[41] They are also capable of directly provoking itch in humans through activating PAR-2 on peripheral nerve fibers.[45] Eosinophils may also be involved in itch in scabies through releasing several proteins (eg, substance P, nerve growth factor, eosinophilic cationic protein, major basic protein, and eosinophil peroxidase). In addition, eosinophils can induce neuron branching in vitro and skin innervation in vivo, thereby contributing to itch in mouse skin following contact toxicant exposure.[46,47] Direct stimulation of peripheral nerve fibers by *Sarcoptes scabiei* var. *hominis* as mechanical itch could be also one of pruritogenic factors. Severe pruritus in scabies may be provoked by multiple and complex immune pathways and pruritogens.

In tick bite lesions, which were used as a control disease in this study, a number of infiltrating CXCR3(+) Th1 cells and IL-17(+) Th17 cells was observed. A recent study showed changes in immune responses during tick bites. Patients with primary tick bite displayed a higher number of macrophages and lower number of basophils in the lesions.[48] In contrast, patients with a history of repeated tick bites (>2) had a smaller number of macrophages but larger numbers of basophils. Infiltrating T cells from patients with repeated tick bites produced a larger amount of type 2 cytokines than those with just one episode of tick bite.[48] Thus, it appeared that skin samples from patients enrolled in this study were possibly first tick bite lesions with Th1/17 immunity. This was further supported by the results that massively infiltrative CD68(+) macrophages were negative for Arg1 and CD163, suggesting that they were M1 phenotype (Figure S4).

In summary, Th2 immunity is predominant in human ordinary scabies lesions and is accompanied by massive infiltration of IL-31(+) M2 macrophages, epidermal expression of TSLP, and dermal deposition of periostin. The key limitations of this study were the small sample sizes, and we have not been able to perform a clinical human study with anti-IL-31 Ab in scabies patients. The present study provides, however, novel insights into the immunopathogenesis of scabies. IL-31 could be a therapeutic target for severe itch in scabies.

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CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

AUTHOR CONTRIBUTIONS

TH designed and conducted the experiments and wrote the manuscript. TS and HY interpreted the data and critically revised the manuscript. All authors have read and approved the final manuscript.

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REFERENCES

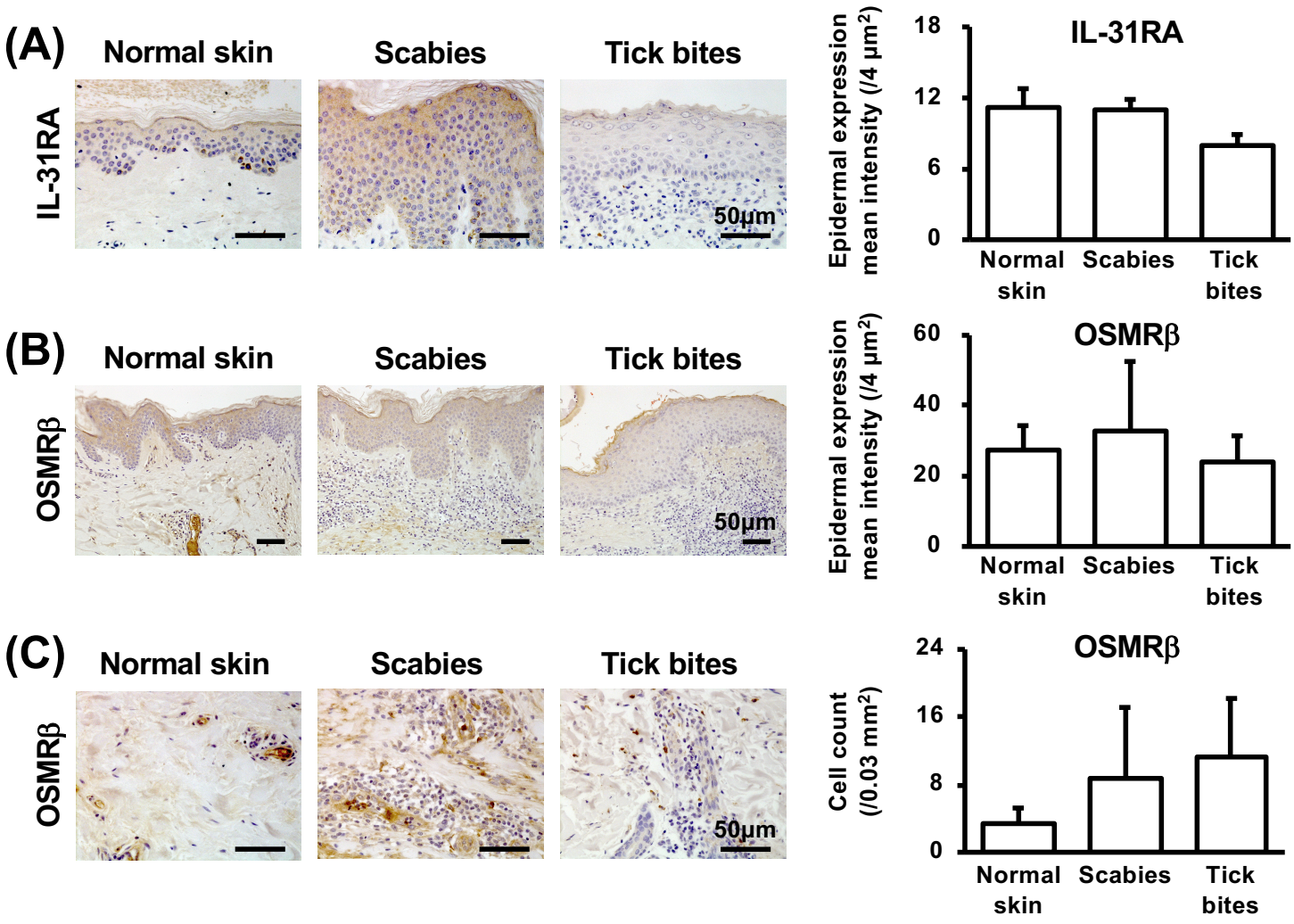
1. Chosidow O. Clinical practices. Scabies. *N Engl J Med*. 2006;354:1718-1727.
2. Fuller LC. Epidemiology of scabies. *Curr Opin Infect Dis*. 2013;26:123-126.
3. Romani L, Steer AC, Whitfeld MJ, Kaldor JM. Prevalence of scabies and impetigo worldwide: a systematic review. *Lancet Infect Dis*. 2015;15:960-967.
4. Murray CJL, Vos T, Lozano R, et al. Disability-adjusted life years (DALYs) for 291 diseases and injuries in 21 regions, 1990–2010: a systematic analysis for the Global Burden of Disease Study 2010. *Lancet*. 2012;380:2197-2223.
5. Puza CJ, Suresh V. Scabies and pruritus-a historical review. *JAMA Dermatol*. 2018;154:536.
6. Bhat SA, Mounsey KE, Liu X, Walton SF. Host immune responses to the itch mite, Sarcoptes scabiei, in humans. *Parasit Vectors*. 2017;10:385.
7. Sonkoly E, Muller A, Lauerma AI, et al. IL-31: a new link between T cells and pruritus in atopic skin inflammation. *J Allergy Clin Immunol*. 2006;117:411-417.
8. Furue M, Yamamura K, Kido-Nakahara M, Nakahara T, Fukui Y. Emerging role of interleukin-31 and interleukin-31 receptor in pruritus in atopic dermatitis. *Allergy*. 2018;73:29-36.
9. Ito Y, Satoh T, Takayama K, Miyagishi C, Walls AF, Yokozeki H. Basophil recruitment and activation in inflammatory skin diseases. *Allergy*. 2011;66:1107-1113.
10. Hashimoto T, Satoh T, Yokozeki H. Protective role of STAT6 in basophil-dependent Prurigo-like allergic skin inflammation. *J Immunol*. 2015;194:4631-4640.
11. Nattkemper L, Martinez-Escala M, Gelman A, et al. Cutaneous T-cell lymphoma and pruritus: the expression of IL-31 and its receptors in the skin. *Acta Derm Venereol*. 2016;96:894-898.
12. Tominaga M, Takamori K. Itch and nerve fibers with special reference to atopic dermatitis: therapeutic implications. *J Dermatol*. 2014;41:205-212.
13. Cevikbas F, Wang X, Akiyama T, et al. A sensory neuron-expressed IL-31 receptor mediates T helper cell-dependent itch: involvement of TRPV1 and TRPA1. *J Allergy Clin Immunol*. 2014;133:448-460.
14. Maier E, Werner D, Duschl A, Bohle B, Horejs-Hoeck J. Human Th2 but not Th9 cells release IL-31 in a STAT6/NF-κB–dependent way. *J Immunol*. 2014;193:645-654.
15. Yoshie O, Matsushima K. CCR15 and its ligands: from bench to bedside. *Int Immunol*. 2015;27:11-20.
16. Masuoka M, Shiraishi H, Ohta S, et al. Periostin promotes chronic allergic inflammation in response to Th2 cytokines. *J Clin Invest*. 2012;122:2590-2600.
17. Kakinuma T, Nakamura K, Wakugawa M, et al. Thymus and activation-regulated chemokine in atopic dermatitis: serum thymus and activation-regulated chemokine level is closely related with disease activity. *J Allergy Clin Immunol*. 2001;107:535-541.
18. Ito T, Liu Y-J, Arima K. Cellular and molecular mechanisms of TSLP function in human allergic disorders - TSLP programs the "Th2 code" in dendritic cells. *Allergol Int*. 2012;61:35-43.
19. Soumelis V, Reche PA, Kanzler H, et al. Human epithelial cells trigger dendritic cell mediated allergic inflammation by producing TSLP. *Nat Immunol*. 2002;3:673-680.
20. Cornelissen C, Brans R, Czaja K, et al. Ultraviolet B radiation and reactive oxygen species modulate interleukin-31 expression in

T lymphocytes, monocytes and dendritic cells. *Br J Dermatol.* 2011;165:966-975.
21. Takamori A, Nambu A, Sato K, et al. IL-31 is crucial for induction of pruritus, but not inflammation, in contact hypersensitivity. *Sci Rep.* 2018;8:6639.
22. Niyonsaba F, Ushio H, Hara M, et al. Antimicrobial peptides human -defensins and cathelicidin LL-37 induce the secretion of a pruritogenic cytokine IL-31 by human mast cells. *J Immunol.* 2010;184:3526-3534.
23. Kunsleben N, Rüdrich U, Gehring M, Novak N, Kapp A, Raap U. IL-31 induces chemotaxis, calcium mobilization, release of reactive oxygen species, and CCL26 in Eosinophils, which are capable to release IL-31. *J Invest Dermatol.* 2015;135:1908-1911.
24. Raap U, Gehring M, Kleiner S, et al. Human basophils are a source of - and are differentially activated by - IL-31. *Clin Exp Allergy.* 2017;47:499-508.
25. Wang N, Liang H, Zen K. Molecular mechanisms that influence the macrophage m1–m2 polarization balance. *Front Immunol.* 2014;5:614.
26. Yamazaki T, Nagata K, Kobayashi Y. Cytokine production by M-CSF- and GM-CSF-induced mouse bone marrow-derived macrophages upon coculturing with late apoptotic cells. *Cell Immunol.* 2008;251:124-130.
27. Furudate S, Fujimura T, Kakizaki A, et al. The possible interaction between periostin expressed by cancer stroma and tumor-associated macrophages in developing mycosis fungoides. *Exp Dermatol.* 2016;25:107-112.
28. Han H, Headley MB, Xu W, Comeau MR, Zhou B, Ziegler SF. Thymic stromal lymphopoietin amplifies the differentiation of alternatively activated macrophages. *J Immunol.* 2013;190:904-912.
29. Mills CD, Kincaid K, Alt JM, Heilman MJ, Hill AM. M-1/M-2 macrophages and the Th1/Th2 paradigm. *J Immunol.* 2000;164:6166-6173.
30. Kitajima M, Lee HC, Nakayama T, Ziegler SF. TSLP enhances the function of helper type 2 cells. *Eur J Immunol.* 2011;41:1862-1871.
31. Takada S, Kou K, Nagashima Y, Ikezawa Z, Aihara M. Aberrant epidermal expression of semaphorin 3A and nerve growth factor in prurigo nodularis. *J Dermatol.* 2013;40:404-406.
32. Schuhknecht B, Marziniak M, Wissel A, et al. Reduced intraepidermal nerve fibre density in lesional and nonlesional prurigo nodularis skin as a potential sign of subclinical cutaneous neuropathy. *Br J Dermatol.* 2011;165:85-91.
33. Pereira MP, Steinke S, Zeidler C, et al. European academy of dermatology and venereology European prurigo project: expert consensus on the definition, classification and terminology of chronic prurigo. *J Eur Acad Dermatol Venereol.* 2018;32:1059-1065.
34. Sanders KM, Nattkemper LA, Rosen JD, et al. Non-histaminergic itch mediators elevated in the skin of a porcine model of scabies and of human scabies patients. *J Invest Dermatol.* 2019;139:971-973.
35. Kato A, Fujii E, Watanabe T, et al. Distribution of IL-31 and its receptor expressing cells in skin of atopic dermatitis. *J Dermatol Sci.* 2014;74:229-235.
36. Walton SF, Pizzutto S, Slender A, et al. Increased allergic immune response to Sarcoptes scabiei antigens in crusted versus ordinary scabies. *Clin Vaccine Immunol.* 2010;17:1428-1438.
37. Walton SF, Oprescu FI. Immunology of scabies and translational outcomes: identifying the missing links. *Curr Opin Infect Dis.* 2013;26:116-122.
38. Mounsey KE, Murray HC, Bielefeldt-Ohmann H, et al. Prospective study in a porcine model of Sarcoptes scabiei indicates the association of Th2 and Th17 pathways with the clinical severity of scabies. Vinetz JM, editor. *PLoS Negl Trop Dis.* 2015;9:e0003498.
39. Fujimura T, Kakizaki A, Furudate S, Aiba S. A possible interaction between periostin and CD163+ skin-resident macrophages in pemphigus vulgaris and bullous pemphigoid. *Exp Dermatol.* 2017;26:1193-1198.
40. Asokananthan N, Graham PT, Fink J, et al. Activation of protease-activated receptor (PAR)-1, PAR-2, and PAR-4 stimulates IL-6, IL-8, and prostaglandin E2 release from human respiratory epithelial cells. *J Immunol.* 2002;168:3577-3585.
41. Wilson S, Thé L, Batia L, et al. The epithelial cell-derived atopic dermatitis cytokine TSLP activates neurons to induce Itch. *Cell.* 2013;155:285-295.
42. Landheer J, Giovannone B, Mattson JD, et al. Epicutaneous application of house dust mite induces thymic stromal lymphopoietin in nonlesional skin of patients with atopic dermatitis. *J Allergy Clin Immunol.* 2013;132:1252-1254.
43. Tatsuno K, Fujiyama T, Yamaguchi H, Waki M, Tokura Y. TSLP directly interacts with skin-homing Th2 cells highly expressing its receptor to enhance IL-4 production in atopic dermatitis. *J Invest Dermatol.* 2015;135:3017-3024.
44. Karasuyama H, Miyake K, Yoshikawa S, Yamanishi Y. Multifaceted roles of basophils in health and disease. *J Allergy Clin Immunol.* 2018;142:370-380.
45. Steinhoff M, Neisius U, Ikoma A, et al. Proteinase-activated receptor-2 mediates itch: a novel pathway for pruritus in human skin. *J Neurosci.* 2003;23:6176-6180.
46. Fujisawa D, Kashiwakura J-I, Kita H, et al. Expression of Mas-related gene X2 on mast cells is upregulated in the skin of patients with severe chronic urticaria. *J Allergy Clin Immunol.* 2014;134(3):622-633.
47. Lee JJ, Protheroe CA, Luo H, et al. Eosinophil-dependent skin innervation and itching following contact toxicant exposure in mice. *J Allergy Clin Immunol.* 2015;135:477-487.
48. Hashizume H, Fujiyama T, Umayahara T, Kageyama R, Walls AF, Satoh T. Repeated Amblyomma testudinarium tick bites are associated with increased galactose-α-1,3-galactose carbohydrate IgE antibody levels: a retrospective cohort study in a single institution. *J Am Acad Dermatol.* 2018;78:1135-1141.

SUPPORTING INFORMATION

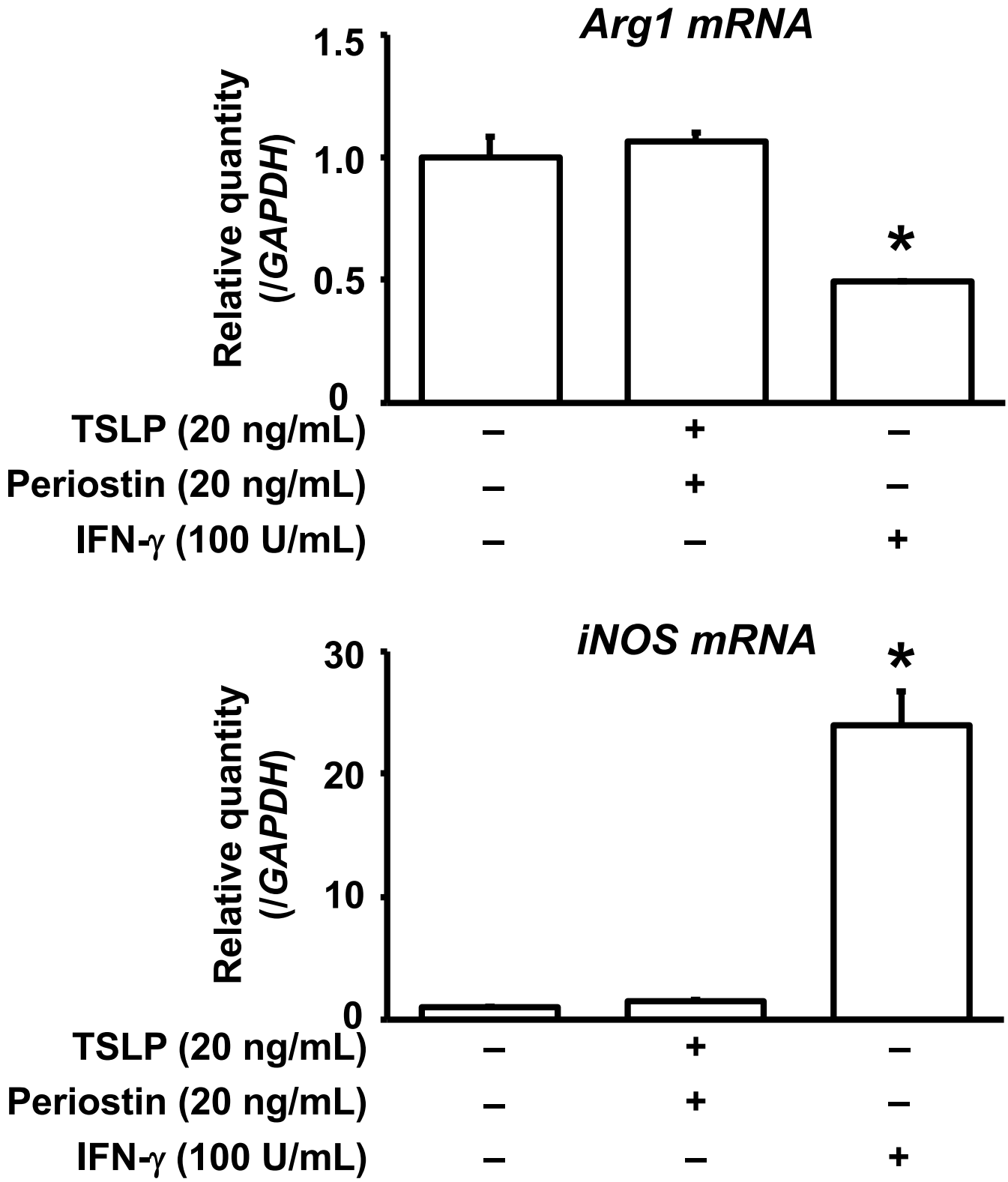
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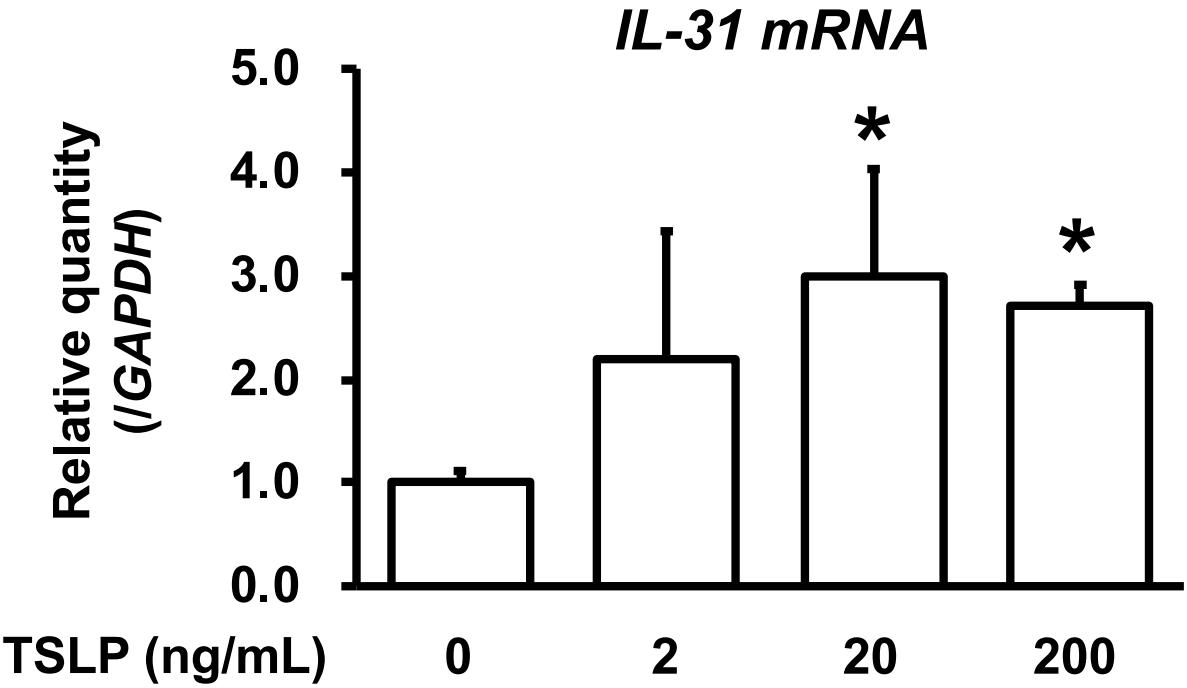
Supplemental Figure #1. Epidermal expression of IL-31RA and OSMRβ and dermal infiltrating OSMRβ (+) cells.

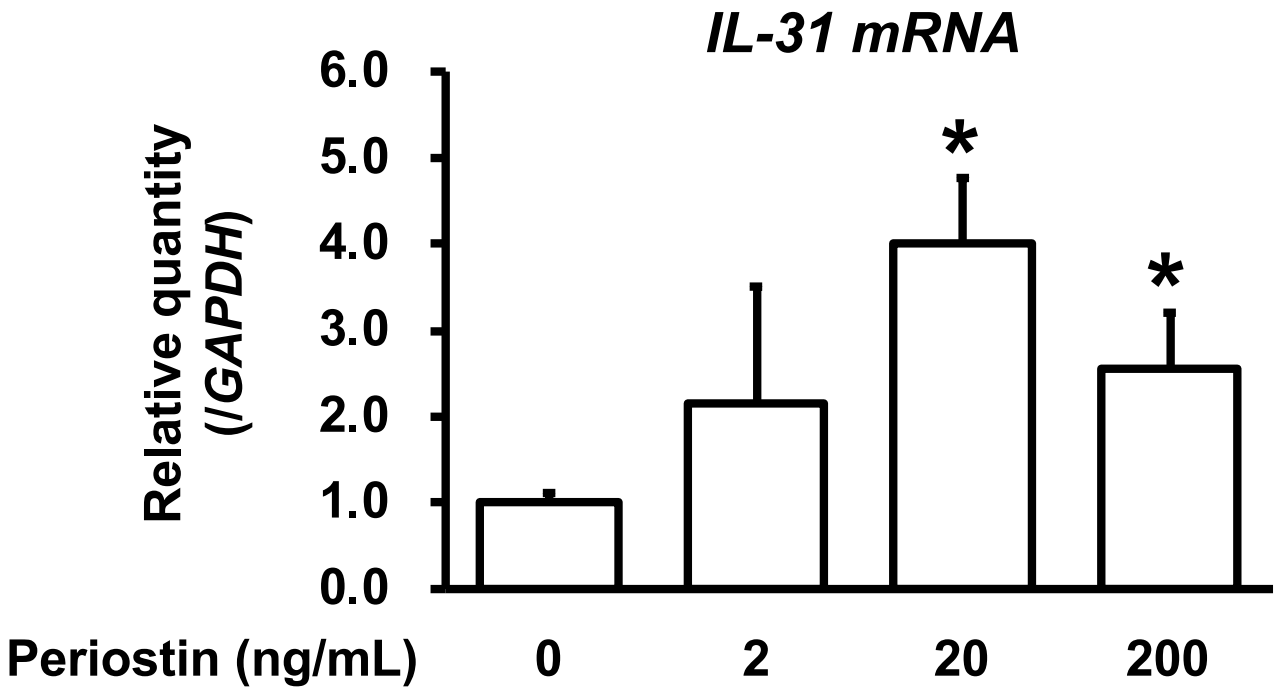
Values represent mean + SD.



Supplemental Figure #2. mRNA expression of *Arg1* and *iNOS* by murine peritoneal macrophages in response to TSLP/periostin or IFN-γ.

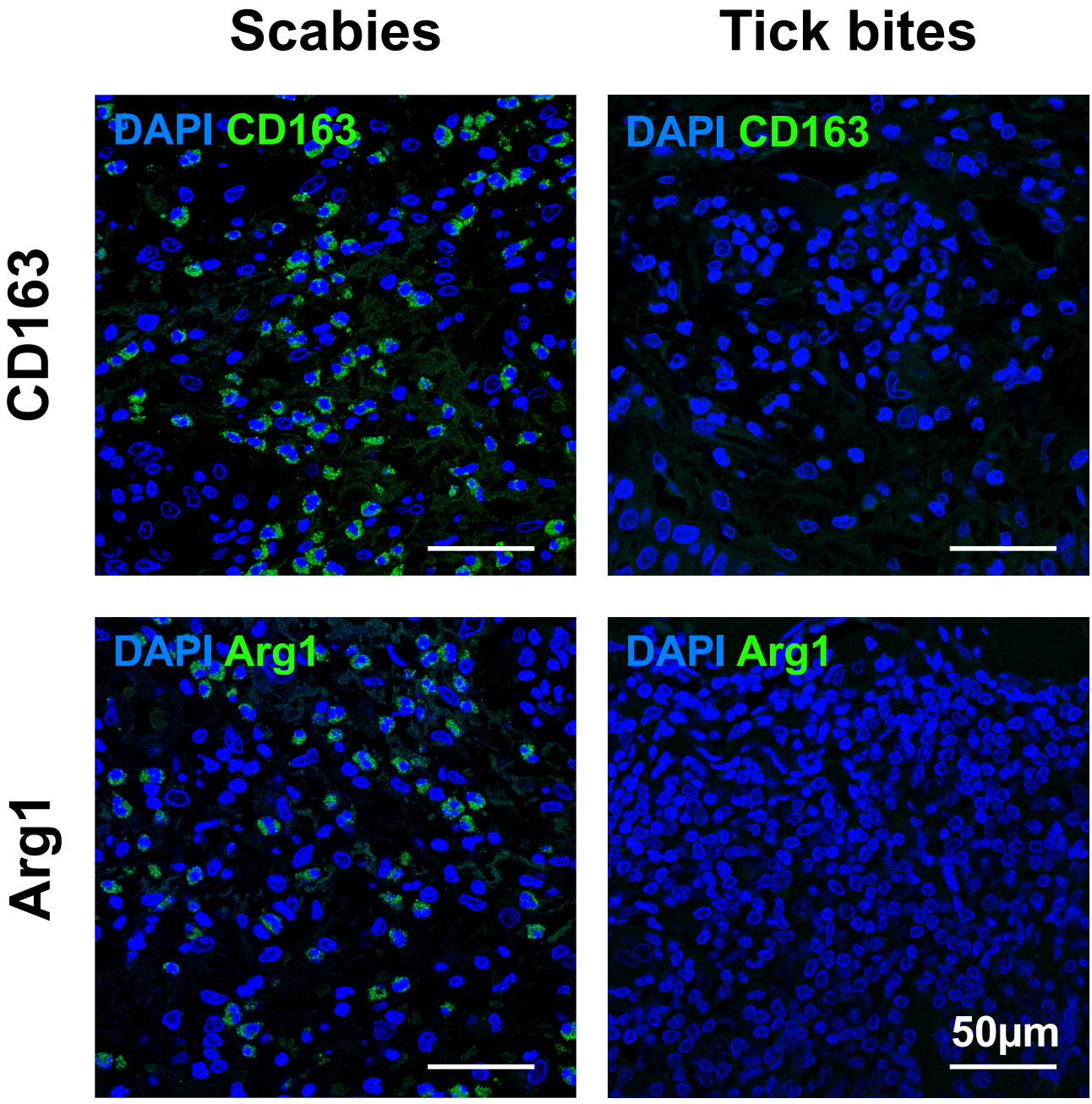
Representative results of at two independent experiments are shown. Values represent mean + SD of three samples. *$p < 0.05$ compared with unstimulated cells.





Supplemental Figure #3. *IL-31* mRNA expression of murine peritoneal macrophages in response to TSLP or Periostin.

Representative results of at two independent experiments are shown. Values represent mean + SD of three samples. *$p < 0.05$ compared with unstimulated cells.



Supplemental Figure #4. CD163(+) or Arg1(+) M2 macrophages in the lesions of scabies and tick bites.