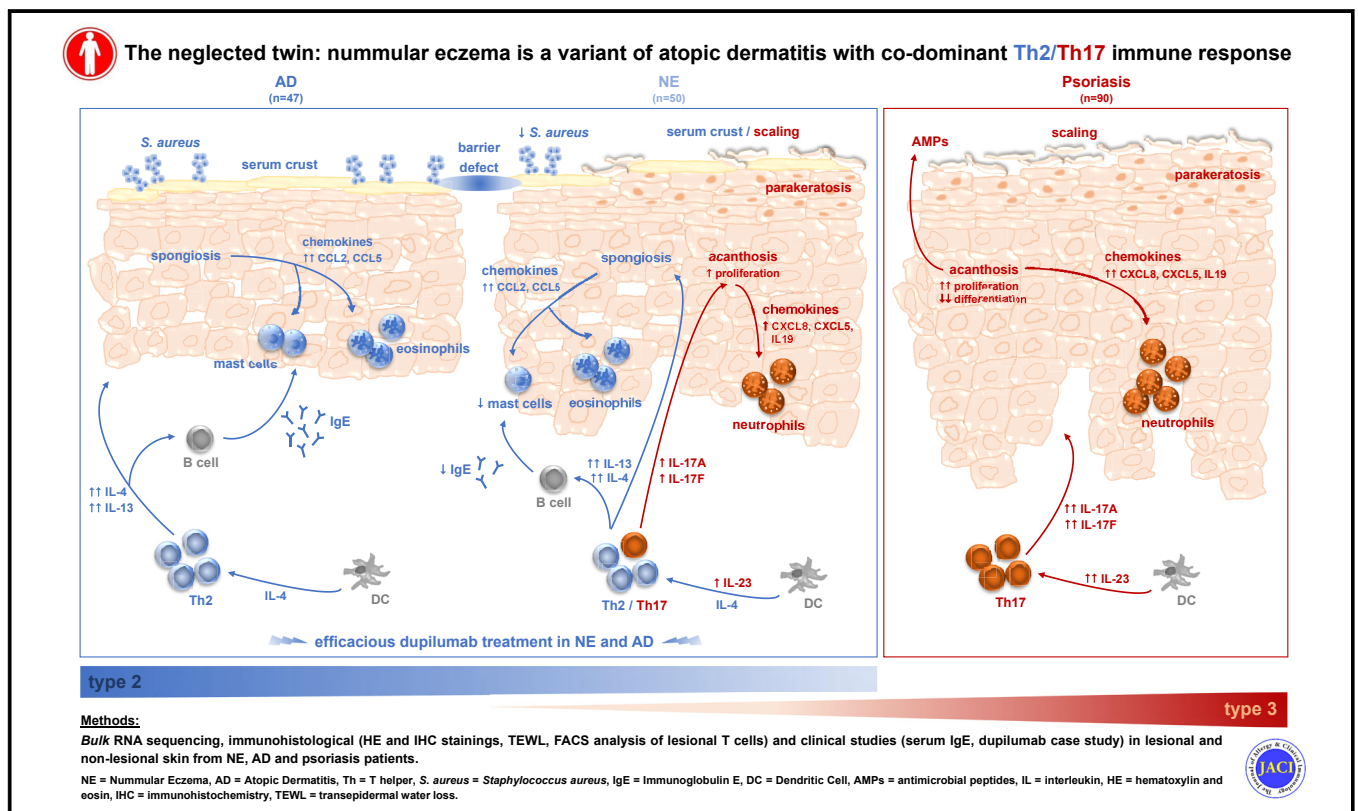


The neglected twin: Nummular eczema is a variant of atopic dermatitis with codominant T_H2/T_H17 immune response



Alexander Böhner, MD,^a Manja Jargosch, PhD,^{a,b} Nikola S. Müller, PhD,^e Natalie Garzorz-Stark, PhD, MD,^{a,c} Caroline Pilz, MD,^{b,d} Felix Lauffer, PhD, MD,^a Rosi Wang, MD,^a Sophie Roenneberg, MD,^a Alexander Zink, PhD, MD, MPH,^a Jenny Thomas, PhD,^{a,c} Fabian J. Theis, PhD,^{e,f} Tilo Biedermann, MD,^a Stefanie Eyerich, PhD,^b and Kilian Eyerich, PhD, MD^{c,d} Munich, Freiburg, Neuherberg, and Garching, Germany; and Stockholm, Sweden

GRAPHICAL ABSTRACT



From ^athe Department of Dermatology and Allergy, Technical University of Munich, Munich; ^bZAUM—Center of Allergy and Environment, Technical University and Helmholtz Center, Munich; ^cthe Department of Medicine, Division of Dermatology and Venerology, Karolinska Institute, Stockholm; ^dthe Department of Dermatology and Venerology, Medical Center, University of Freiburg, Freiburg; ^ethe Institute of Computational Biology, Helmholtz Center Munich, Neuherberg; and ^fthe Department of Mathematics, Technical University of Munich, Garching.

The first 2 authors contributed equally to this article, and both should be considered first author.

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of dupilumab in nummular eczema (ClinicalTrials.gov NCT04600362). The rest of the authors declare that they have no relevant conflicts of interest.

Data sets related to this article can be found at GEO (Gene Expression Omnibus, www.ncbi.nlm.nih.gov/geo/), an open-source online data repository hosted at the National Center for Biotechnology Information under accession number GSE154200.

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Corresponding author: Alexander Böhner, MD, Department of Dermatology and Allergy, Technical University of Munich, Biedersteinerstraße 29, 80802 Munich, Germany. E-mail: alexander.boehner@tum.de.

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Background: Nummular eczema (NE) is a common chronic inflammatory skin disease characterized by multiple, pruritic, discoid-shaped lesions. Since the underlying immune mechanisms are not fully understood, it is unclear whether NE should be regarded as variant of atopic dermatitis (AD) or a distinct disease.

Objective: We compared the clinical, histopathologic, and molecular signatures of NE with that of type 2 and type 3 skin diseases.

Methods: We performed bulk RNA sequencing as well as histologic and clinical studies in lesional and nonlesional skin biopsy specimens from NE (n = 50), AD (n = 47), and psoriasis (n = 90) patients.

Results: NE displayed typical hallmarks of AD, such as an impaired epidermal barrier, microbial colonization, spongiosis, and eosinophil infiltration, but also aspects of psoriasis, including increased epidermal thickness, number of Ki-67⁺ cells, and neutrophilic infiltration. At the gene expression level, neutrophil-attracting cytokines (*IL19*, *CXCL8*, *CXCL5*) were upregulated, whereas T_H2-related cytokines (*IL13*, *CCL17*, *CCL18*, *CCL26*, *CCL27*) were similarly expressed in NE compared to AD. Principal component analysis of transcriptome data from lesional skin showed that AD and NE cluster together distinct of psoriasis. In line with this, an established molecular classifier identified NE as AD rather than psoriasis. Finally, we demonstrated clinical and molecular efficacy of dupilumab treatment in NE.

Conclusion: NE shows overlapping type 2 and type 3 immune signatures, while type 2 immunity predominates and should be the primary target of specific therapeutic interventions. This supports the view of NE as a variant of AD. (J Allergy Clin Immunol 2023;152:408-19.)

Key words: Inflammatory skin diseases, nummular eczema, atopic dermatitis, dupilumab

Noncommunicable inflammatory skin diseases (ncISD) are a frequently occurring heterogeneous group of skin disorders with complex immune-mediated pathogenesis accompanied by impaired quality of life and social stigmatization. Atopic dermatitis (AD), a major representative of ncISD, comprises a diverse group of diseases in terms of clinical, histologic, and molecular hallmarks, with common overlapping morphologic features.¹ However, we lack a clear definition for most of those entities, including nummular eczema (NE).

NE, also called discoid eczema, is an idiopathic chronic inflammatory skin disease that is characterized by multiple, pruritic, discoid-shaped eczematous lesions involving most frequently the upper and lower extremities.² NE often takes a chronic and recursive course, is typically hard to treat, is very itchy, and severely affects quality of life.³ Although the exact pathophysiology of NE is not yet fully understood, it is thought to be induced by impaired epidermal barrier, inflammation, and microbial skin colonization.⁴ Although epidemiologic data are nearly missing, prevalence is 9% in alcoholic patients and around 2% in the normal population in Brazil, and potentially even higher in Asia.² Besides atopy, trigger factors include chronic contact dermatitis, microbial skin colonization, treatment with systemic retinoids, and alcohol abuse.²⁻⁵ Diagnosis is made clinically

Abbreviations used

AD:	Atopic dermatitis
DEG:	Differentially expressed gene
DLQI:	Dermatology life quality index
HPF:	High-power field
ncISD:	Noncommunicable inflammatory skin diseases
NE:	Nummular eczema
NET:	Neutrophil extracellular trap
PCA:	Principal component analysis
SCORAD:	Severity Scoring of AD

according to phenotypic appearance in correlation with histologic findings.

On the basis of the clinical overlap with AD, some studies consider NE to be a clinical variant of AD.⁶⁻⁸ However, NE also shows a substantial clinical and histologic overlap with psoriasis. So far, research on NE has been solely focused on its clinical aspects, leaving the underlying immune response pattern uninvestigated. Understanding the pathogenesis of NE is essential to define treatment targets for clinical trials.

In the past decade, a better understanding of underlying immune signatures in the field of ncISD has led to major therapeutic advances, such as biologics targeting key cytokines orchestrating immune-mediated inflammatory processes. In AD, dupilumab, an antibody targeting the T_H2 immune axis by inhibiting IL-4 and IL-13 signaling, shows high efficacy and an excellent safety profile.⁹ In psoriasis, more than a dozen specific therapies are approved, including antibodies neutralizing IL-23 or IL-17 that reach 90% improvement response rates in up to 75% of patients after 16 weeks.¹⁰

In contrast, for other ncISD such as NE, no approved systemic therapies are available. Therapeutic options for NE are rare and include the use of emollients, topical and systemic corticosteroids, topical calcineurin inhibitors, and phototherapy.¹¹ Still, treatment of this chronic and relapsing disease is difficult.

Therefore, the lack of understanding NE pathogenesis is paralleled by an unmet medical need for effective therapeutic options. In this study, we demonstrate that NE shows a combined type 2 (T_H2) and type 3 (T_H17) immune signature in terms of clinical, histologic, and molecular data. However, comparative investigation of transcriptome revealed that type 2 immunity is dominant while type 3 immunity seems to be secondary. In line with these findings, treatment of NE with dupilumab resulted in rapid clinical and molecular improvement.

METHODS

Study cohort and patient characteristics

A cohort of 50 patients with NE were enrolled in this study and compared to patients with AD (n = 47) and psoriasis (n = 90). To ensure a clear diagnosis, clinical and histologic assessments were performed by independent dermatologists and dermatopathologists. All study subjects were enrolled from the Biobank Biederstein, which ensures high standards by holding regular expert consensus meetings and ensuring standard operating procedures, after written informed consent was obtained. Exclusion criteria were systemic or topical immune suppressive treatment as well as phototherapy within the last 3 months (systemic and phototherapy), or 1 week (topical). Patient characteristics are listed in Table I and in Table E1 in this article's Online Repository available at www.jacionline.org. The study was approved by the local ethics

TABLE I. Patient characteristics

Characteristic	NE (n = 50)	AD (n = 47)	Psoriasis (n = 90)
Age (years), mean (SD)	61.5 (17.0)	47.9 (19.2)	54.5 (16.1)
Sex (%), M/F	58/42	68/32	58/42
SCORAD, mean (SD)	50.0 (18.9)	59.4 (14.5)	
PASI, mean (SD)			19.8 (12.3)
PGA, mean (SD)	2.9 (0.5)	3.4 (0.5)	3.2 (0.8)
DLQI, mean (SD)	10.7 (5.8)	14.0 (6.9)	11.7 (7.1)
BMI, mean (SD)	27.8 (6.6)	25.5 (5.6)	29.2 (6.1)
Atopic comorbidities			
Allergic rhinoconjunctivitis (%)	26	53	
Bronchial asthma (%)	12	37	
Total IgE (kU/L), mean (SD)	320 (767)	5265 (7601)	412 (1177)

BMI, Body mass index; PASI, Psoriasis Area and Severity Index; PGA, physician global assessment.

committee and conducted according to the ethical principles laid down in the Declaration of Helsinki. The Biobank Biederstein follows data protection rules, and the local ethics committee provided approval (Technical University of Munich approval 5590/12).

Punch biopsy specimens

Lesional and autologous nonlesional (>10 cm from active lesion) 6 mm punch biopsy samples were collected under local anesthesia from areas with representative NE, AD, and psoriasis skin lesions. Each biopsy was bisected; half was collected in RNALater stabilization solution (Qiagen) at -80°C until RNA preparation, and the other half was fixed in 4% formalin for histology. For flow cytometry analysis of lesional T cells, additional 4 mm punch biopsy samples were used.

Histology and immunohistochemistry

Formalin-fixed skin tissue was embedded in paraffin. Sections ($2.5\ \mu\text{m}$) were cut and dewaxed. After rehydration, sections were stained with hematoxylin and eosin using standard methods. Immunohistochemistry stainings were performed by an automated BOND system (Leica) according to the manufacturer's instructions using the following monoclonal antibodies: FcεRI (Abcam ab54411, 1:75 in citrate buffer), Ki-67 (Zytomed RBG027, undiluted in EDTA buffer), IL-17 (R&D Systems AF-317-NA, 1:40 in citrate buffer), and neutrophil extracellular traps (NETs) (Abcam ab68672, 1:200 in citrate buffer).

RNA sequencing analysis

RNA was isolated using QIAzol Lysis Reagent (Qiagen) and miRNeasy Mini Kit (Qiagen). RNA sequencing libraries were generated using the TruSeq Stranded Total RNA Kit (Illumina) according to manufacturer's high sample protocol. Samples were sequenced on an Illumina HiSeq4000 device as paired ends with a read length of 2×150 bp and an average output of 40 Mio reads per sample and end. Sequence alignment was performed by STAR aligner with human genome reference hg38.

The RNA sequencing count data were transformed using variance stabilizing transformation (VST) from the Bioconductor package DESeq2 as well as tested and corrected for variances in sex (9%) using a linear mixed effect model from the Bioconductor package variancePartition.¹² Principal component analysis of the transformed data was performed using the top 1000 most varying genes across samples. Variance was computed per gene across the samples, and the 1000 genes with the highest variance were used in principal component analysis. Differentially expressed genes (DEGs) between lesional and nonlesional skin within each disease group were calculated by the Bioconductor package edgeR.¹³ First dispersion of the RNA sequencing count data was estimated, followed by modeling of the counts using negative binomial generalized linear models, and finally differential expression was determined using quasi-likelihood (QL) *F* test.

To identify pathways enriched in DEGs, leading edge analysis was performed from the Bioconductor package 'fgsea'.¹⁴ Genes with nominal $P < .05$ and a $\log_2$ fold change value of >1.5 were ranked by $\log_2$ fold change value for leading edge analysis. Msigdb's compilation of KEGG (Kyoto Encyclopedia of Genes and Genomes; genome.jp/kegg), Reactome, Pathway Interaction Database (PID), and Biocarta's pathways were used as reference.^{15,16} We performed 10K permutations to estimate the significance of enriched pathways. Pathways with nominal $P < .01$ were selected for further analysis.

Transepidermal water loss and epidermal thickness

Transepidermal water loss of lesional and perilesional skin was evaluated via AquaFlux AF200 Biox. All measurements were conducted in temperature-controlled rooms. Epidermal thickness was quantified by analyzing digital histopathologic images with ImageJ software.

Isolation and flow cytometry analysis of lesional T cells

Punch biopsy samples of lesional skin (4 mm) were digested using the Whole Skin Dissociation Kit (130-101-540) and gentleMACS Dissociator from Miltenyi Biotec according to the manufacturer's protocol. The digestion was performed without enzyme P at 37°C for 3 hours.

For intracellular cytokine staining, isolated T cells were stimulated in T-cell media (RPMI 1640 medium; Gibco, 21875-091) supplemented with 5% human serum (Sigma, H4522), 0.1 mmol nonessential amino acid (Gibco, 11140-035), 2 mmol L-glutamine (Gibco, 25030-024), 1 mmol sodium pyruvate (Gibco, 11360-039), and 100 U/mL penicillin/streptomycin (Gibco, 15140-130) with 10 ng/mL phorbol 12-myristate 13-acetate (Sigma, P8139) and 1 $\mu\text{g/mL}$ ionomycin (Sigma, I0634) in the presence of GolgiStop (Becton Dickinson [BD], 554724, 1:1.500) and GolgiPlug (BD, 555029, 1:1.000, only for the last 3 hours) for 5 hours at 37°C and 5% CO_2 . Afterward, T cells were stained with Aqua (Invitrogen, L34957, 1:1.000) for live/dead discrimination, CD3-FITC (Miltenyi Biotec, 130-113-700, 1:50), CD4-APC-Cy7 (BD, 557871, 1:20), and CD8-BV711 (BioLegend, 301044, 1:200). After fixation and permeabilization of cells with the BD Cytofix/Cytoperm Kit (BD, 554714) according to the manufacturer's protocol, cells were stained for intracellular cytokines with IL-4-PerCP-Cy5.5 (BD, 561234, 1:20), IL-13-V450 (BD, 561158, 1:10), and IL-17-PE (BD, 560436, 1:50). Stained samples were acquired with an LSR Fortessa flow cytometer and analyzed by FlowJo v10. The cells were gated for living $\text{CD}3^{+}\text{CD}4^{+}$ cells, and the quotient of $\text{T}_\text{H}17$ cytokines (IL-17-positive cells) to $\text{T}_\text{H}2$ cytokines (IL-4- and IL-13-positive cells) was calculated and visualized.

AD-psoriasis classifier

The molecular classifier to distinguish between psoriasis and AD was conducted by gene expression analysis of *NOS2* and *CCL27* in RNA samples of lesional skin as described before.¹⁷

Real-time quantitative PCR

mRNA was transcribed into cDNA with the Applied Biosystems High Capacity cDNA Reverse Transcription Kit (Thermo Fisher Scientific). Gene expression was measured on an Applied Biosystems ViiA7 Real-Time PCR system (Thermo Fisher Scientific) using Fast Start Universal SYBRGreen Master Rox (Roche). Primers were ordered from Metabion and are listed in Table E2, which is available in the Online Repository available at www.jacionline.org. Data are shown as relative gene expression to the untreated sample using the $2^{-\Delta\Delta C_t}$ method.

Statistical analysis

Data were analyzed and visualized as means $\pm$ SDs by GraphPad Prism v6 software. To test for differences in mean values within disease groups, all data

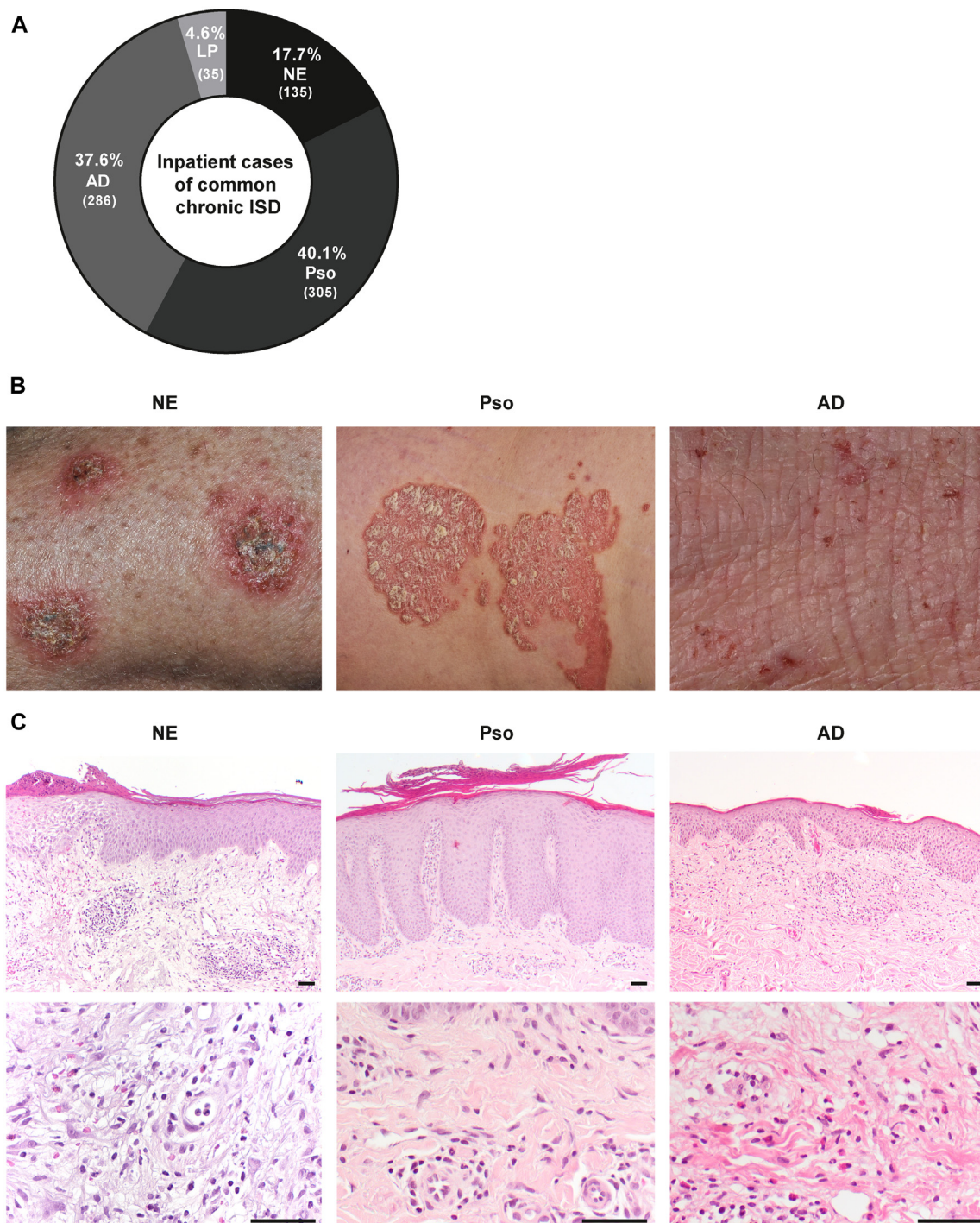


FIG 1. NE shares overlapping clinical and histologic hallmarks with both AD and psoriasis. **(A)** Hospital inpatient records (percentages and counts) of common chronic ISD. Clinical picture **(B)** and histologic hematoxylin and eosin staining **(C)** of lesional skin from NE, psoriasis, and AD patients. Scale bar = 50 μ m. ISD, Inflammatory skin diseases; LP, lichen planus; Pso, psoriasis.

sets were analyzed for Gaussian distribution by the Shapiro-Wilk test. When a Gaussian distribution was given, the parametric ordinary 1-way ANOVA test with Tukey multiple comparison was used, and when not, the nonparametric Kruskal-Wallis test with Dunn multiple comparison was used. To analyze group differences according to observed percentages of patients with specific histologic features, 1-sided chi-square test was applied. Significance level was defined as $*P < .05$, $**P < .01$, $***P < .001$, and $****P < .0001$.

RESULTS

NE shares overlapping clinical and histologic hallmarks with both AD and psoriasis

To estimate the hospitalization rate of NE, we analyzed our hospital inpatient records (ICD-10-CM Code L30.0) from the last 2 years. NE was the third most common ncISD after AD and

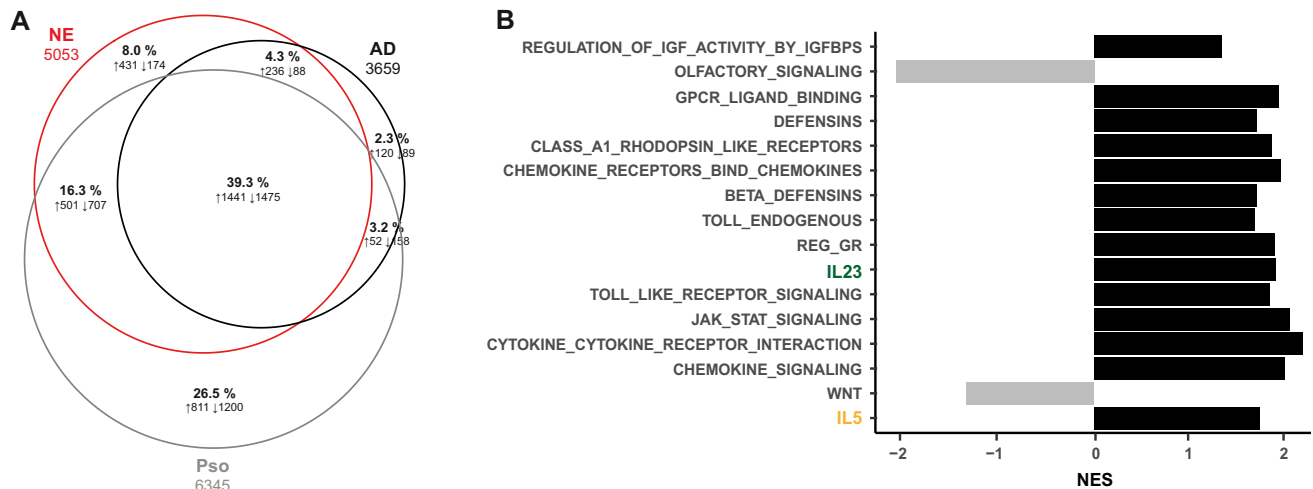


FIG 2. Gene expression analysis of lesional skin demonstrates both T_H2 and T_H17 signatures in NE. RNA sequencing of lesional and nonlesional skin from NE (n = 50), AD (n = 47), and psoriasis (n = 90) patients. **(A)** Venn diagram of DEGs ($|\log_2 FC| > 0.5$, $P < .05$) between lesional and nonlesional skin within NE, AD, and psoriasis. **(B)** Enriched pathways (10K permutations, $P < .01$) in NE DEGs ($|\log_2 FC| > 1.5$, $P < .05$) using KEGG, Reactome, PID, and Biocarta databases. FC, Fold change; NES, normalized enrichment score; Pso, psoriasis.

psoriasis, with 135 patients admitted during this 2-year period (Fig 1, A).

To characterize NE within the group of ncISD, we established a cohort of 50 patients and compared them to AD (n = 47) and psoriasis (n = 90), 2 typical differential diagnoses of NE (Table 1, Table E1). Overall, we mainly included moderate to severe diseases as indicated by a mean physician global assessment (aka PGA) score of 2.9 ± 0.5 (5-point scale; 0-4) for NE, 3.4 ± 0.5 for AD, and 3.2 ± 0.8 for psoriasis. AD patients showed a mean Severity Scoring of AD (SCORAD) of 59.4 ± 14.5 and psoriasis patients a mean Psoriasis Area and Severity Index (aka PASI) of 19.8 ± 12.3 . Quality of life assessed by the dermatology life quality index (DLQI) was equally affected in patients with NE (10.7 ± 5.8) and psoriasis (11.7 ± 7.1) and slightly decreased compared to AD (14.0 ± 6.9).

As previously described, the clinical picture of NE exhibited both morphologic hallmarks of eczema, including seropapules, oozing, and crusting, as well as of psoriasis, exhibiting well-demarcated erythematous plaques and scaling (Fig 1, B). In addition, histologic features of NE displayed typical hallmarks of eczema with spongiosis and eosinophilic infiltrate, but also aspects of psoriasis including acanthosis, parakeratosis, and neutrophilic infiltration (Fig 1, C).

Thus, from a clinical and histologic perspective, NE shows an overlapping phenotype of AD and psoriasis, respectively. To gain insights into the pathogenesis of NE and to develop therapeutic strategies, molecular characterization of NE is needed.

Gene expression analysis of lesional skin demonstrates both T_H2 and T_H17 signatures in NE

Next, we compared the transcriptome of lesional versus nonlesional skin of NE, AD, and psoriasis patients by performing bulk RNA sequencing. As expected, the majority of the DEGs of lesional versus nonlesional skin, with 39.3% (n = 2925), was shared by the 3 entities. Eight percent of the genes (n = 597)

were exclusively regulated in NE compared to 2.3% in AD (n = 174) and 26.5% in psoriasis (n = 1971). NE shared 16.3% of the genes (n = 1208) exclusively with psoriasis and 4.3% (n = 324) with AD (Fig 2, A).

Consistent with the overlap of clinical and histologic hallmarks as well as DEGs, pathway enrichment analysis of DEGs in NE showed both an upregulation of an AD characteristic T_H2 pathway, namely the IL-5 pathway, but also of the IL-23 pathway, which is characteristic for psoriasis (Fig 2, B). This parallel T_H2 and T_H17 signature was specific for NE, as on the one hand the pathway analysis of DEGs in AD did not exhibit an upregulation of the IL-23 pathway, but on the other hand the IL-5 pathway was not enriched in DEGs in psoriasis (see Fig E1 in the Online Repository available at www.jacionline.org).

NE shows a pronounced, yet compared to AD attenuated, T_H2 immunity

To further corroborate that type 2 immunity is upregulated in NE, we analyzed the expression of characteristic type 2 immune genes within NE compared to AD and psoriasis in our bulk RNA sequencing cohort. We identified several signature T_H2 immune genes like *IL13*, *GATA3*, *CCL17*, *CCL18*, *CCL26*, and *CCL27*, which were equally significantly upregulated both in NE and AD compared to psoriasis. Moreover, the type 2-attracting chemokines *CCL2* and *CCL5* were expressed highest in NE compared to psoriasis and AD (Fig 3, A).

Given the activation of T-cell pathways in our gene expression analysis, we further isolated T cells from lesional skin biopsy samples and analyzed the production of T_H2 (IL-4 and IL-13)- and T_H17 (IL-17)-specific cytokines by intracellular fluorescence-activated cell sorting staining. Similar to AD, T cells derived from NE revealed a dominant T_H2 immunity with higher frequencies of IL-4- and IL-13- compared to IL-17-producing cells. Therefore, the quotient of T_H17 to T_H2 cytokines was significantly lower in NE (0.30 ± 0.10 , NE vs psoriasis

$P = .0303$) and AD (0.13 ± 0.03 , AD vs psoriasis $P = .0041$) compared to psoriasis (1.06 ± 0.27) T cells, which showed characteristic T_H17 immunity with higher frequencies of IL-17 compared to IL-4 and IL-13 producing cells (Fig 3, B).

To investigate whether the observed type 2 signature in NE transcriptomics and T cells is clinically relevant, we further investigated functional hallmarks of type 2 immunity in NE and compared them to our AD and psoriasis cohorts. In fact, one of the main characteristics of eczema, namely a significant epidermal barrier defect, was identified in both NE and AD, but not in psoriasis. This was assessed by measuring transepidermal water loss (62.4 ± 17.2 [NE, $P < .0001$] and 53.2 ± 23.7 [AD, $P < .0048$] vs 29.7 ± 6.7 $\text{gm}^{-2}\text{h}^{-1}$ [psoriasis]) (Fig 3, C). In addition, significant infiltration of eosinophils, a typical trait of type 2 immunity, was substantially present in the majority of NE (57%) and AD (64%) while being absent in psoriasis (0%) patients (NE vs psoriasis $P = .0003$; AD vs psoriasis $P = .0002$) (Fig 3, D).

In contrast, various other type 2 immunity features were less pronounced in NE compared to AD. The incidence of blood eosinophilia in NE (41%) and psoriasis (19%) was significantly less frequent compared to AD (71%) (NE vs AD $P = .0058$; psoriasis vs AD $P = .0001$). In addition, colonization with *Staphylococcus aureus* assessed by culture of bacterial swabs—another characteristic for AD (85%)—was less pronounced in NE (37%) while still being significantly more prevalent compared to psoriasis (17%) (NE vs AD $P = .0039$; NE vs psoriasis $P = .0441$; psoriasis vs AD $P < .0001$) (Fig 3, D). Levels of total serum IgE were similar between NE (257 ± 423 kU/L) and psoriasis (207 ± 321 kU/L) while being significantly increased in AD (4407 ± 489 kU/L) ($P < .0001$ to psoriasis, $P = .0003$ to NE) (Fig 3, E). This was accompanied by elevated expression of the IgE receptor FcεRI in lesional skin via immunohistochemistry (Fig 3, F). Here, the number of FcεRI-positive cells was only increased in AD (102.7 ± 58.7 cells), not in NE (52.0 ± 37.2 cells) and psoriasis (57.1 ± 44.9 cells) (NE vs AD $P = .0177$; psoriasis vs AD $P = .0316$).

In summary, humoral aspects of type 2 immunity were significantly less pronounced in NE compared to AD, while cellular components and functional hallmarks of type 2 immunity were comparable between NE and AD.

NE exhibits a skewed IL-23/ T_H17 immunity compared to AD

Considering some clinical and histologic similarities of NE to psoriasis and the upregulation of the IL-23 pathway in both diseases, we next aimed at verifying the presence of T_H17 immunity in NE via investigating the expression of hallmark type 3 immunity genes in our bulk RNA sequencing cohort. We identified a significant upregulation of *IL19* ($P < .0027$) and *CXCL8* ($P = .0205$) in NE compared to AD, both hallmark genes for neutrophil migration in psoriasis. Here, and within various other T_H17 signature genes like *CXCL5*, *IL23A*, and *IL17F*, NE exhibited an intermediate position between AD and psoriasis, highlighting the skewed IL-23/ T_H17 immunity of NE (Fig 4, A). In addition, we also analyzed type 1 immunity genes, as T_H1 -related immune response is an important hallmark of type 3/psoriasis immunity. Indeed, type 1 immunity is also active in NE, and again, NE here occupies an intermediate position between AD and psoriasis (see Fig E2 in the Online Repository available at www.jacionline.org).

To assess whether the skewed type 3 signature in NE transcriptomics is clinically relevant, we further investigated functional hallmarks of type 3 immunity in NE and compared them to our AD and psoriasis cohorts. Typically acanthosis and parakeratosis are more prominent in psoriasis than in AD, and together with neutrophil infiltration are histologic hallmarks of type 3/ T_H17 immunity. In our cohort, histologic analyses revealed a significantly increased epidermal hyperplasia in NE (338 ± 120 μm) compared to AD (239 ± 69 μm), assessed by measuring epidermal thickness ($P = .0003$) (Fig 4, B) and the presence of Ki-67⁺ cells in the epidermis (NE: 188 ± 72 cells; AD: 120 ± 53 cells; $P = .0089$) (Fig 4, C). Furthermore, a trend of more pronounced parakeratosis was observed in NE compared to AD (see Fig E3 in the Online Repository available at www.jacionline.org). In addition, neutrophil infiltration was significantly more present in NE (51%) compared to AD (8%) ($P = .0001$), as judged by a dermatopathologist (Fig 4, D) and via NET-positive cells in the dermis (Fig 4, E, 14.2 ± 4.6 cells [NE] vs 1.8 ± 0.7 cells [AD], $P = .0228$). Yet all 3 T_H17 -related histologic traits were most pronounced in psoriasis than in NE. Accordingly, the number of IL-17⁺ cells in lesional skin was significantly higher in psoriasis (32.8 ± 14.5 cells per high-power field [HPF]) compared to both NE (10.3 ± 6.9 cells per HPF, $P = .0003$) and AD (12.7 ± 6.6 cells per HPF, $P = .0018$) (Fig 4, F).

Type 2 immunity is the dominant underlying immune pathway in NE

To answer the question of whether type 2 or type 3 immunity is the dominant disease-driving pathway in NE, we first performed a principal component analyses (PCA) from our RNA sequencing data comparing NE, AD, and psoriasis. PCA revealed that both NE and AD group together while also clearly being separate from psoriasis, indicating that NE is more similar to AD than to psoriasis at a global level (Fig 5@, A). In addition, a molecular disease classifier¹⁷ for psoriasis and eczema, based on the expression of *NOS2* and *CCL27*, classified disease of 91.7% (33 of 36) of NE patients as eczema, further confirming that type 2 immunity is the dominant immune path in NE (Fig 5, B).

To finally prove this observation, we investigated in a case study whether T_H2 -targeted therapy would demonstrate efficacy on clinical and molecular levels in the treatment of NE. A man in his 50s with a 3-year history of treatment-refractory NE, whose disease failed to respond to previous therapies, including topical steroids, UV therapy, methotrexate, and cyclosporine, was therefore treated with the monoclonal antibody dupilumab, which inhibits the IL-4 and IL-13 pathway by targeting the IL-4 receptor. Our patient's disease quickly responded to dupilumab, with almost complete resolution of skin lesions and pruritus after 16 weeks (Fig 5, C). The SCORAD declined from 24 at baseline to 2.3 after week 16. In addition, quality of life, assessed by the DLQI, improved significantly from 12 to 0 (Fig 5, D). To get a deeper understanding of the underlying mechanisms, we analyzed the gene expression profile before and after dupilumab treatment in the skin. Both type 2 (eg, *IL4*, *IL13*, and *LCE3A*)- and type 3 (eg, *CXCL8*, *CXCL5*, *IL22*, and *DEFB4A*)-related immune genes demonstrated a downregulation of over 90% during treatment with dupilumab. In parallel, a pronounced upregulation of the anti-inflammatory cytokine *IL37* was observed (Fig 5, E).

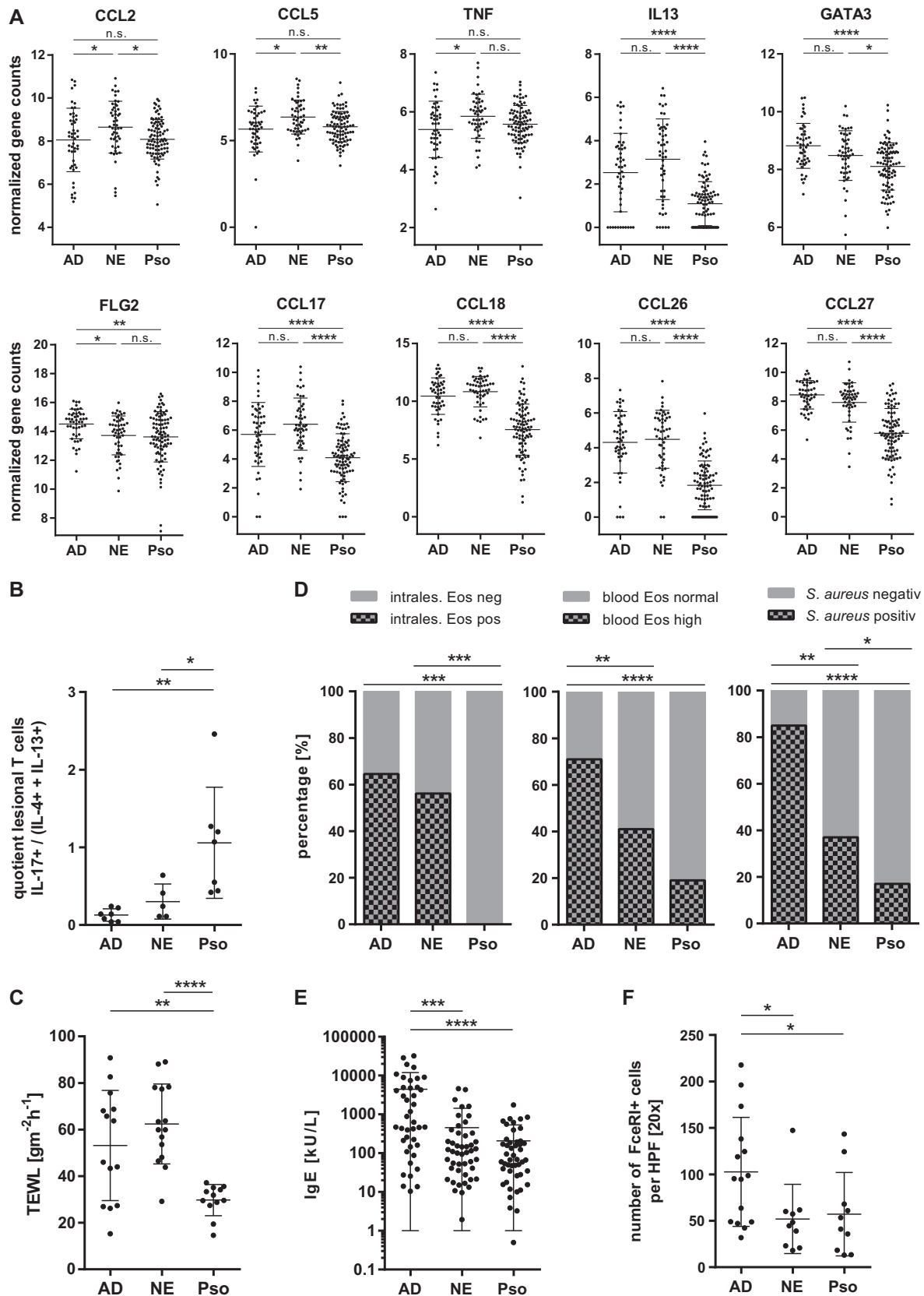


FIG 3. NE shows a pronounced, yet compared to AD attenuated, T_H2 immunity. **(A)** Gene expression of T_H2 immunity genes within NE (n = 50) compared to AD (n = 47) and psoriasis (n = 90) was analyzed by RNA sequencing of lesional skin. **(B)** Flow cytometry analysis of lesional T cells from NE (n = 5), AD (n = 7), and psoriasis (n = 7) skin biopsy samples after 3 hours of skin digestion and 5-hour restimulation with phorbol 12-myristate 13-acetate/ionomycin. Proportion of T_H17 (IL-17⁺) to T_H2 (IL-4⁺ + IL-13⁺) cytokines within

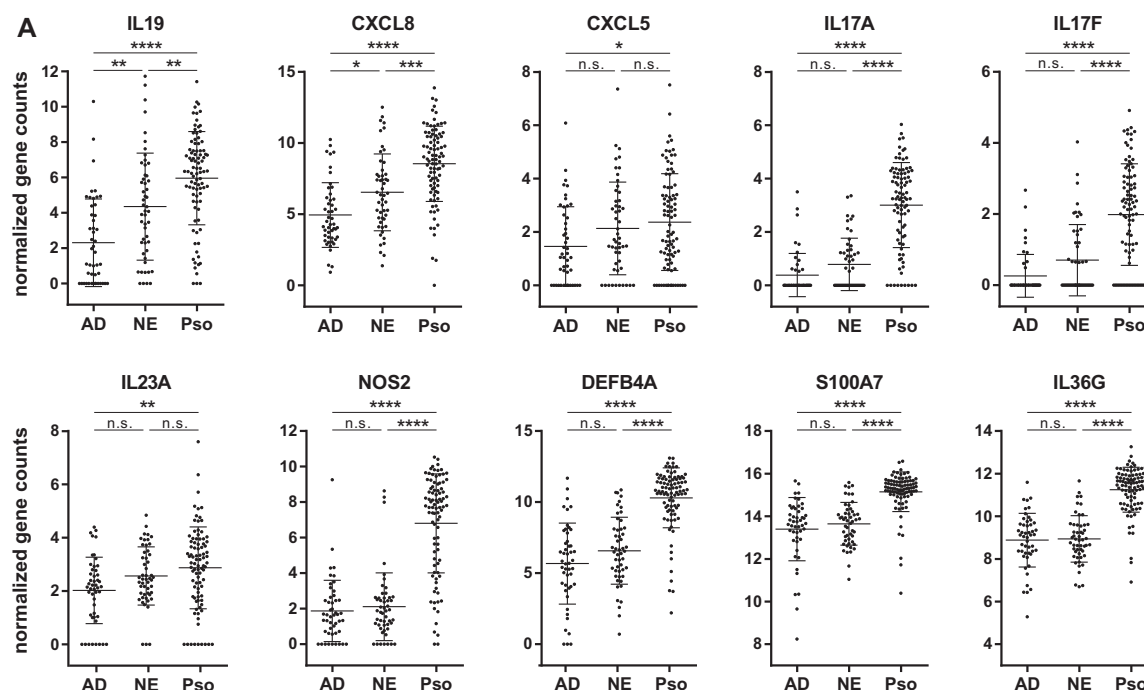


FIG 4. NE exhibits skewed IL-23/T_H17 immunity compared to AD. **(A)** Gene expression of T_H17 immunity genes within NE (n = 50) compared to AD (n = 47) and psoriasis (n = 90) was analyzed by RNA sequencing of lesional skin. **(B)** Epidermal thickness was quantified via analyzing digital histopathologic images with ImageJ software in NE (n = 26), AD (n = 21) and psoriasis (n = 24) lesions. Immunohistochemistry staining and quantification of Ki-67⁺ **(C)**, NET⁺ **(E)**, and IL-17⁺ cells **(F)** in lesional skin of NE (n = 9-10), AD (n = 12-16) and psoriasis (n = 7-12) patients. Scale bar = 50 μ m. **(D)** Percentage of NE (n = 49), AD (n = 25), or psoriasis (n = 47) patients with infiltrated neutrophils in lesional skin judged by a dermatohistopathologist. Group differences based on observed percentages of patients were analyzed by 1-sided chi-square test. Data are visualized as means $\pm$ SDs. Unless otherwise stated, ordinary 1-way ANOVA test with Tukey multiple comparison (for Gaussian distributed data) or nonparametric Kruskal-Wallis test with Dunn multiple comparison (for non-Gaussian distributed data) was used to test for differences between disease groups. **P* < .05, ***P* < .01, ****P* < .001, *****P* < .0001, ns = not significant (*P* > .05). *Pso*, Psoriasis.

In summary, treating NE with a type 2-targeted therapy improved both type 2 and type 3 inflammation in lesional skin, thus corroborating the fact that type 2 immunity is the dominant underlying immune pathogenesis of NE.

DISCUSSION

ncISDs can be classified according to their dominant immune response patterns, and identifying disease-driving immune signatures is essential for the development of highly efficacious and biologic therapeutics. Here, we show that NE is characterized by both T_H2/type 2 and T_H17/type 3 immunity; however, the dominant and relevant immune signature is the type 2 axis.

Recent research suggests that AD is a heterogenous skin disease that can be classified into different endotypes with varying immune signatures.¹⁸ While a pronounced T_H2 immunity is dominant in all endotypes, differentiation can be made with regard to coactivated immune axes.¹⁹ AD was first categorized into 2 types, the classic IgE-associated extrinsic AD (aka eAD) and the intrinsic form without excessive IgE secretion (aka iAD). While initial discrimination between those 2 entities was based on IgE levels and sensitization to allergens,²⁰ advances in research have identified the underlying immune signatures behind these 2 subtypes.^{7,21,22} Hence, although these 2 types share an indistinguishable clinical phenotype, intrinsic AD is characterized by a similar T_H2 but increased T_H17 immune activation compared to extrinsic AD.²¹ Also, chronicity of AD lesions

CD3⁺CD4⁺ population was visualized. **(C)** Epidermal barrier defect of NE (n = 15), AD (n = 14), and psoriasis (n = 12) lesions was measured by TEWL. **(D)** Percentage of NE (n = 49), AD (n = 34), and psoriasis (n = 47) patients with intralesional eosinophils, blood eosinophils, or *Staphylococcus aureus* colonization in lesional skin judged by a dermatohistopathologist. Group differences based on observed percentages of patients was analyzed by 1-sided chi-square test. Concentrations of total serum IgE **(E)** and Fc ϵ RI⁺ cells **(F)** in lesional skin were analyzed by immunohistochemistry in NE (n = 47, 10), AD (n = 41, 10), and psoriasis (n = 47, 15) patients. Data are visualized as means $\pm$ SDs. Unless otherwise stated, ordinary 1-way ANOVA test with Tukey multiple comparison (for Gaussian distributed data) or nonparametric Kruskal-Wallis test with Dunn multiple comparison (for non-Gaussian distributed data) was used to test for differences between disease groups. **P* < .05, ***P* < .01, ****P* < .001, *****P* < .0001, ns = not significant (*P* > .05). *Eos*, Eosinophils; *Pso*, psoriasis; *TEWL*, transepidermal water loss.

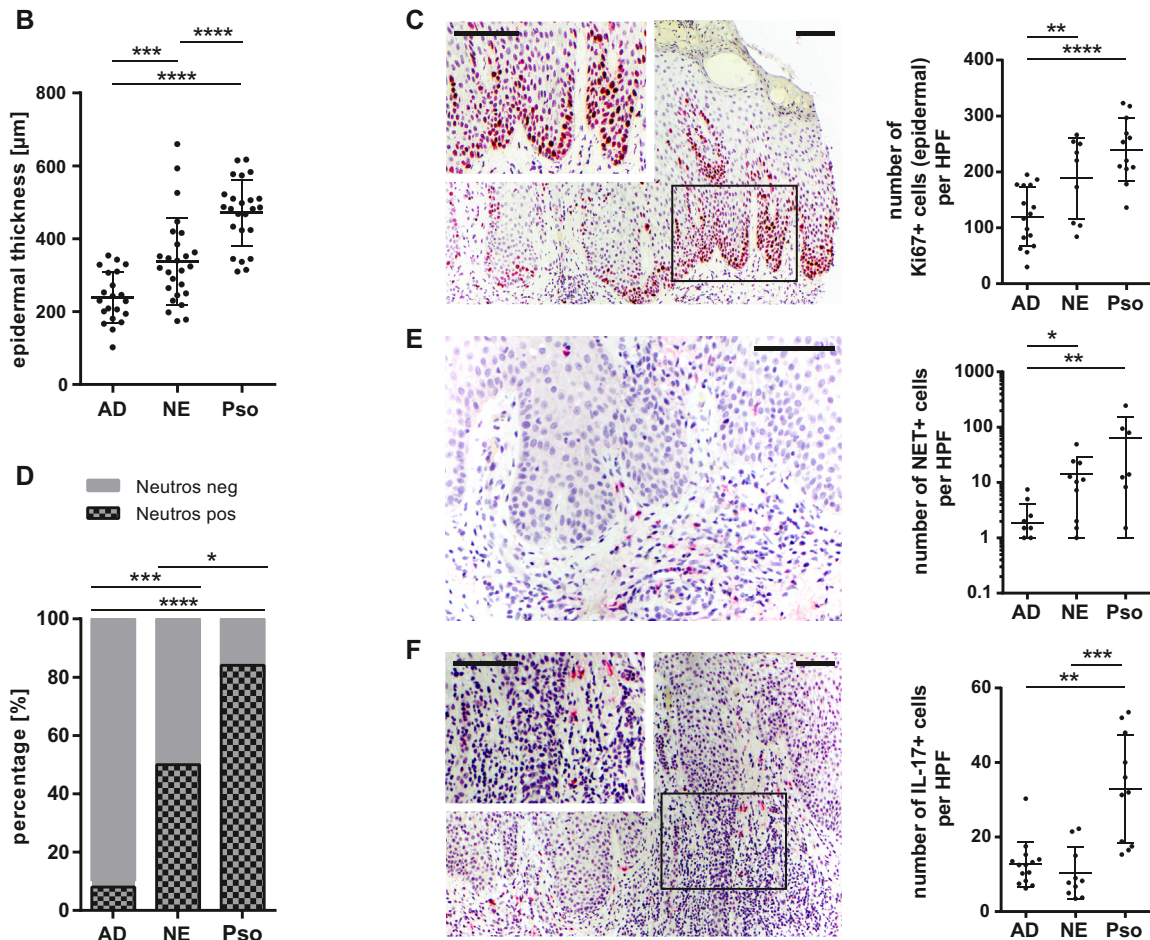


FIG 4. (Continued).

affects both the clinical phenotype and the T-cell infiltrate. On the one hand, while early lesions are characterized by seropapules and oozing, they are almost exclusively driven by type 2 immunity. On the other hand, chronic lesions show signs of lichenification and scaling while simultaneously presenting a mixed type 2, type 1, and type 3 cellular infiltrate.²³ In our patient cohort, chronicity of AD lesions was judged as predominantly acute by the presence of spongiosis and eosinophils as signs for acute AD and increased epidermal thickness and parakeratosis as signs of chronic AD. Also in our patient cohort, we cannot exclude that individual patients with AD of typical onset in childhood had developed NE-like lesions over time. More recently, immune phenotypes for other AD endotypes have been suggested. In early-onset pediatric AD, both type 2 and type 3 immunity are upregulated; in African American AD patients, the T_H2/T_H22 compartment is predominant with a skewed T_H1/T_H17 attenuation.^{24,25} In a landmark study, Noda et al²⁶ characterized patients with AD from Asia compared to European American AD patients and reported an immune phenotype of an increased T_H17 activation with a pronounced T_H2 component. Despite this enhanced type 3 immune axis in Asian AD patients, dupilumab treatment is as efficient as in European AD patients, highlighting the dominance of type 2 immunity for AD pathogenesis.²⁷

NE and Asian AD share clinical (demarcation of lesions, prominent scaling) and histologic (acanthosis, parakeratosis) hallmarks; however, NE is also regarded a distinct disease in Asia. In line with phenotypic similarities, our study identifies both type 2 and type 3 immune signatures in NE. Namely, we observed the upregulation of several type 2 signature genes²⁸ including *IL13*, *CCL17*, and *CLL18* as well as the IL-5 pathway in lesional NE skin. These hallmark AD genes were equally upregulated in NE compared to AD. In line with these findings, T cells isolated from skin biopsy samples exhibited a T_H2 -dominant phenotype. Functional consequences of type 2 immunity such as an impaired epidermal barrier and eosinophilia, which might be attributed to the upregulation of IL-13,^{29,30} were present in NE patients.

Both an *S aureus*-dominant skin microbiome and elevated total IgE are hallmarks of T_H2 immunity in AD.^{31,32} Yet total IgE in NE was not increased, confirming a finding from a previous study comparing IgE levels of NE and AD patients.⁸ In addition, similar to our data, another study confirmed that colonization with *S aureus* is less prevalent in NE.⁴

As an explanation of the observed attenuated T_H2 features, we identified a coactivation of type 3 immunity in NE with an upregulation of the IL-23 pathway and type 3 signature genes like *IL19* and *CXCL8*, typically upregulated in psoriasis.^{33,34} IL-23,

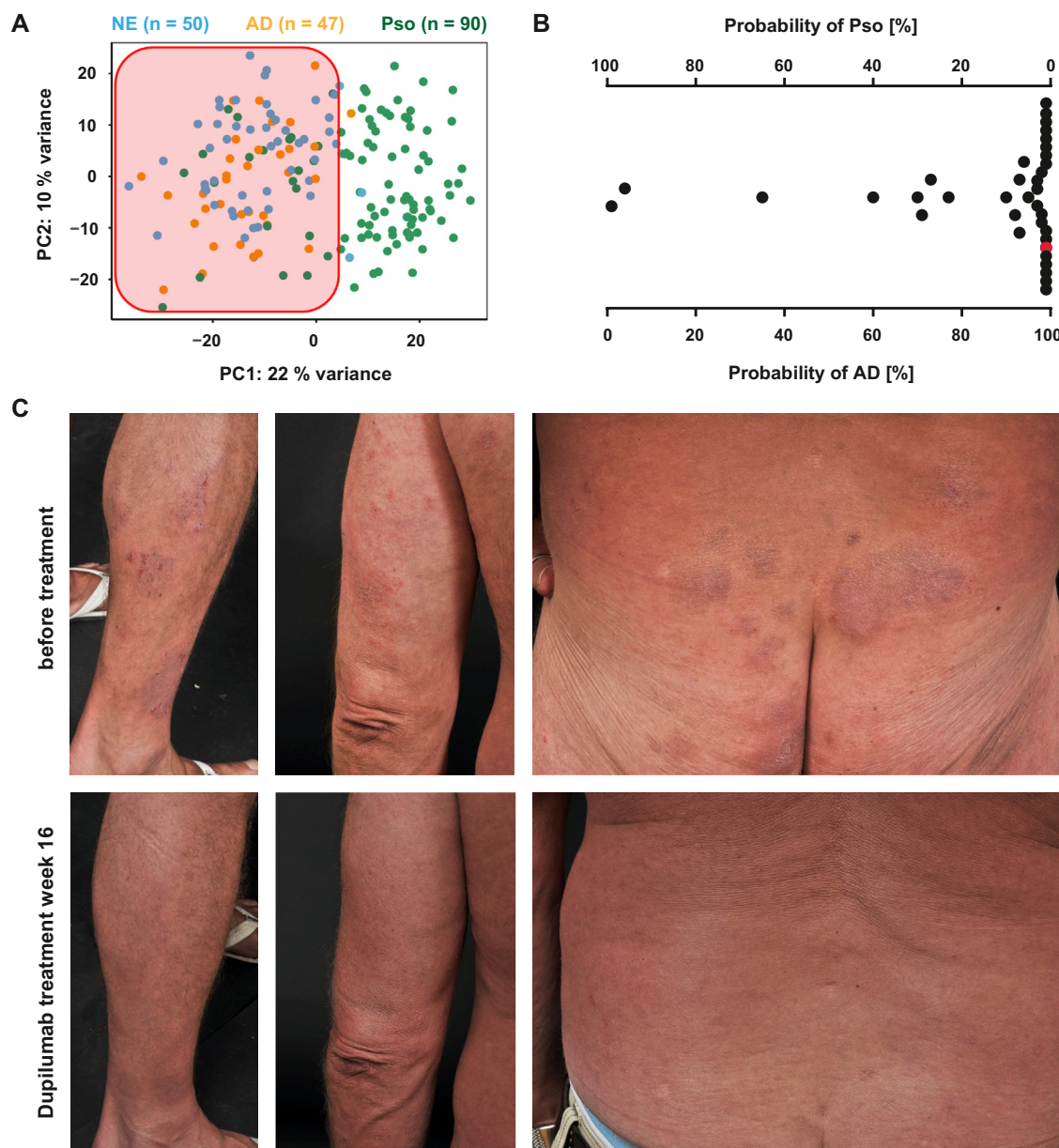


FIG 5. Type 2 immunity is dominant pathogenetic event in NE. **(A)** PCA analysis of gene expression determined by RNA sequencing of lesional skin from NE (n = 50, blue), AD (n = 47, orange), and psoriasis (n = 90, green) patients. **(B)** Probability for AD and psoriasis was calculated in lesional skin of NE patients (n = 36) using a molecular classifier for eczema and psoriasis based on gene expression of *CCL27* and *NOS2*. Patient from dupilumab case study **(C-E)** at week 0 is indicated in red. This case study of a NE patient with dupilumab treatment demonstrates efficacy in treatment of NE. Clinical pictures of skin lesions before and 16 weeks after treatment **(C)**, SCORAD and DLQI during treatment at weeks 0, 8, and 16 **(D)**, and relative gene expression of lesional skin of T_H17 and T_H2 immunity genes 16 weeks after treatment compared to untreated (week 0) **(E)** are shown. PC, Principal component; Pso, psoriasis.

the maintenance factor of IL-17A production, and IL-17 family cytokines are highly expressed in psoriasis³⁵ and mediate keratinocyte proliferation and the migration of neutrophils to psoriatic lesions by chemokines like IL-8 released from IL-17A-stimulated keratinocytes.³⁶ Translating these molecular findings to the clinical and histologic appearance of NE, the epidermal hyperplasia can be explained by IL-19's eliciting keratinocyte proliferation.^{33,37} Additionally, compared to AD, we observed a significantly increased prevalence of neutrophils in NE skin

lesions, which can be attributed to the upregulation of *CXCL8* gene expression, a keratinocyte-derived chemokine that attracts neutrophils to the skin. Apart from IL-17A, IL-19 can also induce *CXCL8* in human skin.^{33,38}

Despite the activated type 3 axis in NE, PCA analysis revealed that NE groups together with AD and is distinct from psoriasis. In addition, a molecular classifier based on the expression of *CCL27* and *NOS2* identified the great majority of NE as AD. Finally, we show that a targeted, type 2 immunity-based treatment—blocking

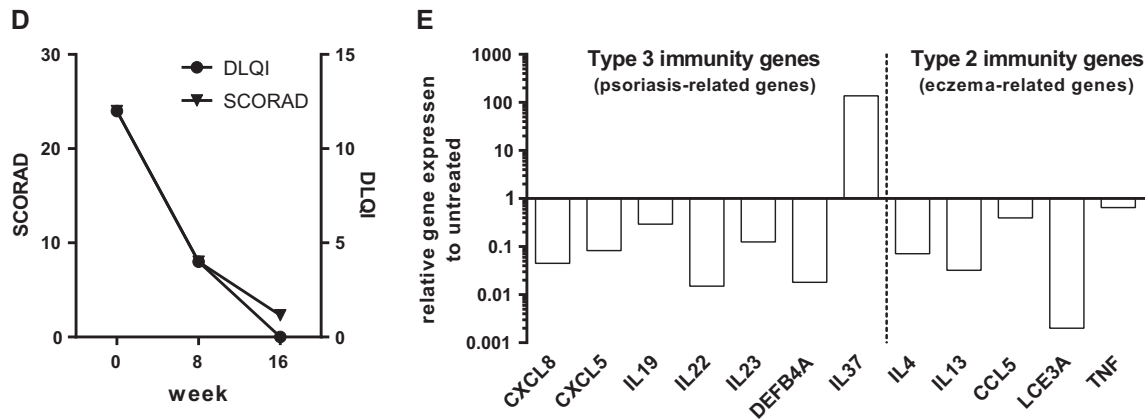


FIG 5. (Continued).

the IL-4/IL-13 receptor—is effective in the management of NE and also leads to a marked reduction of type 3 immunity genes in lesional skin. Of note, this confirms clinical data from an uncontrolled clinical study and a case series of NE successfully treated with dupilumab, highlighting the role of type 2 immunity in NE.^{39,40} To further evaluate the efficacy and safety of type 2 immunity targeted treatment, we started an investigator-initiated, multicenter, prospective, randomized, double-blind, interventional phase 2 study of dupilumab in NE ([ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT04600362) NCT04600362).

In summary, NE is a mixed type 2/type 3 inflammatory skin disease with dominant type 2 immunity. In contrast to the established disease definition of NE as an independent entity,⁸ our results indicate that the NE should be regarded as an endotype of AD with a codominant T_H2/T_H17 immune response.

Bioinformatics analysis was supported by Richa Batra.

Key messages

- NE shares overlapping clinical and histologic hallmarks with both AD (barrier defect, serum crust, spongiosis, eosinophils, IgE) and psoriasis (scaling, parakeratosis, acanthosis, neutrophils).
- Mechanistically, NE is characterized by both a type 2 (T_H2) and type 3 (T_H17) immune signature.
- Efficacy of dupilumab proves that type 2 immunity is the dominant immune pattern and should be the primary target in NE.

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