DOI: 10.1111/all.15064

Conjunctival inflammation in dupilumab-treated atopic dermatitis comprises a multicellular infiltrate with elevated T1/T17 cytokines: A case series study

To the Editor,

Conjunctivitis is a frequently reported adverse event in atopic dermatitis (AD) patients treated with dupilumab, the first antibody-based treatment for AD targeting the interleukin (IL)-4 receptor-alpha-subunit.[1] In a recent study, we demonstrated scarcity of intraepithelial goblet cells (GCs) accompanied by a mixed immune-cell infiltrate in conjunctival biopsies from dupilumab-treated AD patients developing conjunctivitis.[2] In the current case series study, we further specified these infiltrating cells, in order to improve the understanding of this new entity of conjunctivitis.

Conjunctival biopsies from six AD patients (4 male; median age 38.5 years, interquartile range (IQR) 29.0–56.5, median Eczema Area Severity Index (EASI) score before start dupilumab 20.5, IQR 15.4–27.9) with active conjunctivitis developed during dupilumab treatment confirmed by an ophthalmologist (median time from initiation of dupilumab treatment to onset 59.5 days, IQR 51.5–80.5) were evaluated, of which for five histopathology has been described in a previous study[2] (Table S1a). Conjunctival biopsies of two healthy controls (HCs) were included. Biopsies were histologically assessed, and additionally stained with a panel of 27 metal-conjugated antibodies (Figure 1A, Table S2) for imaging mass cytometry (detailed methods in online supplementary).

Most frequently reported ophthalmological characteristics were tarsal and bulbar conjunctivitis (83.3% and 66.7%, respectively), and blepharitis (83.3%). Median goblet cell density was 2.6 cells/mm (IQR 1.1–4.0) in patients compared to 4.1 cells/mm and 9.8 cells/mm in HCs (Table S1b).

The subepithelial cellular infiltrate in inflamed conjunctival tissue of dupilumab-treated AD patients ranged from limited (patient 2) to moderate (patients 1 and 6) to extensive (patients 3, 4, and 5), and comprised a diverse panel of infiltrating immune cells, including CD4+ and CD8+ T cells (Figure 1B–D), CD11c+ dendritic cells, CD14+ monocytes, and CD68+ macrophages in all patients (Figures S1 and S4). Within the T-cell infiltrates high expression of ICOS, Ki67 and HLA-DR were observed in all patients, indicating an activation state accompanied by local proliferation (Figures S2a and S4). In five patients, CD8+ T cells co-expressed granzyme B, indicating cytotoxic activity (Figures S3 and S4). Significantly increased signals of IFNγ, TNFα, IL-10, and IL-17A were observed within subepithelial cell infiltrates in all patient samples compared to non-infiltrated reference regions and HCs (Figure 2). Finally, numerous FOXP3+ regulatory T cells were identified in conjunctival infiltrates of patients 1, 3, 4, and 5 (Figures S2b and S4).

The conjunctival epithelium normally is a GC rich tissue, and IL-13 is the predominant cytokine promoting GC proliferation and mucus secretion.[3] Various types of dry eye diseases have been associated with GC loss, and decreased GC density has been associated with increased IFNγ expression, and increased numbers of HLA-DR+, CD11c+, and CD45+ inflammatory cells in the conjunctiva.[3,4] These results are in line with our findings of GC scarcity combined with an highly activated multicellular infiltrate and increased local Th1-related cytokine production in dupilumab-treated AD patients developing conjunctivitis. Based on these findings,[2] dupilumab might affect GC development and function by inhibiting IL-13, resulting in reduced production of protective mucus and immunoregulatory factors, promoting conjunctival inflammation, which may be further reinforced by the IFNγ-skewing effect of dupilumab through blocking both IL-4/IL-13 signaling in T cells.

The fact that conjunctivitis has only been reported in AD patients who are treated with dupilumab, and not in asthma or chronic rhinosinusitis, might be explained by the high incidence of ocular surface disease in AD and its association with GC cell loss. More severe AD has been associated with lower GC density, implicating that severe AD patients are at higher risk of developing conjunctivitis during dupilumab treatment.[5]

Treatment with ocular cyclosporine A (CsA) emulsion has shown to significantly increase GC density in patients with dry eye syndrome and to reduce conjunctival T-cell infiltration, activation, and cytokine expression of especially IFNγ in atopic keratoconjunctivitis patients.[6,7] In view of our findings, CsA eye drops and/or other calcineurin inhibitors, such as tacrolimus eye ointment, might have the potential to suppress conjunctival inflammation and restore development and function of GCs in patients developing dupilumab-associated conjunctivitis.

A limitation of this study is the relatively small sample size and the lack of baseline samples before initiation of dupilumab due to the

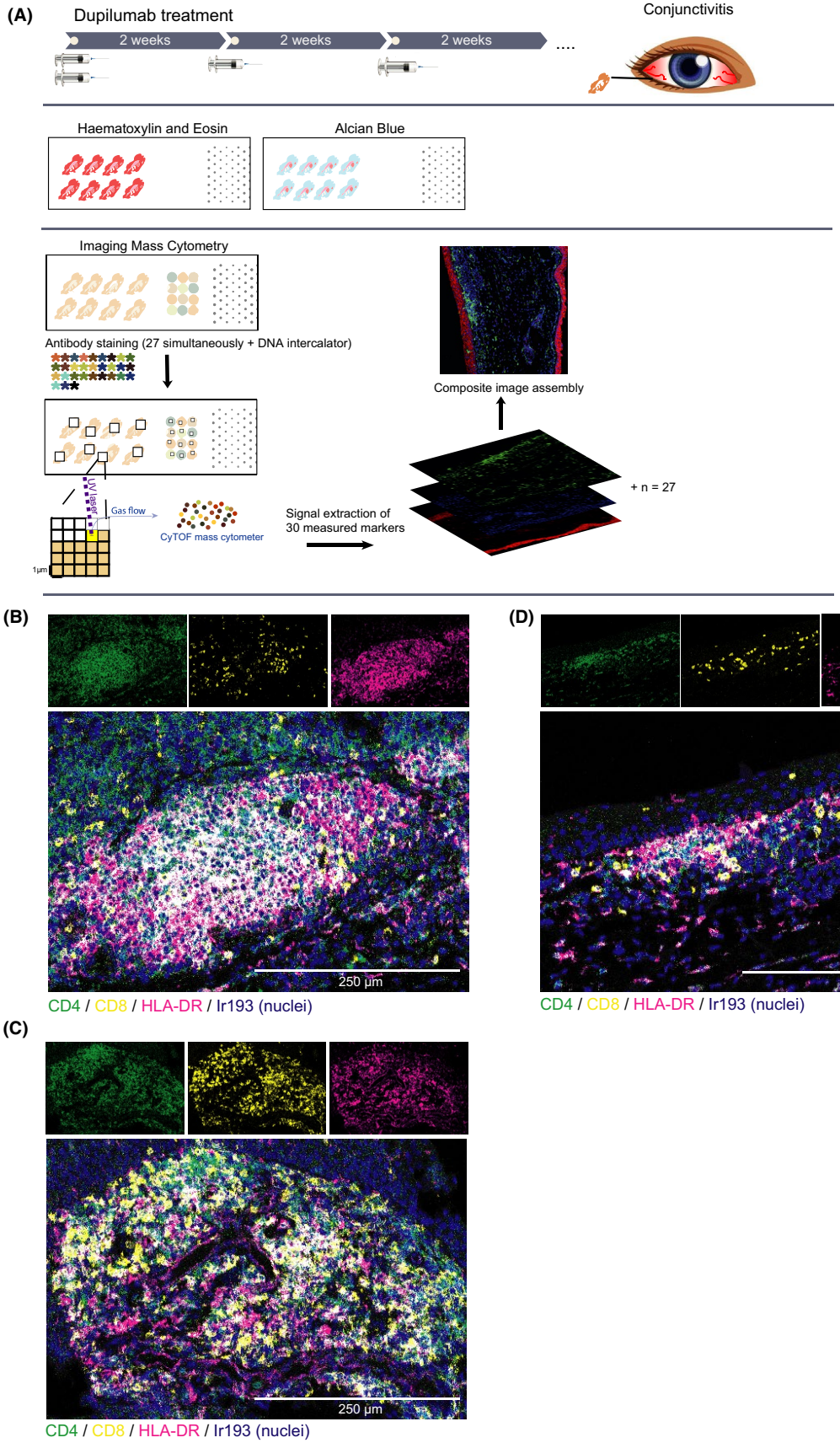

FIGURE 1 Graphical workflow of sample collection, section staining, and Imaging Mass Cytometry. (A). Composite images derived from Imaging Mass Cytometry of conjunctival biopsy samples from AD patients developing conjunctivitis during dupilumab treatment. Representative images of patient 3 (B), patient 4 (C), and patient 5 (D) showing overlay of CD4 (green), CD8 (yellow), HLA-DR (magenta), and DNA intercalator (Ir) 193 (blue)

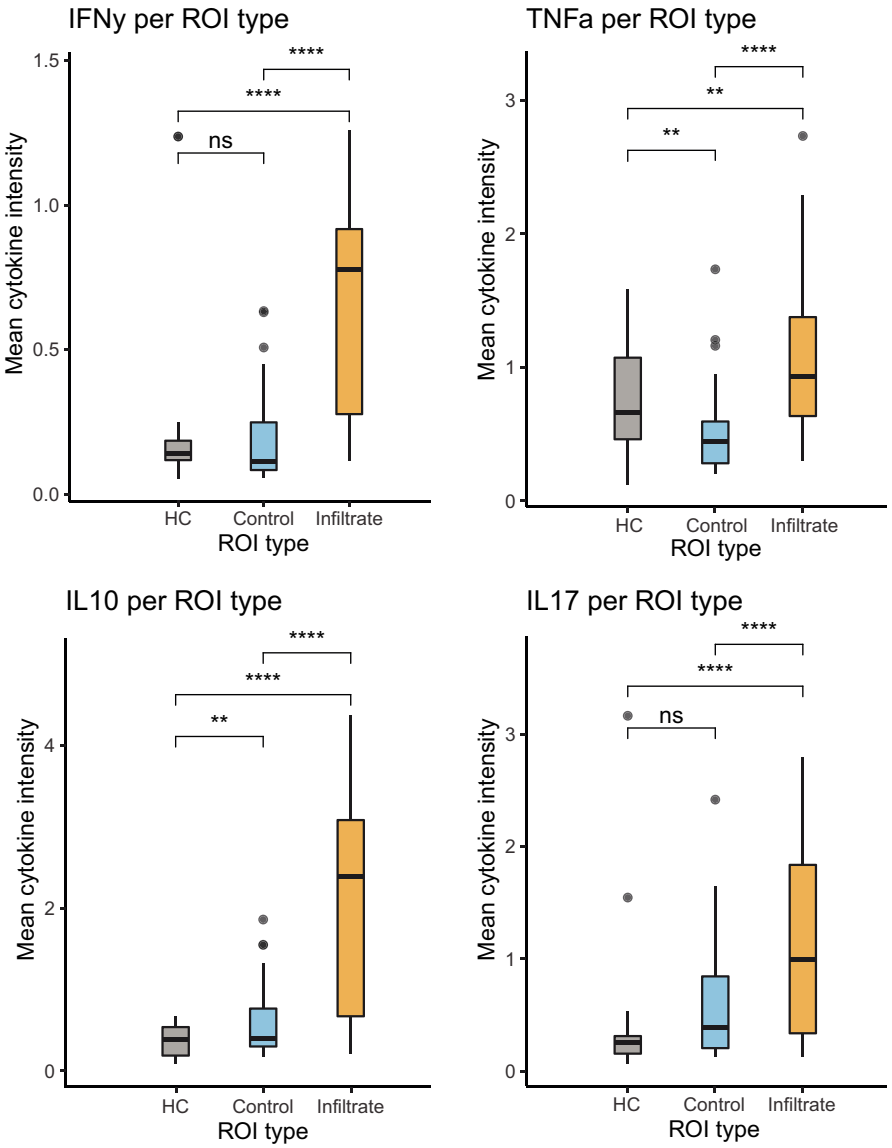

FIGURE 2 Mean cytokine signal intensity plotted for IFNγ, TNFα, IL-10, and IL17. Mean signal intensities per μm were calculated from three types of region of interest (ROI) within the samples: T-cell infiltrated ROIs from patient samples, non-infiltrated reference ROIs from patient samples, and control ROIs from HC samples, based on composite images including CD4, CD8, CD14, and Ecadherin. Boxes represent medians with first and third quartiles (lower and upper hinges). The upper and lower whiskers extend from the hinge to the largest and smallest value, respectively, no further than 1.5* interquartile range. Significance levels correspond to the following p values: $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.005$

difficulty of patient recruitment for conjunctival biopsies. A further limitation is the lack of specific Th2-related cytokines. Nevertheless, we were able to obtain a clear and consistent characterization of the local conjunctival infiltrate. Lastly, the extent of the conjunctival infiltrate was heterogeneous within the 6 patients, which might be explained by the variety in the duration, severity, and location of the conjunctivitis at the moment of sampling. However, our data show that the inflammatory profile of the infiltrates applies to all six patients.

In conclusion, our findings might indicate that dupilumab-associated IL-4/IL-13 signaling inhibition in combination with increased local Th1-related cytokine production can underlie the loss of GCs and their essential immunomodulatory role in the conjunctiva, hence leading to dry eyes, a highly activated multicellular infiltrate, and tissue damage. In the future studies, longitudinal evaluation of conjunctival GC numbers with less invasive techniques such as conjunctival impression cytology and tear IFNγ concentrations could further confirm this.

ACKNOWLEDGMENTS

We would like to thank Matthijs Baars for his help in analyzing the data.

CONFLICTS OF INTEREST

D.S. Bakker reports personal fees from Sanofi/Regeneron, personal fees from Leo Pharma, outside the submitted work. C.M. van Luijk reports personal fees from Sanofi Europe and Santen Europe, outside the submitted work. Dr. de Bruin-Weller reports grants and other from Sanofi-Genzyme, grants and other from Regeneron, grants and other from AbbVie, other from Arena, other from Almirall, grants and other from Eli-Lilly, other from Galderma, other from Janssen, grants and other from Leo Pharma, other from Pfizer, outside the submitted work. Dr. Thijs reports personal fees from Sanofi/Regeneron and Leo Pharma, outside the submitted work. Dr. Vercoulen reports grants from NWO Gravitation 024.001.028 cancergenomicscenter.nl, during the conduct of the study; grants from WKZ fund research grant, grants from TKI-Health Holland grant, grants and non-financial support from TigaTx B.V., grants from Vrienden van het UMC Utrecht, non-financial support from Life Science editors parental

leave grant, outside the submitted work. Dr. van Wijk reports grants from Investigator initiated studies Regeneron and Leo Pharma, outside the submitted work. All other authors have nothing to disclose.

DATA AVAILABILITY STATEMENT

No datasets were generated during this study.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

DOI: 10.1111/all.15070

Real-world evidence of reduced disability costs during the Finnish Allergy Programme 2008–2018

To the Editor,

New insights into immune regulation in modern, urban societies have challenged conventional thinking and motivated implementation of the nationwide, proactive Allergy Programme 2008–2018 in Finland (population ca. 5.5 million).[1] The main aim was to reduce the long-term burden, including costs, of allergic diseases and asthma by improving prevention and care.

Traditional strategy of avoidance was changed to emphasize immunological, psychological and societal resilience. Children and young people were prioritized as were severe clinical manifestations. Implementation was organized and monitored to adapt healthcare for new ideas and improve provision of care.[2] During the 10 years, some 24,000 healthcare professionals took part in 376 educational sessions. An information campaign targeted laypublic and 2.3 million Finns were reached.

Majority of the direct costs could be estimated using national registers. Direct healthcare costs included outpatient visits, hospital days, drugs, rehabilitation, and travel expenses. Drug costs were