

Th17 cells and tissue remodeling in atopic and contact dermatitis

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Abstract

Background: Eczematous skin lesions of atopic dermatitis (AD) as well as allergic and irritant contact dermatitis (ACD, ICD) are characterized by the same typical clinical signs, although due to different causes. In both AD and ACD, the presence of T helper 17 cells which play an important role in host defense, has been reported. Furthermore, IL-17 is involved in tissue repair and remodeling. This study aimed to investigate IL-17 expression in acute eczematous skin lesions and correlate it with markers of remodeling in AD, ACD, and ICD.

Methods: Skin specimens were taken from positive patch test reactions to aeroallergens, contact allergens, and irritants at days 2, 3, and 4. Inflammatory cells as well as the expression of cytokines and extracellular matrix proteins were evaluated by immunofluorescence staining and confocal microscopy.

Results: Allergic contact dermatitis and ICD were characterized by IFN- γ expression, whereas in AD lesions, IL-13 expression and high numbers of eosinophils were the prominent phenotype. Expression of IL-17, but also IL-21 and IL-22, was observed in all eczema subtypes. The number of IL-22+ T cells correlated with the number of eosinophils. Markers of remodeling such as MMP-9, procollagen-3, and tenascin C were observed in all acute eczematous lesions, while a correlation of IL-17+ T cell numbers with tenascin C-expressing cells and MMP-9+ eosinophils was apparent.

Conclusion: The expression of IL-17 and related cytokines, such as IL-22, was demonstrated in acute eczematous lesions independent of their pathogenesis. Our results suggest a potential role for IL-17 in remodeling of the skin.

Eczema delineates a common and heterogeneous group of skin diseases presenting with similar clinical signs, but differing pathogenesis ending up with lichenification. In atopic dermatitis (AD), environmental allergens, such as pollen or the house dust mite allergens, penetrate an impaired skin barrier, resulting in a T helper (Th) 2-driven inflammation (1, 2). With allergic contact dermatitis (ACD) developing as a consequence of contact hypersensitivity to low-molecular-weight allergens (haptens), it is an innate and adaptive, predominantly Th 1 immune response which is involved in both the sensitization and elicitation phases (3). In irritant contact dermatitis (ICD), an exposure to chemicals causes damage of the epidermis, resulting in interleukin (IL)-1, IL-6, IL-8, and tumor necrosis factor (TNF)- α release by keratinocytes and subsequent inflammation (4).

Earlier work characterized the immunophenotype of T cells infiltrating the skin in AD and ACD (5). Recently, Th17 and Th22 have been identified as distinct T-cell subsets being involved in the pathogenesis of various, including allergic, skin diseases (6–10). A role for IL-17 in allergic skin diseases is consistent with the observation that IL-17-deficient mice show impaired contact and delayed-type hypersensitivity responses upon sensitization and challenge with the corresponding allergen (11). In AD patients, the number of peripheral blood IL-17+CD4+ T cells correlates with disease severity (12). Moreover, IL-17+ cells have been detected infiltrating acute lesions (12, 13). Additional exposure to staphylococcal enterotoxin B increased IL-17 expression in *Dermatophagoides pteronyssinus* (Der p)1-challenged skin followed by an upregulation of human beta-defensin 2

(HBD-2) (14). Extracellular vesicles derived from *Staphylococcus aureus*, when applied to the skin, were sufficient to induce AD-like lesions and IL-17 expression (15).

IL-17 and IL-22 expression has also been detected in nickel-challenged skin of allergic patients (16). Nickel-specific T cells were reported to produce IL-17 and, thus, activated keratinocytes to express adhesion molecules and proinflammatory cytokines (6, 17). Furthermore, eosinophils stimulated by IL-17 produced pro-fibrotic cytokines, such as transforming growth factor (TGF)- β and IL-11 (18). In AD, the expression of IL-22 in the skin is higher than in psoriasis that predominantly exhibits IL-17 expression (19). By releasing IL-22, T cells induced keratinocyte hyperplasia and antimicrobial peptide expression (20). In psoriasis, IL-22 was shown to mediate IL-23-induced epidermal hyperproliferation and dermal inflammation (21, 22).

This study aimed to investigate the presence of IL-17 and related cytokines, as well as their association with local tissue remodeling in AD as well as ACD and ICD.

Methods

Immunofluorescence

Punch biopsies were taken from positive patch test (PT) reactions to contact allergens (contact allergen PT; CPT), house dust mite or pollen (atopy PT, APT), and 0.25% sodium

lauryl sulfate (irritant PT; IPT) from patients undergoing diagnostic patch testing as model for ACD, AD, and ICD, respectively. The study was approved by the ethics committee of the Canton of Bern. Written informed consent was obtained from all patients prior to enrollment in the study. Altogether, 27 skin samples (each day and eczema type: $n = 3$) were analyzed. In 9 patients, two biopsies were taken from parallel patch tests using the same allergen or irritant at day 2 (D2; PT 24 hours) and day 3 (D3; PT 48 hours). In other 9 patients, positive reactions were biopsied at day 4 (D4; PT 72 hours). Tissues were fixed in 4% formaldehyde and paraffin-embedded. Cellular infiltration and cytokine expression were analyzed by immunofluorescence techniques as described previously (23) using antibodies directed against CD3, mast cell tryptase (both Dako, Glostrup, Denmark), eosinophil cationic protein (Pharmacia Diagnostics AB, Uppsala, Sweden), IL-8, interferon (IFN)- γ , TGF- β (all R&D Systems, Minneapolis, MN, USA), IL-1 β , IL-6, IL-13, IL-17, metalloproteinase (MMP)-9 (all Santa Cruz Biotechnology, Santa Cruz, CA, USA), IL-21, procollagen-3 (both Millipore, Billerica, MA, USA), IL-22 (Abcam, Cambridge, UK), IL-23 (BioLegend, San Diego, CA, USA), IL-33 (Enzo Life Science, Lausen, Switzerland), tenascin C (Monosan, Am Uden, Netherlands), and TNF- α (Novus Biologicals, Littleton, CO, USA). Appropriate secondary antibodies labeled with Alexa Fluor 488 (Invitrogen Molecular Probes, Paisley, UK) or Cy3 (Jackson ImmunoResearch Ltd, Suffolk, UK) were

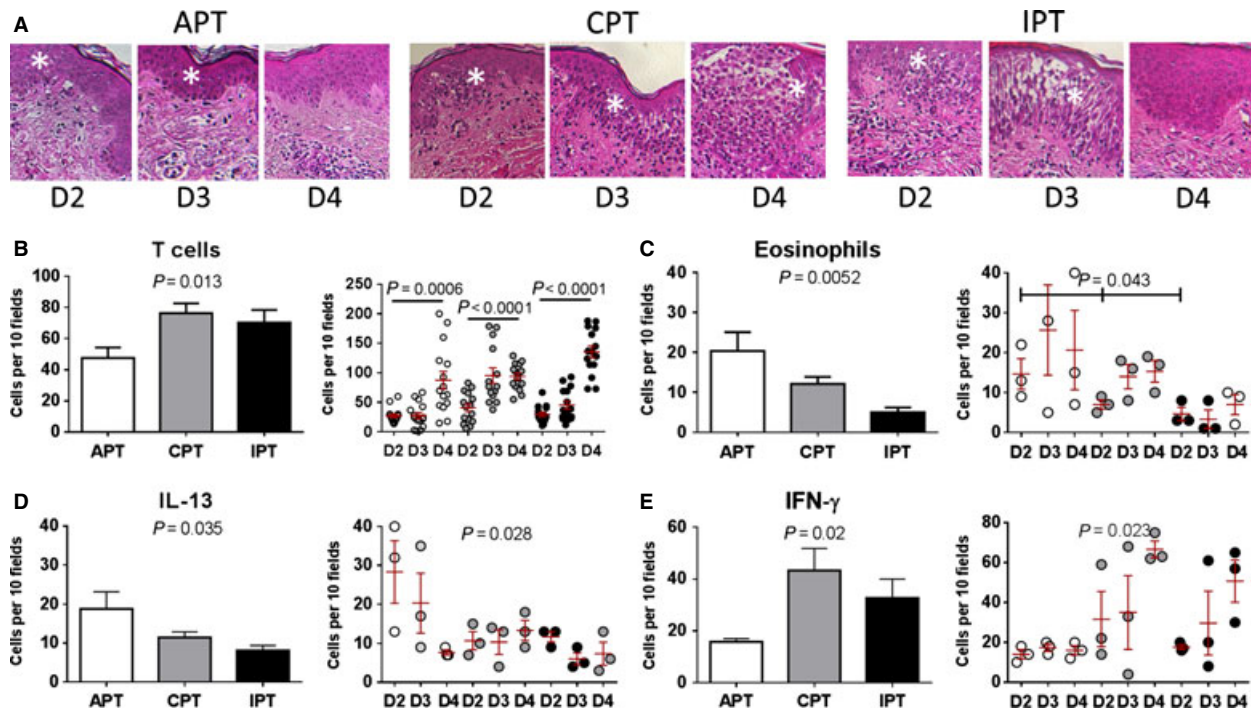


Figure 1 Characterization of inflammation in eczema subtypes. (A) Histopathology revealing time-dependent (D2-4) infiltration of inflammatory cells, spongiosis (*) and acanthosis (thickening of epidermis) in atopy (APT), contact allergen (CPT), and irritant (ICD)

patch test reactions (H&E, magnification $\times 63$). (B-E) Pattern of T cell (B) and eosinophil (C) infiltration as well as IL-13 (D) and IFN- γ (E) cytokine expression in the different eczema subtypes. APT, white; CPT, gray; IPT, black bars and circles.

applied, and confocal laser scanning microscopy performed (Carl Zeiss MicroImaging, Jena, Germany). Positive cells were counted in ten consecutive fields in the upper dermis at a magnification $\times 1000$.

Statistics

Mean values ($\pm$ SEM) are given. For comparisons of different eczema types and time courses, ordinary one-way ANOVA and the Kruskal–Wallis test, respectively, were applied. To describe the correlation between two variables, Pearson's coefficient was determined. P -values <0.05 were considered statistically significant. Statistical analysis was carried out using GraphPad Prism 6 software (La Jolla, CA, USA).

Results

IFN- γ dominates in CPT and IPT, IL-13 in APT

Corresponding to the clinical findings, inflammation, spongiosis, and acanthosis increased from D2 to D4 in CPT, whereas in both APT and IPT, they were seen at D2 and D3 (Fig. 1A). Among inflammatory cells, T cells dominated with a maximum at D3 and D4 in CPT and at D4 in APT and IPT (Fig. 1B). The number of eosinophils was highest in APT ($P = 0.0052$) (Fig. 1C). Mast cell numbers did not differ

between eczema types and time points (data not shown). IL-13-expressing cells were predominantly found in APT reactions ($P = 0.035$) with a peak at D2 and D3 ($P = 0.028$) (Fig. 1D), whereas the numbers of IFN- γ -expressing cells were significantly higher in CPT and IPT than in APT ($P = 0.02$) with a maximum at D4 ($P = 0.023$) (Fig. 1E).

Expression of IL-17, IL-21, and IL-22 in eczema subtypes

Th17 cells have previously been observed in lesional skin of AD and ACD (12, 16). We found IL-17-expressing cells in all eczema types (Fig. 2 A, E). In APT, the expression of IL-17 was highest at D2 and D3 ($P = 0.036$), whereas in IPT, a sharp increase was observed only at D4. Approximately 50% of IL-17-expressing cells were IL-17 $^{+}$ T cells; remainder were larger than lymphocytes and, from the morphology, could have been mast cells, natural killer cells, or macrophages, all of which have been reported to be able to produce IL-17 (24–26). On average, less than 20% of the infiltrating T cells expressed IL-17 (Fig. 2B). However, in APT, the percentage increased to 35% at D2 and D3. Th17 cells were reported to secrete, in addition to IL-17, a panel of cytokines, such as IL-21 and IL-22 (27, 28). In all eczema types, the absolute and relative numbers of both IL-21- and IL-22-expressing T cells were low (Fig. 2C, D). In CPT, an increase in IL-21+ as well as

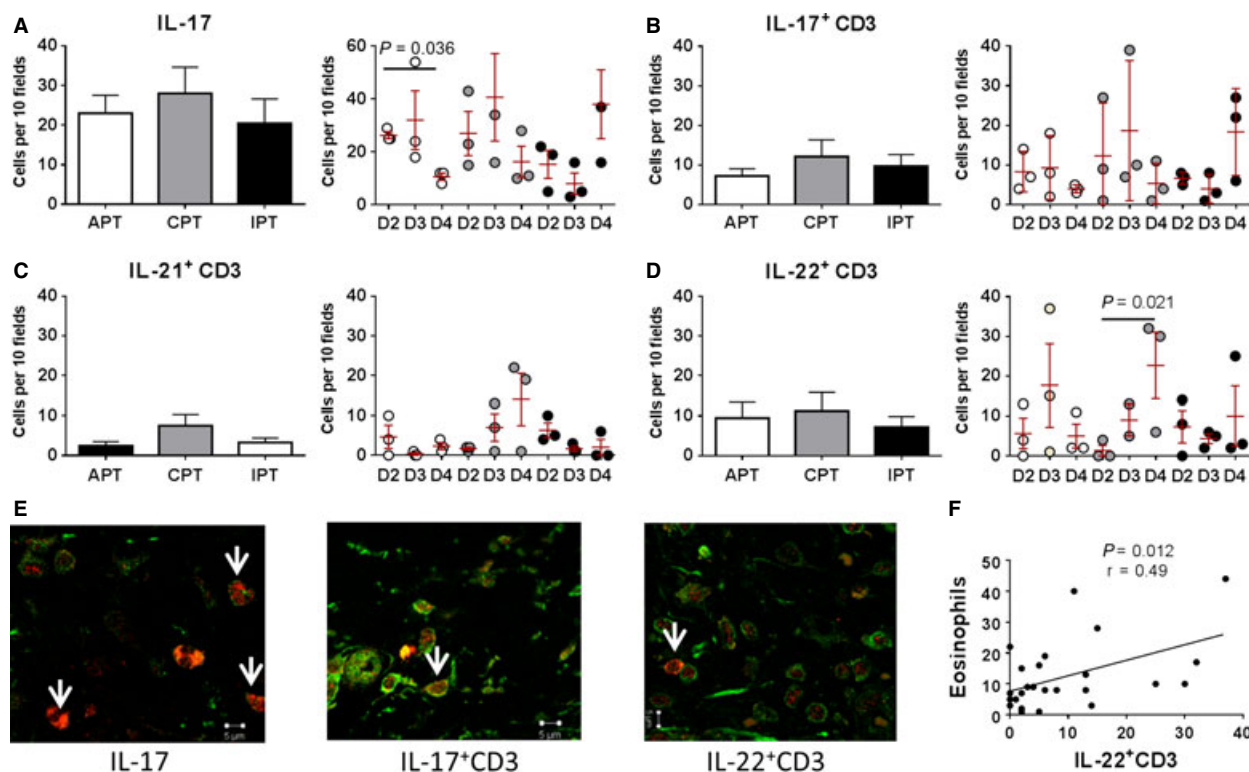


Figure 2 Cytokine expression in eczema subtypes. (A–D) Mean numbers of expressing cells per 10 fields with corresponding time course: (A) Total IL-17 $^{+}$ cells, (B) IL-17 $^{+}$ T cells, (C) IL-21 $^{+}$ T cells, and (D) IL-22 $^{+}$ T cells in the different eczema subgroups. APT, white;

CPT, gray; IPT, black bars and circles. (E) Representative images of IL-17-expressing cells (red) or IL-22-expressing cells (red). CD3 $^{+}$ T cells are labeled green. Magnification $\times 1000$. (F) Positive correlation between numbers of eosinophils and IL-22 $^{+}$ T cells ($n = 27$).

IL-22+ T ($P = 0.021$) cells from D2 to D4 was noticed. Furthermore, a positive correlation between the number of IL-22-expressing T cells and eosinophil numbers was apparent ($r = 0.487$; $P = 0.012$) (Fig. 2F).

Cytokines regulating Th17 differentiation and activation

As inflammation and, in particular, Th17 cell differentiation and activation are regulated by cytokines released by resident and/or inflammatory cells (29–33), the expression of TGF- β , TNF- α , IL-1, IL-6, IL-23, and IL-33 was assessed in the different eczema types over time (Fig. 3A–F). No correlation between the numbers of cells expressing any of these cytokines relative to the number of Th17 cells was detected (data not shown). The number of IL-33-expressing cells, known to promote Th2-type responses (34–36), was significantly higher in APT compared with CPT and IPT ($P = 0.0011$). In APT, higher numbers of TGF- β + as well as TNF- α + ($P = 0.034$) cells were observed as compared with CPT and IPT, with highest expression at the early time points D2 and D3. IL-6-expressing cells were mainly found in CPT ($P = 0.021$). Expression of IL-23, which is produced by dendritic cells and activates Th17 cells (37), was found at approximately the same levels in all eczema subtypes.

IL-17 correlated with fibrosis markers

As both IL-17 and IL-22 have been shown to be involved in tissue repair and remodeling (18, 19, 38, 39), we investigated

whether the expression of these cytokines correlates with the tissue remodeling in eczematous skin lesions as determined by MMP-9, procollagen-3, and tenascin C expression (Fig. 4A–C). Procollagen-3+ cells were increased in IPT compared with APT and CPT, in particular at D4 ($P = 0.021$). MMP-9 and tenascin C-expressing cells were present in all eczema types from D2 to 4 without significant differences. The numbers of IL-17+ T cells correlated with tenascin C-expressing cell numbers, suggesting a role for Th17 cells in remodeling in eczema (Fig. 4D). In CPT and IPT, deposition of tenascin C increased from linear deposits along the basement membrane (score 1) at D2 to deposits filling the papillary dermis (score 2) and in the dermis (score 3) at D4 (Fig. 4E).

Eosinophils, that constitutively bear IL-17 and IL-23 receptors, have previously been reported to release profibrotic cytokines in response to IL-17 and/or IL-23 stimulation (18, 40). Interestingly, we observed a correlation between the number of IL-17+ T cells present and the number of eosinophils expressing MMP-9 ($r = 0.41$; $P = 0.03$).

Discussion

This study investigated the expression of IL-17 in pathogenically distinct eczema subtypes such as AD, ACD, and ICD and a possible role of IL-17 in tissue remodeling. By analyzing patch test reactions, we could demonstrate a time-dependent expression of IL-17 and related cytokines, as well as Th17-regulating cytokines. We observed IL-17 expression in AD and ACD consistent with previous reports (12, 16), and as a

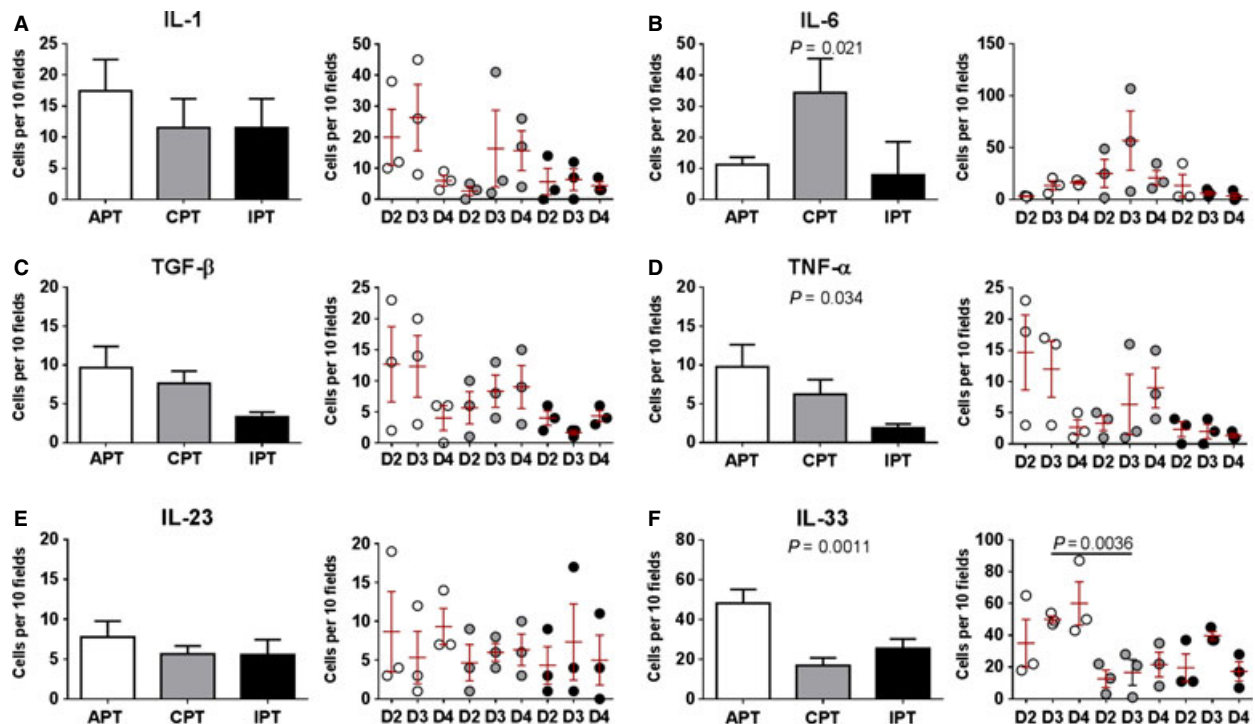


Figure 3 Expression of cytokines regulating IL-17 production in eczema subtypes. Differential expression in the different eczema

subtypes and over time of (A) IL-1, (B) IL-6, (C) TGF- β , (D) TNF- α , (E) IL-23, and (F) IL-33. APT, white; CPT, gray; IPT, black bars and circles.

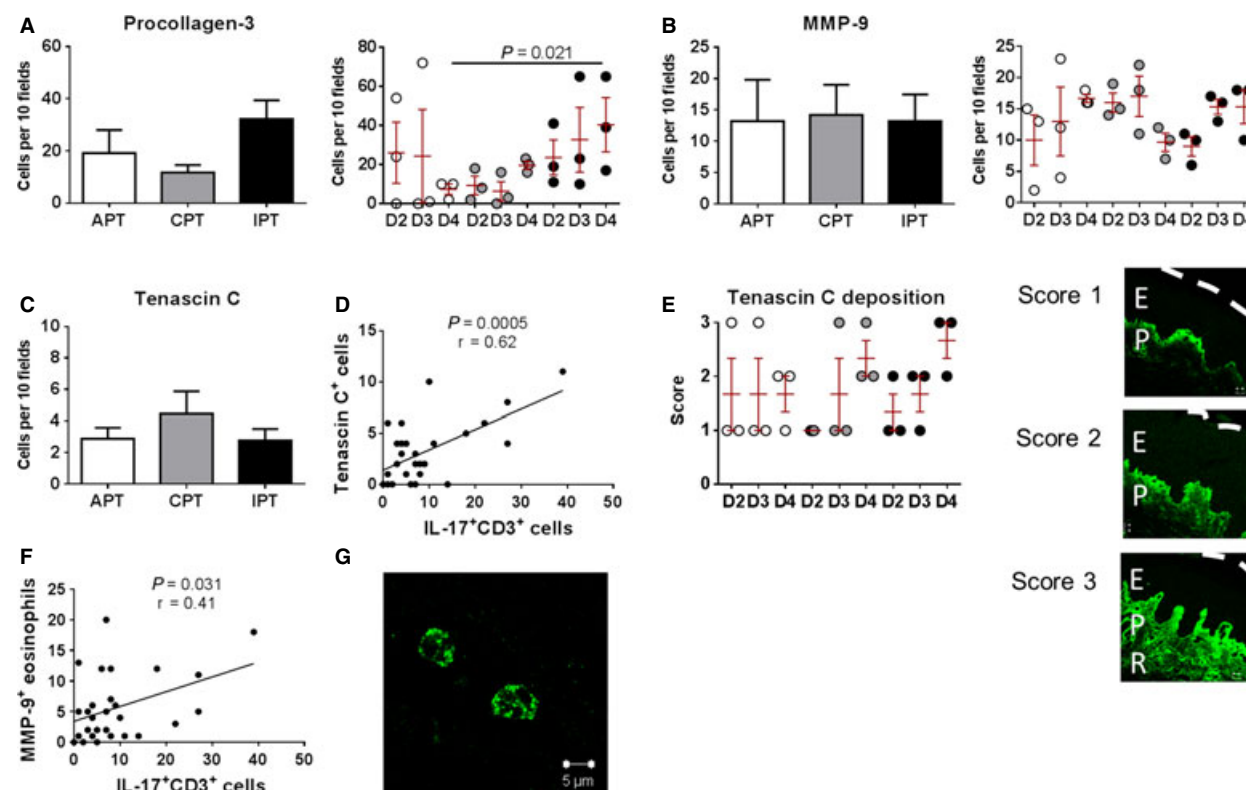


Figure 4 Association of IL-17 expression and tissue remodeling. Eczema subtypes show distinct expression of (A) procollagen-3, (B) MMP-9, and (C) tenascin C. APT, white; CPT, gray; IPT, black bars and circles. (D) Correlation between IL-17⁺ T cell and tenascin C⁺ cell numbers ($n = 27$). (E) Increase of tenascin C deposition in CPT and IPT over time (Score: 1, linear deposits along dermal-epidermal

junction; 2, deposits in papillary dermis; 3, deposits in papillary and reticular dermis; E, epidermis; P, papillary dermis; R, reticular dermis). (F) Number of MMP-9⁺ eosinophils correlated with numbers of IL-17⁺ T cells ($n = 27$). (G) Image of MMP-9⁺ eosinophils. Magnification $\times 1000$.

novel finding also in ICD. Interestingly, in IPT, the expression of IL-17 drastically increased at D4, whereas it was highest at earlier time points in APT and CPT. Higher numbers of IL-17⁺ lymphocytes in acute erythematous, as compared with chronic lichenified AD lesions, have been previously reported (12). IL-22 expression did not seem restricted to AD, as IL-22⁺ T cells could also be detected in CPT and IPT representing ACD and ICD, respectively. In AD, the number of IL-22⁺CD8⁺ T cells in the skin has been reported to correlate with disease severity (19). IL-22 has been demonstrated to regulate eosinophilic inflammation in an asthma model (41). Therefore, it seems probable that IL-22 is involved in eosinophil recruitment in eczematous skin lesions.

In AD, the expression of IL-17 and IL-22 has been reported to relate to host defense, in particular the production of antimicrobial peptides (14, 15, 42, 43). By releasing IL-17, T cells from nickel-induced ACD were able to stimulate proinflammatory cytokine, chemokine, and adhesion molecule production in keratinocytes (6, 17). IL-17 may further amplify ACD, as IL-17 together with IFN- γ has been shown to induce T-cell-mediated keratinocyte killing in an antigen-independent manner (44). Eczema shares histological

features, such as acanthosis and dermal inflammation, with psoriasis, which in both cases are driven by IL-23 (21). However, we found no correlation of IL-23 expression with infiltrating cell numbers or remodeling markers in eczematous PT reactions.

Interestingly, in eczematous lesions, both IL-17⁺ T cells and eosinophils were found. So far, it remains unclear whether the presence of IL-17⁺ T cells and eosinophils, both of which are known to be involved in innate and adaptive immune responses (29, 44–52), was coincidental or interdependent in eczematous lesions. IL-17 and IL-23 have been reported to activate eosinophils to release pro-fibrotic cytokines, suggesting a role of Th17 cytokines in tissue remodeling (18, 40). In line with these reports, we observed a correlation between the number of IL-17⁺ T cells and the number of eosinophils expressing MMP-9, a mediator that has been implicated in tissue remodeling (53), similar to the role of tenascin C expression as a remodeling marker (54–56).

Skin lesions following PT with allergens or irritants in sensitized and sensitive patients, respectively, resemble their clinical counterparts AD, ACD, or ICD and often are used as model for the study of skin inflammation. Even in the short period of PT over 3 days (from D2 to D4), we noticed

a time-dependent inflammatory cell infiltration and cytokine expression with distinct patterns characteristic for different eczematous subtypes. These time courses of eczematous reactions with variations in cell and cytokine expression should be considered when interpreting and comparing studies.

In summary, we have investigated the expression of IL-17 and related cytokines in eczema subtypes, including, in addition to AD and ACD, also ICD. We report that IL-17 and IL-22 appear to play a role in tissue remodeling and eosinophil recruitment, respectively, and therefore represent promising therapeutic targets in eczema.

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Conflicts of interest

The authors declare no conflicts of interest.

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