Single-cell immunopathology of recurrent acute generalized exanthematous pustulosis associated with vancomycin

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Background: Acute generalized exanthematous pustulosis (AGEP) is a severe cutaneous adverse reaction to medication that presents within 72 hours of exposure with erythematous papules and plaques with overlying pustules. The immunopathogenesis and predisposing factors of AGEP are not well characterized.

Objective: To better understand the genetic risk factors and single-cell immunopathogenesis of AGEP, we longitudinally characterized a patient with recurrent AGEP after an initial episode triggered by vancomycin.

Methods: A clinical timeline over an 8-year period was paired with skin testing, histopathology, and immunogenetic and other testing at 3 time points. Skin biopsies performed on affected skin (positive vancomycin-delayed intradermal testing [IDT]) and unaffected control skin 8 years after the initial event were subjected to single-cell sequencing to measure gene and protein expression.

Results: The patient was HLA-A*32:01 positive, which has been associated with vancomycin-induced drug reaction with eosinophilia and systemic symptoms. IDT remained positive over time, despite recurrent reactions without drug exposure. Clinical features and histopathology of IDT-positive skin were consistent with AGEP. Single-cell analysis of affected skin showed polyclonal T_H17-like cells with gene expression signatures similar to T-cell response during prevalent infectious diseases.

Conclusions: This patient exhibited persistent vancomycin-positive IDT despite distinct nondrug episodes of ALEP/AGEP. This suggests that AGEP may be triggered by both antigen-specific and non–antigen-specific factors. AGEP-affected skin showed an inflammatory infiltrate with a T_H17-like effector population, which may represent potentially actionable targets for therapeutic intervention. The presence of HLA-A*32:01, a defined risk factor for vancomycin-induced drug reaction with eosinophilia and systemic symptoms, may indicate a shared predisposition, warranting further study. (J Allergy Clin Immunol Global 2025;4:100426.)

Key words: *Acute generalized exanthematous pustulosis, vancomycin, drug reaction analysis, multiomic analysis, T-cell response*

Acute generalized exanthematous pustulosis (AGEP) is a severe cutaneous adverse reaction (SCAR) with an annual incidence of 1 to 5 cases per million.[1] The condition presents with a generalized eruption of erythematous papules and plaques, with overlying pinpoint sterile neutrophilic pustules. AGEP has been reported to be associated with medications, vaccines, viral infections, and arthropod bites. When AGEP is drug induced, as in most cases, it typically presents within 24 to 72 hours of dosing, distinguishing it from other SCAR, which typically present weeks or even months after exposure. Systemically, AGEP presents with fever, neutrophilia, and mild eosinophilia; less commonly, it can cause mucosal lesions and have hepatic, renal, lung, or bone marrow involvement.[2] Although the overall mortality rate is low (<5%) and most cases are self-limiting, up to 20% have systemic involvement, and severe cases of distributive shock have been reported, requiring intensive care.[3,4] Ninety percent of cases are caused by medication, most frequently β-lactams, antifungals, and calcium channel blockers.[4,5] Prompt withdrawal of the offending drug is critical. Topical steroids are the mainstay of treatment, although more severe cases (particularly those requiring intensive care) require the use of systemic corticosteroids or other immunosuppressants like IL-17 inhibitors.[6] Unlike drug reaction with eosinophilia and systemic symptoms (DRESS), relapse of AGEP on weaning corticosteroids and after drug discontinuation has not been reported. However, prolonged cases due to hydroxychloroquine and recurrent cases due to drug reexposure have been reported.[7-9]

The pathogenesis of AGEP likely begins with drug-reactive $CD8^+$ T cells inducing keratinocyte apoptosis, followed by the recruitment of neutrophils by drug-reactive IL-17– and CXCL8-secreting $CD4^+$ T cells.[10] The fact that patch test and intradermal test (IDT) results are often positive in AGEP suggests the presence of antigen-specific T cells resident in the skin.[11] Unlike other SCAR, like DRESS and Stevens-Johnson syndrome/

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Abbreviations used

AGEP: Acute generalized exanthematous pustulosis
ALEP: Acute localized exanthematous pustulosis
CITE-Seq: Cellular indexing of transcriptomes and epitopes sequencing
CLA: Cutaneous lymphocyte antigen
COVID-19: Coronavirus disease 2019
DRESS: Drug reaction with eosinophilia and systemic symptoms
ELISPOT: Enzyme-linked immunospot
HLA: Human leukocyte antigen
IDT: Intradermal testing
IHC: Immunohistochemistry
PBMC: Peripheral blood mononuclear cell
RTU: Ready to use
SCAR: Severe cutaneous adverse reaction
scRNA-seq: Single-cell RNA sequencing
TMP-SMX: Trimethoprim–sulfamethoxazole

toxic epidermal necrolysis, only a handful of human leukocyte antigen (HLA) markers have been associated with AGEP, and none has been repeatedly reproduced.[12,13] Furthermore, there has been no high-resolution phenotyping of AGEP-affected tissue to better understand its pathogenesis at the single-cell level.

We present a complex case of vancomycin-induced AGEP (EuroSCAR score 9) and subsequent recurrent episodes of acute localized exanthematous pustulosis (ALEP) and AGEP in the absence of vancomycin reexposure.[1] Histopathology, delayed IDT, HLA typing, and single-cell multiomics were conducted to better characterize this unusual reaction at multiple time points.

METHODS

Deidentified case and control samples from Vanderbilt University were collected under guidelines of the Vanderbilt University institutional review board (study 150754). Patient consent for photographs was obtained as part of enrollment.

Pathology

Histology during acute episodes and of IDT-positive skin was taken using 4 mm or 5 mm punch biopsy samples of affected skin, followed by sectioning and staining by hematoxylin and eosin according to institutional protocols. Retrospectively, for research purposes, additional sections were taken from paraffin-embedded blocks for immunohistochemistry (IHC).

CD162 (PSGL-1/CLA/SELPLG) IHC

Slides were placed on either the Leica BOND Max or BOND RX IHC stainer (Leica BioSystems, Deer Park, Ill). All steps besides dehydration, clearing, and coverslipping are performed on the BOND stainer. Slides were v deparaffinized. Heat-induced antigen retrieval was performed on the BOND stainer using the manufacturer's Epitope Retrieval 2 solution for 20 minutes. Slides were placed in a Protein Block (x0909; Dako, Carpinteria, Calif) for 10 minutes. Slides were incubated with anti–cutaneous lymphocyte-associated antigen (CLA)/CD162 (NB100-78039; Novus, Centennial, Colo) for 1 hour at 1:100 dilution, followed by a rabbit–anti-rat antigen (BA-4000; Vector Laboratories, Burlingame, Calif) at 1:2000 dilution for 15 minutes. The BOND Refine (DS9800; Leica BioSystems) detection system was used for visualization. Slides were then dehydrated, cleared, and coverslipped.

IL-17 IHC

Slides were placed on the BOND Max IHC stainer. All steps besides dehydration, clearing, and coverslipping were performed on the BOND Max. Slides were deparaffinized, and a heat-induced antigen retrieval was performed on the BOND Max using their Epitope Retrieval 2 solution for 20 minutes. Slides were placed in DakoCytomation Biotin Blocking System (x0590, Dako) for 15 minutes for each solution. Slides were placed in a Protein Block (x0909, Dako) for 10 minutes. Slides were incubated with anti–IL-17 (AF-317-NA; R&D Systems, Minneapolis, Minn) for 1 hour at a 1:200 dilution, followed by biotinylated anti-goat (BA-5000, Vector Laboratories) for 30 minutes at 1:200 dilution. The BOND Polymer Refine detection system was used for visualization. Slides were then dehydrated, cleared, and coverslipped.

CD8 (RTU) IHC

Slides were placed on the BOND Max IHC stainer. All steps besides dehydration, clearing, and coverslipping were performed on the BOND Max. Slides were deparaffinized. Heat-induced antigen retrieval was performed on the BOND Max using their Epitope Retrieval 2 solution for 20 minutes. Slides were incubated with Ready-To-Use (RTU) anti-CD8 (MM39-10; StatLab, McKinney, Tex) for 15 minutes. The BOND Polymer Refine detection system was used for visualization. Slides were then dehydrated, cleared, and coverslipped.

CD4 (RTU) IHC

Slides were placed on the BOND Max IHC stainer. All steps besides dehydration, clearing, and coverslipping were performed on the BOND Max. Slides were deparaffinized. Heat-induced antigen retrieval was performed on the BOND Max using their Epitope Retrieval 2 solution for 20 minutes. Slides were incubated with RTU anti-CD4 (PA0427, Leica BioSystems) for 1 hour. The BOND Polymer Refine detection system was used for visualization. Slides were then dehydrated, cleared, and coverslipped.

IDT

IDT was performed with validated and sterile concentrations of the relevant antibiotics, injecting 0.02 mL intradermally for all reagents.[14,15]

ELISPOT

Enzyme-linked immunospot (ELISPOT) assay for IFN-γ release on peripheral blood mononuclear cells (PBMCs) was conducted as previously described.[15]

Single-cell sequencing

Combined single-cell RNA sequencing (scRNA-seq), cellular indexing of transcriptomes and epitopes (CITE-seq), and T-cell receptor sequencing (TCR-seq) were conducted as follows. Punch biopsy samples were taken from affected and unaffected

skin 48 hours after positive reaction to 5 mg and 50 mg vancomycin IDT 8 years after the original reaction. An established collagenase P–based protocol optimized for lymphoid cell recovery was used to process skin into single-cell suspensions.[16,17] For CITE-seq, suspensions were stained following the manufacturer's protocol for up to 137 functional and surface lineage markers using the TotalSeq-C Human Universal Cocktail v1.0 (BioLegend, San Diego, Calif). Individual cells were captured on barcoded beads for cDNA library preparation using the 10x Chromium Next GEM chip K (10x Genomics, Pleasanton, Calif), then sequenced with an Illumina NovaSeq 6000 device (Illumina, San Diego, Calif). Raw data were normalized by the Seurat v4.1.1 software package.[18] Cells with a low number of unique molecular identifiers (<500 identifiers), <100 genes, and/or >10% mitochondrial content were removed to filter low-quality or dead populations, with a 10% mitochondrial cutoff recommended for scRNA-seq of human tissue including skin.[19] Subsets with <50 scRNA-seq–defined cells were removed. Visual Genomics Analysis Studio was used for differential analyses and visualization.[20]

HLA typing

High-resolution HLA-A, HLA-B, HLA-C, HLA-DP, HLA-DR, and HLA-DQ typing was performed using sequence-based typing on the Illumina MiSeq instrument as previously validated.[21]

RESULTS

The patient was a 64-year-old woman at time of presentation, of self-identified White race, with a history of hypothyroidism, long-term levothyroxine therapy, asthma, anxiety, and depression receiving long-term medications. She reported no history of systemic dermatologic or inflammatory disease (including psoriasis, eczema, or previous episodes of AGEP), but she mentioned a remote rash to amoxicillin. The patient's first episode occurred after bilateral knee arthroplasty with gentamicin-impregnated spacers, for which she received perioperative vancomycin (Fig 1, *A*). Ten days later, a papulopustular eruption developed over the surgical sites in a flexural distribution. She received empirical trimethoprim–sulfamethoxazole (TMP-SMX) and an additional dose of vancomycin for presumed incision-site infection. Symptoms progressed to a diffuse erythematous papulopustular eruption with some tense bullae in dependent areas, followed by desquamation (Fig 1, *B*). Our initial differential included AGEP, pustular variants of DRESS or Stevens-Johnson syndrome/toxic epidermal necrolysis, pustular psoriasis, and subcorneal pustular dermatosis (Sneddon-Wilkinson syndrome). However, the distribution and the timing relative to drug exposure were far more consistent with AGEP. Studies revealed neutrophilic leukocytosis and no evidence of liver, kidney, or other systemic involvement. She subsequently received short courses of methylprednisolone and clindamycin. After transferring to our tertiary-care center, histopathology revealed subcorneal neutrophilic abscesses, perivascular lymphocytic infiltrate with neutrophils, and a paucity of eosinophils—results most consistent with AGEP (Fig 1, *C*). Direct immunofluorescence was negative, ruling out autoimmune bullous disease.

She subsequently had multiple episodes of ALEP. One episode occurred 7 days after receipt of levofloxacin for a respiratory infection and resolved within 2.5 weeks. That episode was accompanied by throat swelling and shortness of breath. She also experienced episodes after 5 days of clindamycin prophylaxis for back surgery, another on day 5 of a course of amoxicillin for a dental abscess, and several without any known drug exposure. Episodes were accompanied by fever, headaches, and nausea, and generally resolved within 3 weeks, sometimes requiring short courses of oral methylprednisolone. Histopathology from the amoxicillin episode revealed dermal infiltrate with neutrophils, consistent with resolving ALEP (see Fig E1 in the Online Repository available at www.jaci-global.org). IHC from skin taken during this episode was positive for IL-17, CLA, and CD4 in the infiltrate, suggesting a skin-homing $CD4^+$ T-cell population. Later, the patient had 4 episodes of ALEP without any clear drug trigger that resolved on their own.

Before the availability of coronavirus disease 2019 (COVID-19) vaccines, she subsequently had a more severe episode, consisting of a generalized papulopustular eruption requiring hospital admission, 38 days after testing positive for COVID-19 and 3 days after beginning nystatin for oral candidiasis (Fig 2, *A* and *B*). She initiated a short course of oral prednisone on day 41, then subsequently increased to a 6-week oral steroid taper, after which the eruption resolved. A biopsy sample from an erythematous papule without overlying pustules, taken during the resolution stage of the eruption, demonstrated a dermal infiltrate with scant neutrophils, with IHC positive for CD4, CLA, and IL-17 in the infiltrate (Fig 2, *C*).

In the Vanderbilt University Medical Center drug allergy clinic, the original AGEP episode was investigated by performing IDT to vancomycin, clindamycin, and fluoroquinolones (Fig 3, *A*) as well as epicutaneous patch testing with TMP-SMX (Fig 3, *B*), all conducted approximately 5 years after the initial episode. To shed light on the etiology of the multiple episodes of ALEP/AGEP that occurred in the 5 years after this initial skin test investigation and 8 years after the initial episode, repeat IDT to vancomycin and β-lactams was performed, along with patch testing β-lactams and nystatin, after the resolution of the COVID-19–related episode (Fig 3, *C*). Vancomycin IDT was positive on both occasions 3 years apart; on both occasions, it triggered a spreading, pustular AGEP-like reaction. In the latter setting, performed 2 months after developing non–drug-related AGEP, the patient experienced the start of a generalized rash, which was stopped by topical corticosteroids. Skin testing to penicillins and other β-lactams was negative on both occasions despite the later episode of ALEP having a temporal association with amoxicillin exposure. IFN-γ ELISPOT on PBMCs was negative to vancomycin (see Fig E2 in the Online Repository available at www.jaci-global.org). HLA typing showed that the patient carries HLA-A*32:01 (Fig 3, *D*), which is expressed in 6.8% of individuals with European ancestry and strongly predisposes to vancomycin-induced DRESS.[15]

To better understand the tissue-specific immunopathogenesis of AGEP, we used single-cell multiomics to define the clonality, transcriptome, and surface proteome of affected (positive IDT to vancomycin) and unaffected skin taken using punch biopsy samples.[22] Patient PBMCs were also included. Unbiased transcriptome-inferred clustering identified diverse immune cell populations in affected skin (Fig 4, *A*), validated by single-cell cellular indexing of transcriptomes and epitopes sequencing (CITE-Seq) expression of subset-defining surface proteins for T cells (CD3, CD4, CD8), natural killer cells (CD56), dendritic cells (CD1c), monocytes (CD14, CD11c), and B cells (CD19,

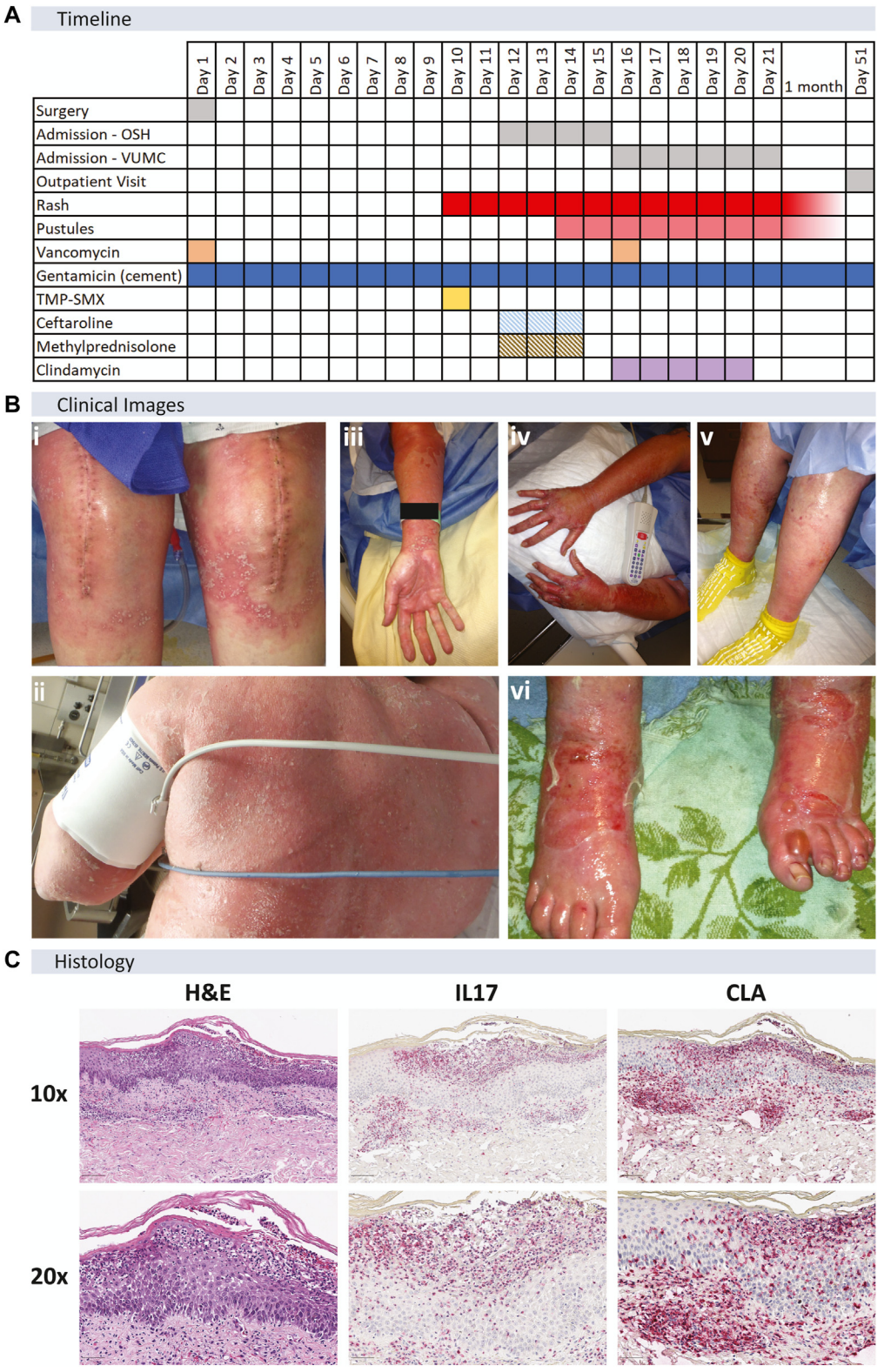


FIG 1. Initial episode. **(A)** Clinical timeline. Patient developed generalized eruption 10 days after vancomycin exposure and knee arthroplasty with gentamicin-impregnated spacer. **(B)** Patient sought care on day 16 with generalized pustular eruption *(i, ii, iii),* which evolved into superficial erosions *(iv, v),* and several bullae *(vi).* At outpatient follow-up on day 51, eruption had resolved. Photographs were taken on day 16 *(i, ii),* day 17 *(iii),* day 20 *(iv, v),* and day 22 (*vi,* from home). **(C)** Histology shows neutrophilic pustules. Infiltrate is positive for IL-17 and CLA, marker of skin-homing T cells.

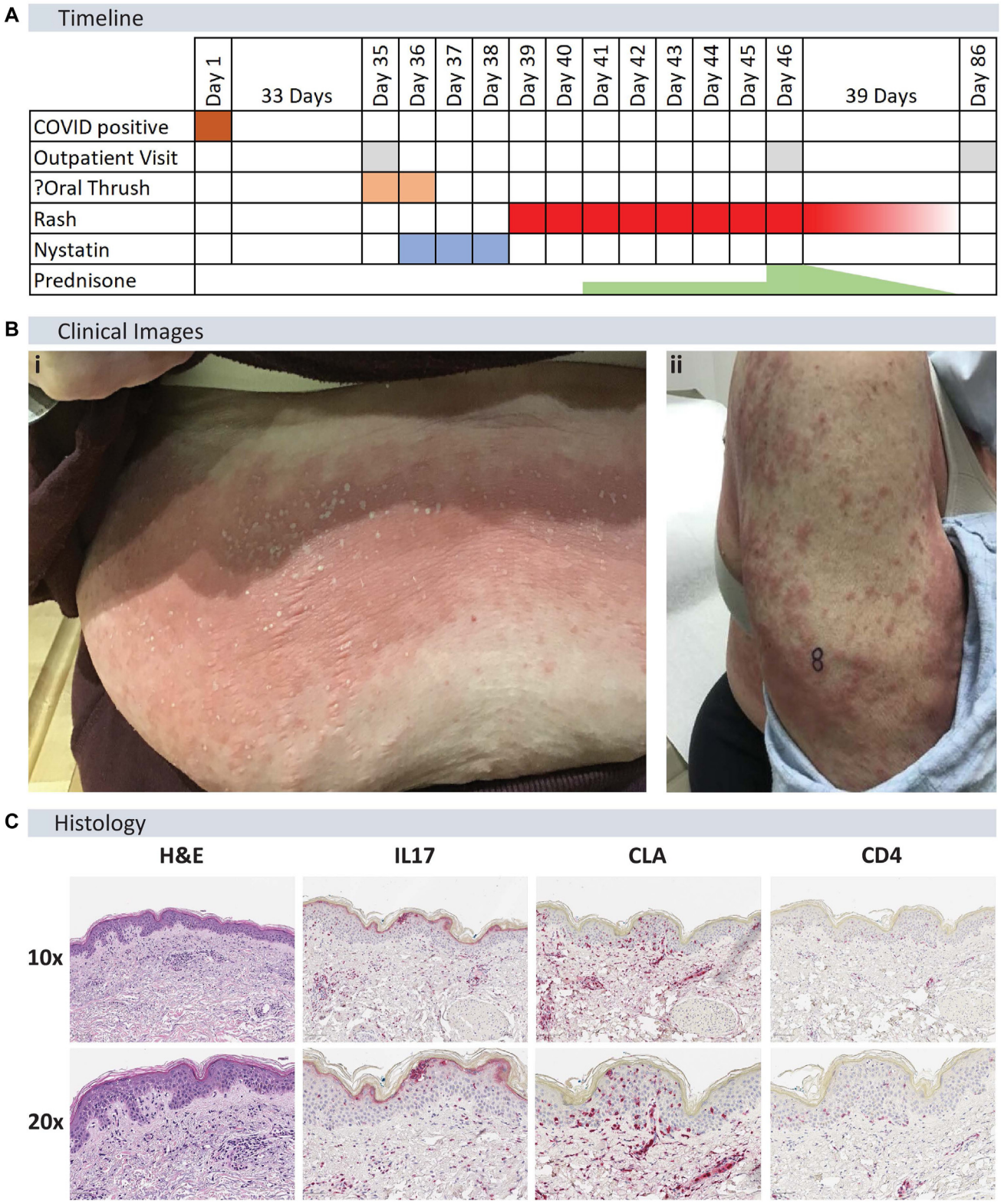
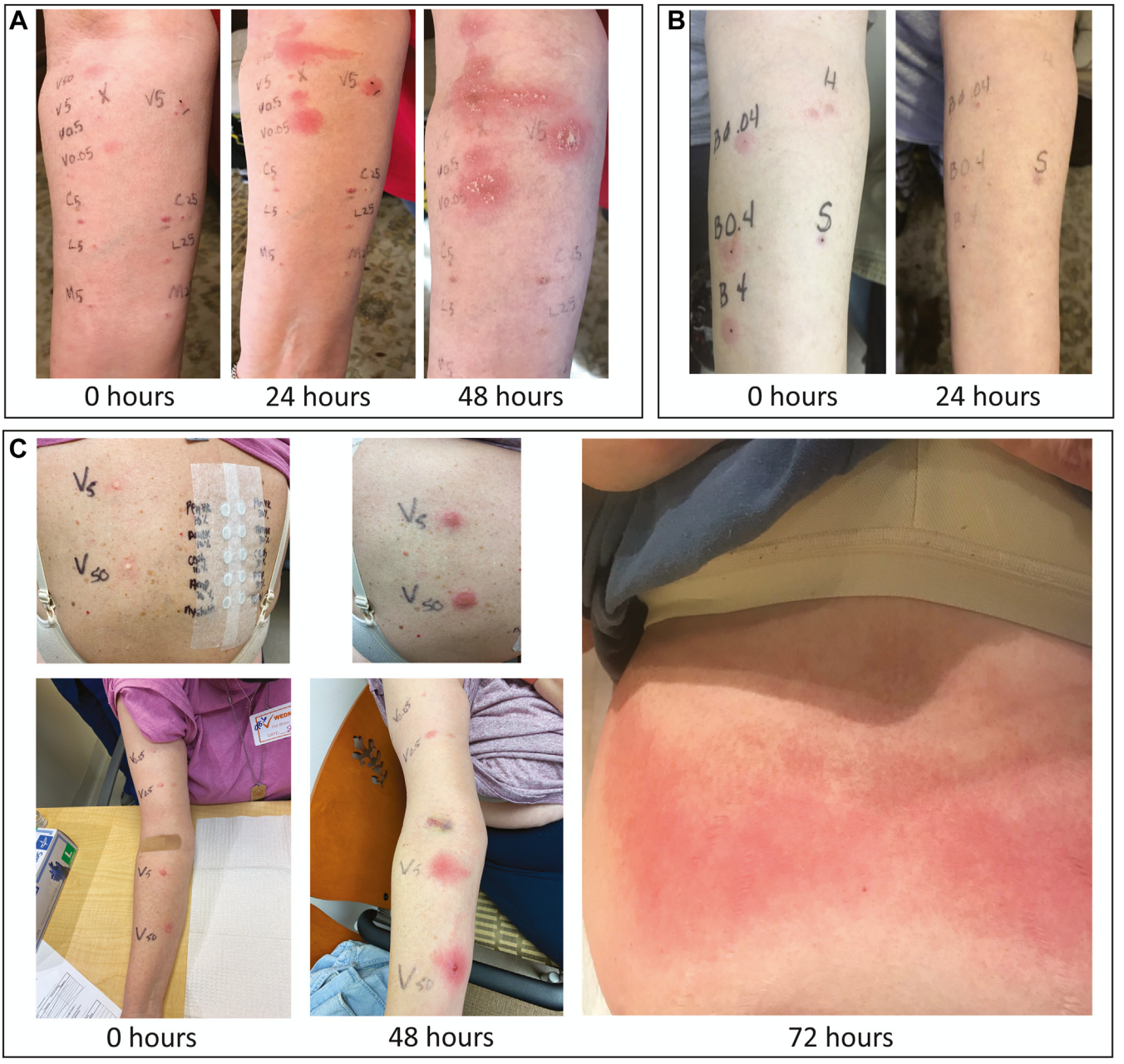


FIG 2. Post–COVID-19 recurrence. **(A)** Patient developed generalized eruption 38 days after exposure to COVID-19 and 3 days after exposure to nystatin to treat thrush. Patient was prescribed prednisone 20 mg twice daily for 5 days, then increased to 80 mg daily, tapering over 3 weeks, which cleared eruption by day 86. **(B)** Pustules appeared on background of erythematous plaques (*i,* day 42), which improved to erythematous papules and plaques (*ii,* day 46) without pustules after initial steroid receipt. **(C)** Biopsy on day 46 from upper arm (site shown in *(B), ii*) revealed scant dermal infiltrate with neutrophils, without pustules, consistent with resolving AGEP. Infiltrate is positive for IL-17, CLA, and CD4.



D

HLA-A	HLA-B	HLA-C	HLA-DPB1	HLA-DQA1	HLA-DQB1	HLA-DRB1
29:02:01G	40:02:01G	02:02:02G	04:01:01G	01:02:01G	02:02:01i	07:01:01i
32:01:01G	44:03:01G	16:01:01G	11:01:01G	02:01:01G	06:02:01i	15:01:01i

FIG 3. Allergy testing. **(A)** Pustular reaction to vancomycin IDT at 48 hours accompanied by fever and malaise, resolving in 2 or 3 weeks. Fluoroquinolone IDT was negative. Clindamycin IDT showed more muted pustular reaction (not shown). *V* indicates vancomycin (0.05, 0.5, 5, 50 mg); *C,* ciprofloxacin (5 and 25 mg/L); *L,* levofloxacin (5 and 25 mg/L); and *M,* moxifloxacin (5 and 25 mg/L). **(B)** No reaction to TMP-SMX IDT at 24 hours. Patch testing to TMP 5% and SMX 10% in petrolatum was also negative (not shown). *B* indicates TMP-SMX (0.04, 0.4, 4 mg/mL); *H,* histamine (positive control); and *S,* saline (negative control). **(C)** Vancomycin IDT (*V;* 0.05, 0.5, 5, 50 mg) results in pustular reaction that spread locally. Penicillin IDT was negative (not shown). Patch testing to penicillin VK, amoxicillin, ampicillin, cephalexin, and nystatin was negative.

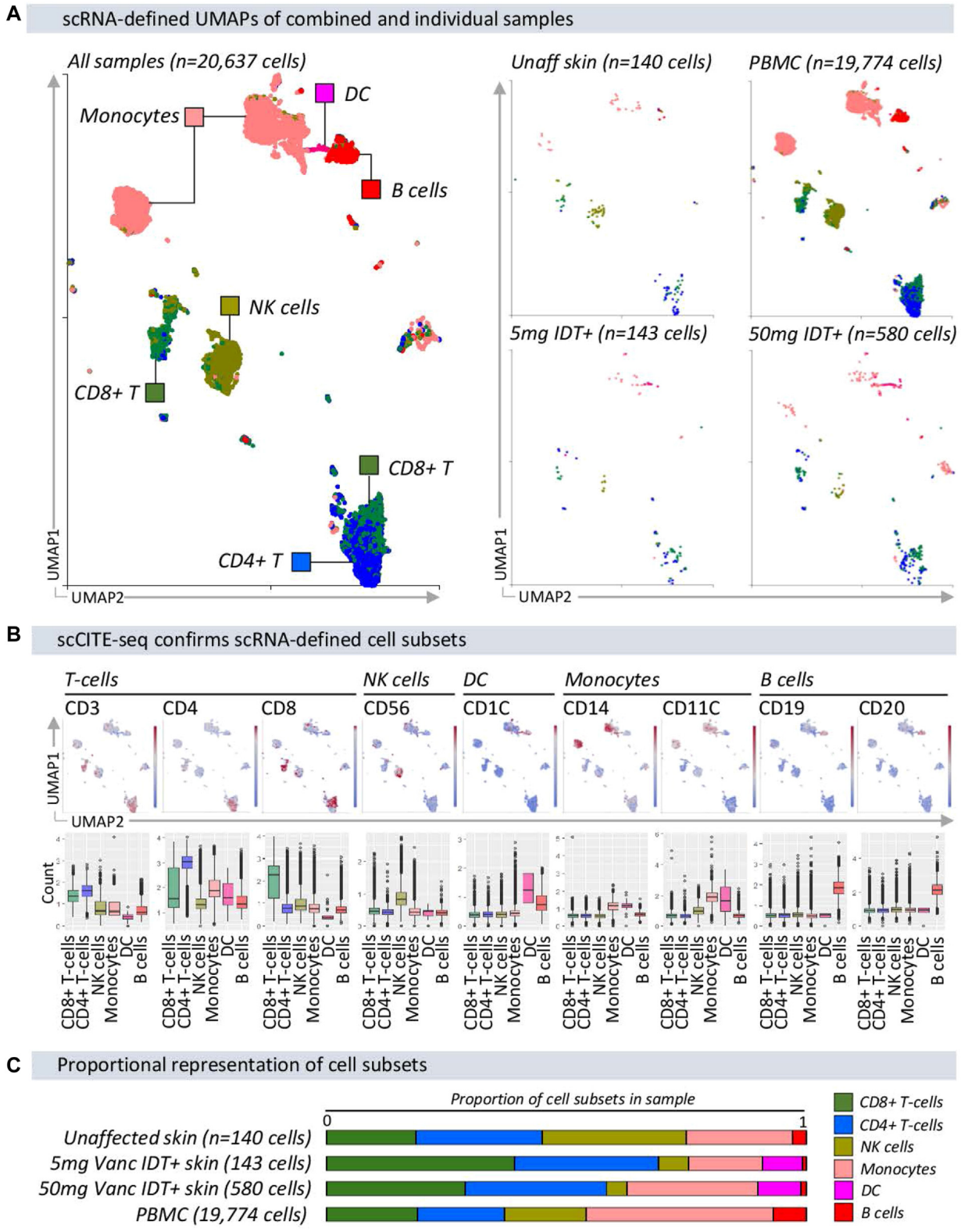


FIG 4. Single-cell multiomics of IDT-affected skin **(A)** scRNA-seq clusters cells into T cells; monocytes, macrophages, and neutrophils; and some others. **(B)** CITE-Seq of surface markers reproduces clusters defined by transcriptomics alone. **(C)** scRNA-seq–defined representation of cell subsets. Compared to healthy and unaffected skin, IDT-affected skin shows increased representation of $CD4^+$ and $CD8^+$ T cells, with decreased presentation of NK cells. *DC,* dendritic cell; *HSC,* hematopoietic stem cell; *ILC,* innate-like lymphoid cell; *MAIT,* mucosal-associated invariant T; *MSC,* mesenchymal stromal cell; *NK,* natural killer; *UMAP,* uniform manifold approximation and projection; *Unaff,* unaffected; *Vanc,* vancomycin.

CD20; Fig 4, *B*). Affected skin was primarily composed of $CD4^+$ and $CD8^+$ T cells and monocytes, but also dendritic cells, which were only found in affected skin. There was a 1.2-fold and 2.1-fold increase in $CD4^+$ and $CD8^+$ T cells, respectively, in affected compared to unaffected skin (Fig 4, *C*). In contrast, the proportion of natural killer cells reduced by 6-fold in affected skin, and only a few B cells were identified.[18] In $CD4^+$ and $CD8^+$ T-cell populations of PBMCs and 50 mg IDT^+ skin, >70% of cells expressed a productive TCR αβ pair, respectively. The TCR repertoire was diverse, without clonal expansion and limited overlap between samples (see Fig E3 in the Online Repository available at www.jaci-global.org). Indeed, just 8 and 24 TCR CDR3 αβ clonotypes identified on $CD4^+$ T cells or $CD8^+$ T cells of 50 mg IDT^+ skin, respectively, were similarly identified among the 3535 (0.2%) and 3308 (0.7%) total clonotypes on $CD4^+$ T cells or $CD8^+$ T cells in PBMCs. These same TCR were not identified on T cells in unaffected skin, so the diluted peripheral repertoire of TCR driving disease in the skin may provide a reason for the lack of ELISPOT response.

Differential analyses (ANOVA, false discovery rate–adjusted $P < .05$) showed that $CD4^+$ and $CD8^+$ T cells in IDT-affected skin from high- and low-dose vancomycin samples shared similar differentially expressed gene signatures (see Fig E4, *A,* in the Online Repository available at www.jaci-global.org). Both $CD4^+$ and $CD8^+$ T cells on 50 mg IDT^+ skin were significantly enriched for genes aligned with T_H17 and Tc17-like differentiation *(JUNB, CREM, FOS, NR4A2, KLF6).* Further, among the extended signature of these cells, both $CD4^+$ and $CD8^+$ T cells expressed *SOCS1*, which modulates T effector to regulatory T cell differentiation,[23] and *PTGER4,* the EP4 receptor for prostaglandin E_2, which facilitates T_H1/T_H17 expansion and inflammation in autoimmune diseases like ankylosing spondylitis.[24] $CD8^+$ T cells were enriched for *CALM2* and *HSP90AB1,* suggesting recent activation. $CD4^+$ T cells were enriched for *ICOS* and the transcription factors *BATF* and *KLF6,* suggesting an activated T_H17-like population.

Monocytes also shared signatures associated with inflammation *(IL1β, IFI30, LITAF),* leukocyte adhesion *(PLAUR, ICAM1),* and HLA class II antigen presentation *(HLA-DQA1). CXCL8* expression was also enriched in monocytes, but not T cells, in the 50 mg vancomycin-positive IDT sample. Pathway analysis, performed using Enrichr, aligned $CD4^+$ and $CD8^+$ T cells with IL-17 signaling, and $CD8^+$ T cells and monocytes with antigen processing; it also highlighted overlapping signatures similar to pathways upregulated during infection (Fig E4, *B*).[25] Consistent with these findings, IHC showed a mixed infiltrate of $CD4^+$ and $CD8^+$ cells, with positive staining for CLA and IL-17 in the infiltrate (Fig E4, *C*).

DISCUSSION

We describe an HLA-A*32:01-positive woman with multiple episodes of AGEP/ALEP in both the presence and absence of the initial culprit drug, vancomycin, after an initial episode of vancomycin IDT–confirmed AGEP. Because the patient carried a risk allele for vancomycin DRESS, we performed an ELISPOT, the results of which was negative. However, the sensitivity of this assay in AGEP is lower than that in DRESS; one study showed it is only 43% sensitive for β-lactam–induced AGEP.[26] Additionally, the histopathology and clinical course were more consistent with AGEP over DRESS. IDT was durably positive over time to vancomycin and negative to other drugs like amoxicillin (despite an episode of ALEP temporally related to amoxicillin). Recurrent AGEP to multiple structurally disparate drugs has been described in the same patient. However, to our knowledge, episodes of AGEP and ALEP in the absence of the drug over time in the same patient, like that which developed with our patient, has not been previously described.[8,9,27] Repeated episodes in the absence of known drug triggers may suggest the possibility of epitope spreading, making the patient susceptible to subsequent non–antigen-specific reactions.

It may also be the case that the initial episode caused long-lasting derangement of T_H17-related pathways, creating a picture reminiscent of recurrent pustular psoriasis (which is difficult to distinguish from AGEP).[28] Some studies have shown shared risk with generalized pustular psoriasis and AGEP via overactivation of, and/or mutations in, the IL-36 pathway.[29,30] Other studies have demonstrated increased staining for IL-36 isoforms in lesional skin and release of IL-36 from PBMCs obtained from AGEP patients in response to culprit drugs.[31] However, our CITE-Seq data did not demonstrate IL-36 signaling in affected skin, and the patient did not carry the risk alleles for pustular (HLA-C*01:02) or early-onset (HLA-C*06:02) psoriasis.[32,33] Furthermore, the patient did not have a history of plaque psoriasis, which often precedes pustular psoriasis, and histopathology of this patient showed scattered eosinophils, which is more consistent with AGEP than psoriasis.

Biopsy samples taken within 48 hours of IDT showed both $CD4^+$ and $CD8^+$ cells, and stained positive for IL-17 and CLA, supporting a model in which $CD8^+$ T cells drive an initial reaction that is then sustained by $CD4^+$ T cells. Single-cell multiomics on affected skin identified a polyclonal population of T_H17-like $CD4^+$ and $CD8^+$ T cells sharing signatures with pathogenic effectors driving infectious disease. Previous studies have demonstrated increased circulating T_H17 cells and IL-22 (an IL-17–pathway cytokine) in AGEP, and the anti–IL-17A antibody secukinumab has successfully treated steroid-refractory AGEP.[34,35] Another study succeeded in isolating hapten-specific T_H17 cells in the peripheral blood of AGEP patients.[36] To our knowledge, this is the first demonstration of T_H17 predominance in AGEP-affected skin. We have also identified novel differentially expressed genes in AGEP, which may be additional therapeutic targets; for example, modulators of *PTGER4,* upregulated in our data, are under development and may present a novel therapeutic approach to AGEP treatment.[37]

Previous studies have not conclusively determined genetic risk factors for AGEP. Besides the aforementioned association with IL-36, another study showed a possible association with HLA-B51, -DR11, and -Dq3, none of which was present in our patient.[38] However, another previous report showed a potential association between β-lactam–induced AGEP and HLA-DRB1*15:01, which our patient possesses.[12] Additionally, this patient carries HLA-A*32:01, which is strongly associated with vancomycin-induced DRESS, suggesting the possibility of shared susceptibility between SCAR.[15,39] Interestingly, there exists a potentially overlapping condition, a form of DRESS with pustular lesions, linked to vancomycin, other antibiotics, and antiepileptics.[40] Our patient did not meet the criteria for DRESS during any episode, making this diagnosis less likely.

The generalizability of our work is limited by the fact that we report a single case. However, our approach demonstrates the utility of detailed assessment of an individual case pairing the clinical timeline with histopathology, IDT, and tissue-specific single-cell multiomics for studying SCAR causality and immunopathogenesis. Future studies of AGEP and other SCAR using these methods will potentially drive the discovery of risk factors and biomarkers for diagnosis and treatment.

DISCLOSURE STATEMENT

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Disclosure of potential conflict of interest: E. J. Phillips receives royalties and consulting fees from UpToDate and consulting fees from Janssen, Verve, Elion, Servier, Esperion, and Rapt; is codirector of IIID Pty Ltd, which holds a patent for HLA-B*57:01 testing for abacavir hypersensitivity; and holds a patent with AC and KCK (without financial remuneration) for detection of HLA-A*32:01 in connection with diagnosing drug reaction with eosinophilia and systemic symptoms. The rest of the authors declare that they have no relevant conflicts of interest.

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All raw and normalized data generated by single-cell sequencing and used in this study have been deposited to the NCBI sequence read archive PRJNA1070820 and GEO database under accession code GSE275871 user identifer “SCAR patient 26”. All other data are available in the article and its supplementary files or from the corresponding article upon request.

Key messages

- **Recurrent AGEP-like reaction may be driven by an autoreactive T_H17 population, triggered by multiple drugs.**
- **The combination of IDT and single-cell multiomics can be used to study the pathogenesis of certain drug hypersensitivity reactions.**

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