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Superficial Conjunctival Cells from Dupilumab-Treated Patients with Atopic Dermatitis with Ocular Adverse Events Display a Transcriptomic Psoriasis Signature

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Dupilumab has demonstrated efficacy in the treatment of atopic dermatitis. However, a subset of patients experiences ocular adverse events (OAEs), including conjunctivitis and dry eye syndrome, the pathological mechanisms of which are still unknown. In a bicentric study, we used DNA microarray analysis to compare the transcriptome of conjunctival cells of patients with atopic dermatitis collected by impression cytology before (M0) and 4 months after (M4) initiating dupilumab treatment. Thirty-six patients were included and divided in 2 groups according to their ophthalmological status at M4: 12 with OAEs (OAE+) and 24 without (OAE-). The analysis revealed 52 differentially expressed genes between OAE+ and OAE- patients at M0 and 113 at M4. Ingenuity Pathway Analysis enrichment revealed a psoriasis signature in OAE+ patients, both before and after OAE outcomes. In addition, we noticed the overexpression of several genes involved in keratinocyte differentiation, particularly encoding cornified envelope components. Among the 16 differentially expressed genes selected for real-time RT-PCR validation, 9 were confirmed as upregulated at M4 in OAE+ versus OAE- patients, validating the psoriasis signature, whereas *MUC7* was downregulated. In conclusion, these results suggest that a conjunctival transcriptomic profile predisposes some patients with atopic dermatitis to developing OAEs upon dupilumab treatment.

Keywords: Atopic dermatitis, Conjunctival impression, Dupilumab, Conjunctivitis, Transcriptomics

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INTRODUCTION

Atopic dermatitis (AD) is a chronic pruritic inflammatory dermatosis that affects approximately 4% of the population, with an increasing prevalence in Western countries (Richard et al, 2018). Its origin is multifactorial, involving environmental and genetic factors as well as immune dysfunctions and skin barrier defects. The skin abnormalities of affected

patients promote the transcutaneous penetration of allergens inducing an exacerbated T helper (Th)2 type immune response. Therefore, Th2 type cytokines such as IL-4 and IL-13 are overexpressed. Other cytokines of the Th1, Th17, and Th22 pathways are also involved in the pathophysiology of AD (Langan et al, 2020). Among other comorbidities, patients with AD have an increased risk of ophthalmological complications compared with the general population (Beck et al, 2019). The main ones are seasonal or perennial allergic conjunctivitis, vernal or atopic keratoconjunctivitis, and keratoconus.

Dupilumab was the first biologic approved for AD treatment. This recombinant human mAb specifically binds to the shared subunit (IL-4R α) of the IL-4 and IL-13 receptors, inhibiting IL-4 and IL-13 signaling. Dupilumab is used to treat adults and children with moderate to severe forms of the disease. Dupilumab is also indicated in patients with asthma, severe nasosinus polyposis in adults, and eosinophilic esophagitis (Hirano et al, 2020). Many clinical trials have shown a significant reduction in pruritus and an improvement in lesion scores and in QOL in patients with treated AD (Wollenberg et al, 2020).

Despite its efficacy, dupilumab is associated with ocular adverse events (OAEs) in up to 26.1% of patients with treated AD, as we and others have reported (Blauvelt et al, 2017;

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Abbreviations: AD, atopic dermatitis; DEG, differentially expressed gene; OAE, ocular adverse event; Th, T helper

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Kychygina et al, 2022; Maudinet et al, 2019; Simpson et al, 2016; Touhouche et al, 2021; Wollenberg et al, 2018). In particular, a meta-analysis including 7578 patients revealed a higher risk (2.44, 95% confidence interval = 1.82–3.26) of developing conjunctivitis in the dupilumab-treated group than in the placebo-treated group (Chen et al, 2021). The average time to onset of these complications is 3 months. It is usually a de novo conjunctivitis rather than exacerbation of a pre-existing one (Costedoat et al, 2023; Maudinet et al, 2019). The severity is usually mild and easily controlled, but 1–3% of patients have to discontinue the dupilumab treatment because of this adverse effect (Akinlade et al, 2019; Touhouche et al, 2021). Surprisingly, the OAEs appear to be limited to patients with AD because they did not occur in clinical trials for the management of asthma, nasal polyposis, and eosinophilic esophagitis (Agache et al, 2020; Akinlade et al, 2019; Boyle et al, 2020; Castro et al, 2018; Hirano et al, 2020; Rabe et al, 2018; Wenzel et al, 2016).

The origin of dupilumab-induced OAEs is not yet known, and no biological markers are available to predict their onset. Their occurrence has been associated with AD severity; disease duration; pre-existing dry eye syndrome, eyelid eczema, or conjunctivitis; high eosinophils and serum IgE levels at baseline; and a history of food allergy (Achten et al, 2022; Akinlade et al, 2019; Thormann et al, 2024; Touhouche et al, 2021; Treister et al, 2018). The hypothesis of a defect in the ocular epithelial barrier, similar to what exists at the epidermal level, has also been proposed (Akinlade et al, 2019; Thormann et al, 2024). A relatively high expression of Th2/Th17 cytokines at baseline and a shift toward Th1/Th17 under

dupilumab were revealed in tear fluids of patients who developed OAEs compared with the fluids of those who did not (Thormann et al, 2024).

In this study, we investigated the molecular mechanisms involved in dupilumab-induced OAEs. A comparative transcriptomic analysis of superficial conjunctival cells of adult patients with AD was performed between baseline (M0) and 4 months after dupilumab administration (M4), regardless of whether they presented with OAEs or not.

RESULTS

Patient characteristics

A total of 36 patients with AD were included in this study: 12 with dupilumab-induced OAEs (OAE+) and 24 without (OAE–), as assessed by ophthalmological examinations at M4 compared with baseline examination. The baseline characteristics of the patients at M0 and the developed OAEs are detailed in Table 1. In particular, in the OAE+ group, 50% of patients had conjunctivitis, and 66.7% had corneal superficial punctate keratitis. At M0 and M4, superficial cells from the patients' conjunctiva were obtained by impression cytology. Immunostaining of the membranes at M4 showed that less than 4% of collected cells (mean values of $2.53 \pm 1.42\%$ for OAE+ patients and $1.40 \pm 0.28\%$ for OAE– patients) were CD45+ immune cells (Supplementary Figure S1a and b).

A psoriasis signature is highly enriched in patients developing OAEs at both M0 and M4

RNA was extracted from the membranes, and a full transcriptome DNA microarray analysis was performed from 19 patients without OAE (OAE–/M0 and OAE–/M4 mRNA

Table 1. Patient Characteristics at Baseline (M0) and at Week 16 (M4)

Characteristic	Without OAE (n = 24)	With OAE (n = 12)	P-Value
Sex (male/female)	15/9	10/2	.201
Age ¹ (y)	42.2 ± 15.6	39.8 ± 18.1	.624
History of familial atopy ²	12 (50%)	8 (67%)	.400
History of food allergy ²	6 (25%)	4 (33%)	.832
History of asthma ²	10 (42%)	5 (42%)	.916
History of allergic rhinitis ²	18 (75%)	8 (67%)	.896
History of atopic kerato-conjunctivitis ²	8 (33%)	5 (42)	.676
Keratoconus at M0 ²	2 (8%)	2 (17%)	.851
Eyelid atopic dermatitis at M0 ²	1 (4%)	4 (33%)	.061
SCORAD ¹ at M0	40.18 ± 15.52	44.66 ± 18.18	.461
at M4	15.67 ± 8.02	18.87 ± 11.24	.744
SCORAD variation ¹ (M4–M0)	-24.51 ± 15.43	-25.79 ± 19.92	.693
Break-up time ¹ at M0 (s)	8.17 ± 3.09	7.13 ± 3.76	.214
Break-up time ¹ at M4 (s)	9.10 ± 3.28	9.36 ± 2.54	.744
Shirmer test value at M0 ¹ (mm)	23.33 ± 10.63	23.36 ± 11.75	.989
Shirmer test value at M4 ¹ (mm)	24.9 ± 11.20	25.17 ± 8.45	.921
SPK at M0 ²	2 (8.3%)	4 (33.3%)	.019
SPK at M4 ²	1.5 (6.25%) ³	8 (66.7%)	<.00001
Oxford grading scale ¹ at M0	0.10 ± 0.37	0.54 ± 0.93	.058
at M4	0.08 ± 0.20	1.33 ± 0.49	<.00001
Conjunctivitis ² at M0	1 (4%)	2 (17%)	.522
at M4	0 (0%)	6 (50%)	.0009

Abbreviations: OAE, ocular adverse event; SCORAD, SCORing Atopic Dermatitis; SPK, superficial punctate keratitis.

Bold indicates statistically significant P values.

¹Mean value.

²n (%).

³One patient showed SPK for only 1 eye.

samples) and 11 patients who developed OAE (OAE+/M0 and OAE+/M4 mRNA samples).

When an unsupervised principal component analysis was performed on the entire transcriptomic dataset (Figure 1a), samples from the same patient generally clustered closely. This suggested that the transcriptomic changes are mild between M0 and M4. We observed a more or less clear segregation of OAE+ versus OAE- patients, particularly considering that 3 patients developed OAEs a few months after M4 (indicated by the arrows in Figure 1a). When the differentially expressed genes (DEGs) (\log_2 fold change ≥ 1 , $P < .05$) were evaluated

between the 4 groups, this segregation weakly appeared by the hierarchical clustering at M4 and M0 (Figure 1b and Supplementary Figure S2, respectively).

A multiple analysis of the different conditions was nevertheless performed. At M4 versus M0, only 2 genes (namely *PMP22* and *NOX1*) were overexpressed in OAE+ patients and, as expected, none in OAE- patients. As shown by a Volcano plot representation, we found 52 DEGs between OAE+ and OAE- patients at M0 (Figure 1c) and 113 at M4 (Figure 1d). A full list of the DEGs is available in Supplementary Table S1. Lists of the top 10 DEGs

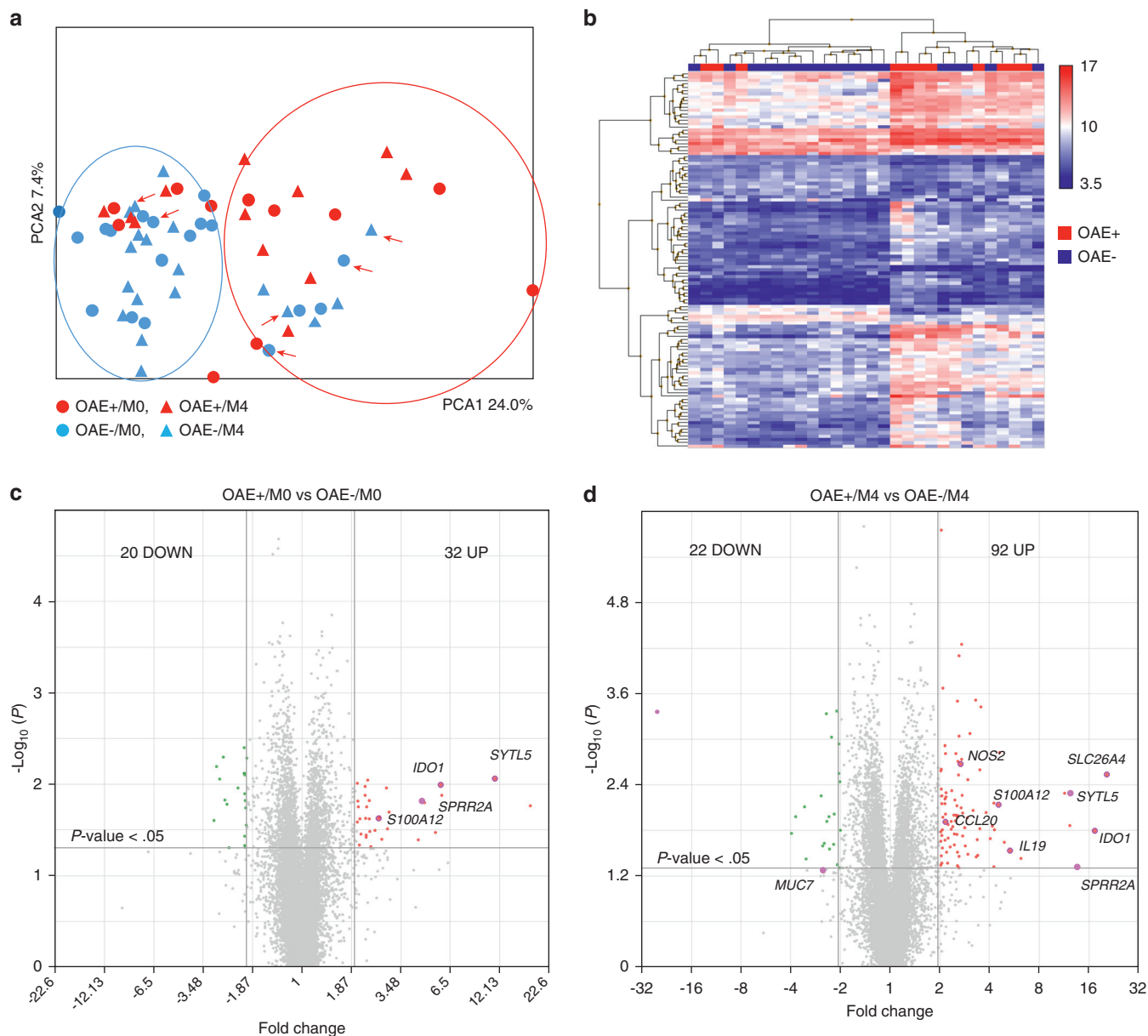


Figure 1. Transcriptomic analysis of superficial conjunctiva samples of patients with AD before dupilumab treatment (M0) and 4 months after start of treatment (M4); some patients with OAEs (OAE+) some without (OAE-). (a) PCA. OAE- samples are indicated in blue, and OAE+ are in red. Samples at M0 are shown by dots, and samples at M4 are shown by triangles. Red arrows indicate patients who displayed OAEs in a 2-month period after M4. (b) Heat map of DEGs between OAE+/M4 and OAE-/M4 samples. Each column corresponds to 1 sample, and each row corresponds to a DEG. DEG expression color system is from dark blue (low signal) to dark red (strong signal). (c, d) Volcano plots showing P -value ($-\log_{10}$) and fold change of all transcripts (c) from OAE+/M0 versus OAE-/M0 and (d) from OAE+/M4 versus OAE-/M4. Overexpressed DEGs are colored in red, whereas underexpressed DEGs are colored in green. Some DEGs of interest are annotated on the graphs. AD, atopic dermatitis; DEG, differentially expressed gene; OAE, ocular adverse event; PCA, principal component analysis.

(upregulated and downregulated) for OAE+/M4 versus OAE-/M4 and for OAE+/M0 versus OAE-/M0 are presented in Tables 2 and 3, respectively. Interestingly, the same 6 genes (*DEFB4A*, *HLA-DQB1*, *IDO1*, *KRT78*, *SPRR2A*, and *SYTL5*) were upregulated in OAE+ patients on both occasions. An effect on goblet cell proliferation and function has been proposed to explain dupilumab-induced OAEs (Bakker et al, 2019; Barnett and Afshari, 2020). When we checked for the expression of goblet cells key markers, we only observed overexpression of the *SPDEF* gene at M4 (fold change = 3.32) in patients with OAE compared with that in patients without, whereas *MUC5AC*, *CLCA1*, *SPINK4*, and *TFF3* genes were not differentially expressed.

To further investigate the molecular mechanisms underlying OAE susceptibility, biological functions were examined using Ingenuity Pathway Analysis enrichment (full list is available in Supplementary Materials), with a focus on diseases (Table 4). The results showed that a psoriasis signature was significantly enriched in OAE+ patients at both M0 and M4 (eg, *ADM*, *IDO1*, *PDZK1IP1*, *S100A12*, *S100P*, etc) as well as a viral infection-related one (*ADM*, *CDKN1A*, *HLA-DPB1*, *HLA-DQB1*, etc) at M0. We noticed the overexpression of several genes involved in epidermal keratinocyte differentiation in OAE+ patients, in particular, genes involved in cornified envelope formation: *SPRR2A*, *SPRR3*, *SPRR1A*, *ABCA12*, *DEFB4A*, *ANXA9*, and *KRT78* at M0 and *SPRR2A*, *ABCA12*, *DEFB4A/4B*, *KRT78*, and keratin 39 gene *K39* at M4.

CCL20 protein is more abundant in OAE+ patients

Fifteen overexpressed genes in OAE+ patients (Supplementary Table S2) were selected for real-time RT-PCR assays, either

among the top 10 upregulated genes or among the genes highlighted by the Ingenuity Pathway Analysis. Given its high relevance in dry eye diseases and its antimicrobial properties, we also assessed the expression of *MUC7* because its decreased expression (0.398-fold) in OAE+ patients at M4 was close to being statistically significant ($P = .0558$).

The overexpression of *NOX1* in OAE+ patients at M4 compared with that at M0 was confirmed. In addition, RT-PCR analysis revealed the upregulation of *CCL20*. At M4, 5 DEGs (*CCL20*, *IL19*, *NOS2*, *S100A12*, and *SLC26A4*) were confirmed to be overexpressed in OAE+ patients compared with those in OAE- patients, and there was a trend ($P < .01$) for a higher expression of 4 other genes (*SYTL5*, *NOX1*, *IDO1*, and *SPRR2A*). These data confirmed the enrichment of the psoriasis signature in patients with OAEs. *MUC7* downregulation was also confirmed (Figure 2a and b). At M0, unfortunately, none of the tested genes were confirmed to be overexpressed in OAE+ patients compared with those in OAE- patients. Slight increases in the expression of *FCER1A*, encoding the IgE high-affinity receptor subunit alpha, and *IDO1* were observed (1.64- and 1.68-fold, respectively) but did not reach statistical significance.

Differential *CCL20* expression was then analyzed at the protein level by ELISA (Figure 2c). To tentatively increase the statistical values of our analysis, because we were mindful of the relatively small size of our cohort, additional patient samples were included in the ELISA analysis in addition to the previous ones: 2 from 1 patient with OAE and 10 from 5 patients without. Between M0 and M4, *CCL20* was increased 3.21-fold in OAE- patients (from 0.019 ± 0.006 to 0.061 ± 0.016 pg/ μ g of total proteins) and 2.96-fold in OAE+ patients (from 0.024 ± 0.014 to 0.071 ± 0.015 pg/ μ g of total

Table 2. Top 10 Genes Upregulated and Downregulated at M4 between Patients with (OAE+) and without (OAE-) OAEs

Gene Symbol	OAE+/M4 ¹	OAE-/M4 ¹	Fold Change	P-Value
<i>SLC26A4</i>	10.42 ± 3.39	6.05 ± 2.68	20.62	.0029
<i>IDO1</i>	13.33 ± 3.09	9.21 ± 2.36	17.37	.0161
<i>SPRR2A</i>	11.74 ± 2.14	7.97 ± 2.52	13.7	.0496
<i>SYTL5</i>	9.68 ± 2.04	6.06 ± 1.85	12.31	.0051
<i>DUOXA2</i>	11.36 ± 2.67	7.74 ± 2.51	12.26	.0137
<i>HLA-DQB1</i>	11.45 ± 2.39	7.93 ± 2.42	11.43	.0051
<i>KRT78</i>	11.64 ± 1.88	9.00 ± 1.51	6.19	.0371
<i>IL19</i>	11.12 ± 2.51	8.71 ± 2.37	5.33	.029
<i>RND1</i>	9.35 ± 1.95	7.05 ± 1.7	4.92	.0227
<i>DEFB4A</i>	7.97 ± 1.5	5.77 ± 1.34	4.61	.0015
<i>SPDYE5</i>	6.7 ± 1.07	7.94 ± 0.8	0.43	.0105
<i>PIWIL4</i>	5.91 ± 0.99	7.18 ± 0.78	0.41	.0121
<i>MLANA</i>	6.63 ± 0.65	7.92 ± 0.73	0.41	.0005
<i>ZFP42</i>	4.93 ± 1.1	6.25 ± 0.91	0.40	.0233
<i>SOX5</i>	8.42 ± 0.93	9.78 ± 1.16	0.39	.0252
<i>SYCP3</i>	6.19 ± 0.87	7.58 ± 0.92	0.38	.0056
<i>LYPD1</i>	7.99 ± 1.82	9.68 ± 1.71	0.31	.0373
<i>KANK4</i>	6.38 ± 1.02	8.1 ± 1.27	0.30	.0077
<i>ATP6V1B1</i>	10.89 ± 1.36	12.79 ± 1.44	0.27	.0104
<i>KCNMA1</i>	8.24 ± 1.68	10.23 ± 1.63	0.25	.0173

Abbreviations: K8, keratin 8; OAE, ocular adverse event.

¹Mean (±SD) expression levels (log₂).

Table 3. Top 10 Genes Upregulated and Downregulated at M0 between Patients with (OAE+) and without (OAE-) OAEs

Gene Symbol	OAE+ /M4 ¹	OAE- /M4 ¹	Fold Change	P-Value
<i>HLA-DQB1</i>	11.83 ± 2.44	7.67 ± 2.6	17.83	.0171
<i>SYTL5</i>	9.09 ± 2.15	5.57 ± 1.83	11.45	.0087
<i>KRT78</i>	11.21 ± 2.21	8.66 ± 1.98	5.83	.0132
<i>IDO1</i>	11.42 ± 2.86	8.9 ± 2.3	5.75	.0102
<i>SPRR3</i>	11.95 ± 2.88	9.52 ± 2.58	5.39	.0337
<i>IRAK3</i>	9.95 ± 1.58	7.73 ± 1.81	4.69	.0159
<i>SPRR2A</i>	10.33 ± 2.97	8.22 ± 2.41	4.33	.0404
<i>APOBEC3A</i>	16.67 ± 1.36	15.09 ± 1.49	2.99	.0202
<i>DEFB4A</i>	6.99 ± 2.1	5.44 ± 1.12	2.93	.0306
<i>MERTK</i>	9.87 ± 1.72	8.33 ± 1.33	2.91	.011
<i>ZBTB10</i>	8.1 ± 1.02	9.16 ± 0.71	0.48	.004
<i>FRMD4B</i>	9.67 ± 1.28	10.73 ± 0.83	0.48	.0076
<i>ST6GAL2</i>	6.18 ± 1.01	7.34 ± 1.25	0.44	.0109
<i>ACSF2</i>	8.65 ± 1.13	9.98 ± 0.86	0.39	.0496
<i>MT1F</i>	9.5 ± 1.64	10.86 ± 1.14	0.39	.0164
<i>STATH</i>	6.68 ± 1.24	8.09 ± 1.29	0.38	.0149
<i>PROM1</i>	9.16 ± 1.72	10.59 ± 1.36	0.37	.005
<i>GNG7</i>	8.37 ± 1.35	9.87 ± 1.13	0.35	.0087
<i>KANK4</i>	7.06 ± 1.19	8.62 ± 1.47	0.34	.0064
<i>KCNMA1</i>	8.8 ± 2.2	10.4 ± 1.34	0.33	.0249

Abbreviations: K78, keratin 78; OAE, ocular adverse event.

¹Mean (±SD) expression levels (log₂).

proteins). *CCL20* tended to increase at M4 in OAE+ compared with that in OAE- patients.

IL-19 protein expression was analyzed by indirect immunofluorescence on a subgroup of patients at M4 and was shown to be slightly increased in OAE+ patients (Supplementary Figure S2c and d).

DISCUSSION

We have previously reported an association between dupilumab treatment and occurrence of OAEs in patients with AD, including conjunctivitis (Costedoat et al, 2023; Touhouche et al, 2021). In this study, we aimed to unravel the biological pathways that might explain these OAEs.

We collected ocular cell samples from the conjunctiva surface of patients before and after 4 months of dupilumab therapy, and we performed a transcriptomic analysis in patients who developed OAEs and in those who did not.

As expected, because no clinical sign changes were observed, no genes were differentially expressed between OAE-/M4 and OAE-/M0 samples.

We expected to see several DEGs between OAE+/M4 and OAE+/M0 considering that ocular inflammation has increased over those 4 months. However, this was not the case: only 2 DEGs, namely, *PMP22* and *NOX1*, were identified. The fact that immune cell infiltrate constitutes only a very small percentage of cells captured by impression cytology, as shown by immunostaining of the membranes, probably explains this result. In contrast, conjunctival smears obtained by wiping with a plastic loop showed massive neutrophil infiltration (Patra et al, 2024). *PMP22* is mainly expressed by Schwann cells of the peripheral nervous system, and there are no data to date concerning its detection at the

ocular surface. *NOX1*, a protein that produces ROS, is involved in numerous inflammatory processes. In 2-dimensional AD and psoriasis models, *NOX1* inhibition has been shown to rescue elevated oxidative stress (Emmert et al, 2020). Its expression has been observed in vitro in corneal fibroblasts, but so far, no study has linked *NOX1* to ocular surface diseases. In addition, an increased expression of *CCL20*, which was not detected using the DNA microarray experiment, was shown at mRNA (4.9-fold) and at protein (2.96-fold) levels. *CCL20* relevance will be discussed below.

Among OAE+ and OAE- patient samples, 52 genes were differentially expressed at M0 and 113 genes at M4. Six genes were found among the top 10 upregulated genes at both time points, confirming their possible involvement in the OAE outcome. Among these, 3 genes encode proteins related to keratinocyte differentiation, namely, keratin 78, *DEFB4A*, and *SPRR2A*. In addition, the *SPRR3* gene, which also encodes an epidermis structural protein, was highly overexpressed at M0.

Ingenuity Pathway Analysis of the DEGs revealed a psoriasis signature. This is consistent with the fact that psoriasis is a possible complication of dupilumab treatment in patients with AD (Jaulent et al, 2021; Kim and Yeung, 2020; Varma and Levitt, 2020). It has already been proposed that the IL-17 pathway is involved in dupilumab-induced OAEs in patients with AD (Bridgewood et al, 2022; Thormann et al, 2024). Notably, this pathway drives psoriasis and musculoskeletal disorders, another type of dupilumab-associated adverse event. Consistent with this hypothesis, we observed an increased expression of *IL19* at M4 in OAE+ patients. Indeed, the expression of IL-19, a proinflammatory cytokine highly expressed in the lesional skin of patients with AD and psoriasis, is increased by IL-17A. In turn, IL-19 stimulates the

Table 4. Ingenuity Pathway Analysis of Differentially Expressed Genes between Patients without and with OAEs At M0 (Gray Boxes) and M4 (Blue Boxes) and Enriched for Diseases

Description	P-Value	Genes in Overlap (n)	Gene Names ¹
Psoriasis	2.37 e ⁻¹⁰	15	<i>ABCA12, ADM, ANXA8/ANXA8L1, APOBEC3A, CLEC7A, DEFB4A/DEFB4B, IDO1, KYNU, MT1L, NR4A2, PDZK1IP1, S100A12, S100P, SPRR1A, SPRR2A</i>
Immune mediated inflammatory diseases	1.28 e ⁻⁷	19	<i>ADA2, ADM, APOBEC3A, CLC, CLEC7A, CYP2S1, DEFB4A/DEFB4B, FCGBP, GNG7, GSDMC, HLA-DPB1, HLA-DQB1, IDO1, KCNMA1, MERTK, NR4A2, PROM1, S100A12, SPRR2A</i>
Infection by coronavirus	7.63 e ⁻⁷	11	<i>ADM, CDKN1A, DYNC2H1, HLA-DPB1, HLA-DQB1, IDO1, MSMB, NR4A2, S100A12, SPRR2A, STATH</i>
Chronic skin disorder	1.17 e ⁻⁵	7	<i>CLEC7A, DEFB4A/B, KYNU, PDZK1IP1, S100A12, SPRR1A, SPRR2A</i>
Allergy	1.68 e ⁻⁵	8	<i>DEFB4A/B, FCGP, HLA-DPB1, HLA-DQB1, KCNMA1, NR4A2, PROM1, SPRR2A</i>
Eosinophilia of esophagus	3.49 e ⁻⁵	4	<i>APOBEC3A, CLC, CLEC7A, KCNMA1</i>
Plaque psoriasis	4.48 e ⁻⁵	5	<i>SPRR2A, S100A12, PDZK1IP1, DEFB4A/B, SPRR1A</i>
Viral infection	5.79 e ⁻⁵	18	<i>ADM, CDKN1A, CLC, DEFB4A/B, DYNC2H1, FRMD4B, H1-2, HLA-DPB1, HLA-DQB1, IDO1, MERTK, MSMB, NR4A2, PRSS27, S100A12, S100P, SPRR2A, STATH</i>
Esophageal lesion	7.88 e ⁻⁵	11	<i>ACSF2, ADA2, APOBEC3A, CDKN1A, CLC, CLEC7A, DYNC2H1, FRMD4B, KCNMA1, RHCG, SPRR3</i>
Immune mediated inflammatory diseases	1.60 e ⁻¹¹	37	<i>ADA2, ADM, AIF1, ARHGAP15, BCL2A1, CCL20, CPVL, CYP2S1, DEFB4A/DEFB4B, DHRS9, DSE, FCER1A, FCER1G, FCGBP, FGL2, FPR3, GSDMC, HLA-DPA1, HLA-DPB1, HLA-DQB1, HLA-DQB2, HLA-DRA, IDO1, IL4R, KCNMA1, KDR, LAPTM5, LCPI, MERTK, NOS2, RDH10, S100A12, SLC26A4, SPRR2A, TMEM51, TRIM31, VSIG4</i>
Psoriasis	2.96 e ⁻⁸	18	<i>ABCA12, ADM, CCL20, DEFB4A/DEFB4B, ELOVL4, EPHX3, ID1, IDO1, IL19, IL4R, KDR, KYNU, NOS2, PDZK1IP1, PI3, S100A12, S100P, SPRR2A</i>
Lichen planus	7.49 e ⁻⁸	8	<i>CCL20, DEFB4A/4B, HLA-DRA, HLA-DPB1, HLA-DQB1, HLA-DPA1, LAPTM5, LCPI</i>
Systemic autoimmune syndrome	2.69 e ⁻⁷	28	<i>ADA2, ADM, AIF1, ARHGAP15, CA1, CCL20, CYP2S1, DHRS9, DSE, FCER1A, FGL2, GSDMC, HLA-DMA, HLA-DPA1, HLA-DPB1, HLA-DQB1, HLA-DQB2, HLA-DRA, IL4R, KDR, KYNU, LCPI, MERTK, RDH10, S100A12, TMEM51, TRIM31, VSIG4</i>
Allergy	1.63 e ⁻⁶	13	<i>CCL20, CPVL, DEFB4A/B, FCER1A, FCER1G, FCGBP, HLA-DPB1, HLA-DQB1, HLA-DRA, IL4R, KCNMA1, NOS2, SPRR2A</i>
Abnormality of skin morphology	4.00 e ⁻⁶	10	<i>ABCA12, CCL20, DEFB4A/DEFB4B, DHRS9, ELOVL4, FPR3, HLA-DPA1, HLA-DPB1, HLA-DQB1, HLA-DRA, IL4R, LAPTM5, LCPI, MLANA, PI3</i>
Chronic skin disorder	9.38 e ⁻⁶	10	<i>CCL20, DEFB4A/DEFB4B, ID1, IL4R, KYNU, NOS2, PDZK1IP1, PI3, S100A12, SPRR2A</i>
Hypersensitive reaction	1.07 e ⁻⁵	11	<i>CCL20, CPVL, DEFB4A/DEFB4B, FCER1A, FCER1G, FCGBP, HLA-DRA, IL4R, NOS2, PI3, SPRR2A</i>

Abbreviations: K78, keratin 78; OAE, ocular adverse event.

¹Genes investigated by real-time RT-PCR are listed in red.

production of Th2 cytokines, suggesting that IL-19 may link Th17 and Th2 pathways (Oka et al, 2017). A recent study showed overexpression of *IL19* in the conjunctiva of patients with Sjögren syndrome keratoconjunctivitis sicca (de Paiva et al, 2021). The increased levels of *S100A12*, *DEFB4A*, and *SPRR1A/2A/3* genes in OAE+ samples at M0 and of *S100A12*, *DEFB4A/4B*, *SPRR2A*, *NOS2/iNOS*, *IL19*, and *CCL20* genes at M4 are also consistent with upregulation of Th17 immunity (Böhner et al, 2023; Haider et al, 2008). *NOS2* is weakly expressed in the normal conjunctival epithelium, and its expression gradually increases with the severity of dry eye symptoms (Cejková et al, 2007). Notably, *NOS2* is also expressed by macrophages at the ocular surface in experimental murine dry eye disease (You et al, 2015). *CCL20* plays an important role in mucosal immune surveillance by orchestrating the trafficking of dendritic cells and T cells expressing the CCR6 receptor, including Th17 lymphocytes (Singh et al, 2008). Inappropriate recruitment of T cells by *CCL20* contributes to various dermatoses, including psoriasis and AD. In psoriasis, *CCL20* is the most upregulated chemokine in active lesions (Kagami et al, 2010). This *CCL20*–CCR6 axis also mediates the migration of Th17

lymphocytes to the ocular surface in dry eye syndrome (Dohlman et al, 2013). In addition, *CCL20* displays antibacterial activity, and its expression is induced by bacteria surface molecules such as lipopolysaccharide (Yang et al, 2003), establishing a link between the psoriasis and antimicrobial signatures. Related to the antimicrobial signature, a unique microbial landscape differentiates patients with AD with dupilumab-induced OAEs, both at baseline and during the conjunctivitis episode (Patra et al, 2024).

Our transcriptomic data showed that *MUC7* mRNA is less expressed in OAE+ than in OAE– patients at M4. *MUC7* is a secreted non–gel-forming mucin that has been studied mainly in the saliva and esophagus, but its expression has been reported in the conjunctival cells (Jumblatt et al, 2003). It has been proposed as a microbe-clustering agent in saliva (Ghosh et al, 2023). Similar to other mucins, *MUC7* may also keep the ocular surface moist and protected from environmental aggressions. It has been described as a factor of poor prognosis in Sjögren syndrome (Fusconi et al, 2021). Whether *MUC7* expression is reduced at the protein level remains to be demonstrated. Because *MUC7* activity is usually modulated by enzymes, including glycosidases (Fusconi

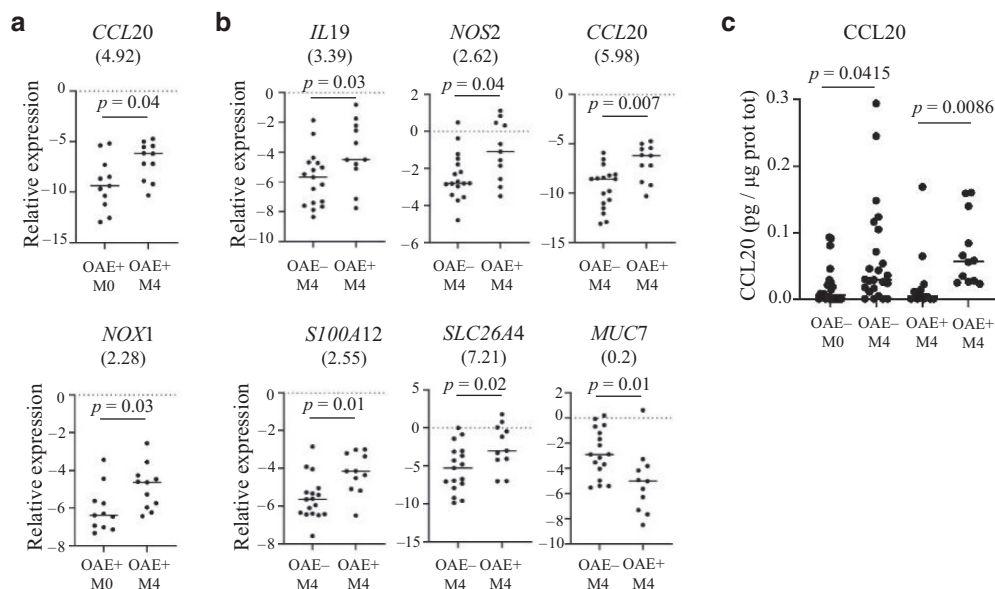


Figure 2. Overexpression of CCL20 in patients with OAEs. (a, b) Whisker box plots showing relative mRNA expression of selected genes in the conjunctival samples analyzed by real-time RT-PCR. Fold-change means are indicated in brackets under the gene names. Nonparametric Mann–Whitney test was used. (a) Patients ($n = 11$) with OAEs at baseline (OAE+/M0) and 4 months after start of treatment (OAE+/M4). (b) At M4, patients with OAEs (OAE+/M4, $n = 11$) and patients without OAEs (OAE-/M4, $n = 19$). (c) CCL20 protein quantification by ELISA in conjunctival samples from OAE- ($n = 24$) and OAE+ ($n = 12$) patients. CCL20 quantity was normalized to the amount of total protein and expressed as pg of CCL20 per μg of total protein. Kruskal–Wallis test with a Dunn's correction for multiple comparisons was used. $P < .05$ was considered statistically significant for all tests. The bars represent the median values. OAE, ocular adverse event.

et al, 2021), its post-translational modifications should also be investigated. Further work will be necessary to evaluate the potential clinical benefit of MUC7 in dupilumab-induced OAEs, in particular, a link between its reduced function and ocular dryness.

The main limitation of our study is the limited number of patients involved. In particular, this is probably the reason why the real-time RT-PCR experiments only partially confirmed the DNA-microarray data. Indeed, of the 16 selected DEGs between OAE+ and OAE- patients, 10 were confirmed at M4 but none at M0. A large replicative cohort and validation by another technical approach, including cell sorting and single-cell RNA sequencing, will be necessary in the future to consolidate our results. A second limitation is the absence of deep conjunctiva cells on the impression membrane. Deeper cells should provide more transcriptomic changes. However, it is not ethically possible to collect deeper cells using the imprinting method because repeated membrane applications would be necessary. Biopsies would be the gold technic, but again, this is not ethically recommended. A similar analysis, including dupilumab-treated patients with asthma, who do not develop OAEs, would also be beneficial.

In conclusion, this study, to our knowledge, investigated the previously unreported transcriptomic differences in the superficial conjunctival cells at the ocular surface of patients with AD developing OAEs under dupilumab therapy. On the basis of the reported data, dupilumab seems to exert negligible effects on superficial conjunctival epithelial cells, suggesting that dupilumab-associated OAEs might be driven by effects on other cell types (such as immune, goblet, or deep conjunctival epithelial cells). Our data also suggest that

patients who develop OAEs may already have a predisposing immunological profile oriented toward the Th17 pathway. Whether a similar pre-existing Th17/psoriasis immunophenotype exists in peripheral blood remains to be investigated. Under dupilumab, this switch is enhanced as shown by an increased expression of the genes encoding CCL20 and other inflammatory mediators such as S100A12, NOS2, and IL-19. In addition, reduced MUC7 may contribute to the ocular inflammation. The identification of patients with AD at risk of developing eye complaints is crucial to preventing adverse outcomes through adapted care. Our study suggests that a transcriptomic analysis from a minimally invasive conjunctiva sample may be of help.

MATERIALS AND METHODS

Study design

We conducted a bicentric clinical study between Toulouse and Nantes Hospitals. It is ancillary to the clinical study in Toulouse from 2017 and the multicentric DUPIOEIL study from 2019 in Toulouse and Nantes. This study was conducted in accordance with the declaration of Helsinki. Written informed consent was obtained from all participants at M0. Ethical approval was not necessary because samples were collected for primary diagnosis and clinical characterization objectives.

Population and groups

All patients who needed to be treated with dupilumab were aged >18 years. Dermatological and ophthalmological examinations were performed on the same day, before treatment introduction. Four months after the beginning of dupilumab treatment (M4), the same ophthalmologist (MC in Toulouse and MW in Nantes) examined the patients again. We excluded patients who could not be

consulted before treatment or at M4. The ophthalmological examinations concerned the ocular surface status. The slit lamp examination with photos provides a stereoscopic view of the eye. The Schirmer test I determines the presence of dry eye; tears are collected, without anesthesia, using a blotting paper placed into the inferior eyelid, and the length of moisturized paper is expressed in millimeter; a normal value corresponds to more than 10 mm in 5 minutes (Gontier and Fish, 1976). The tear film break-up time assesses the stability of tear film; it corresponds to the time in seconds that elapses between the last blink and the appearance of a break of the tear film; a normal value is at least 10 seconds (Sweeney et al, 2013). The Oxford scale is used to estimate surface damage in dry eye; it corresponds to the extent of ocular surface stained after fluorescein instillation and ranges from 0 (absent) to 5 (severe), according to the number of superficial punctate keratitis (Bron et al, 2003). The McMonnies scale graduates conjunctival hyperemia from 0 to 5 (McMonnies and Chapman-Davies, 1987). Occurrence of OAEs was defined as an aggravation of the Oxford scale or development of conjunctivitis at M4 compared with that at M0, according to the McMonnies scale and photos. At M4, we divided the patients in 2 groups: one group who developed OAEs (OAE+) and a control group who did not (OAE–).

Conjunctival cell collection

At M0 and M4 examinations, a conjunctival impression was performed in routine examination to analyze ocular surface. The conjunctival impression was collected by the same practitioner (MC in Toulouse and MW in Nantes) for all patients at M0 and M4. After application of anesthetic drops (oxybuprocain, Théa Pharma), a conjunctival impression device (Eyeprim, Opia technologies SAS) was used to collect cells from the superior temporal quadrant at 2 mm from the limbus. The samples were immediately preserved in liquid nitrogen and then stored at -80°C for later evaluation.

RNA extraction and isolation

Total RNA was directly extracted from the membranes with a Qiagen MicroRNA extraction kit in accordance with the manufacturer's instructions (Qiagen S.A.S.). Reverse transcriptase was performed using the Invitrogen Superscript III VILO kit, in accordance with the manufacturer's recommendations (Thermo Fisher Scientific). RNA quantity and quality were determined using QBIT system (Thermo Fisher Scientific) and Agilent (Agilent) systems. Selected samples had an RNA integrity number above 8 and RNA concentration above 25 ng/ μl .

Microarray analysis

A full transcriptome microarray analysis was performed using the human Clariom S Assay platform (Affymetrix, Thermo Fisher Scientific) at the Genotoul Get Biopuces facility (Toulouse, France). A total of 5 ng per sample was processed. The Transcriptome Analysis Console software (version 4.0) was used to process the results of the microarray (DEG analysis, including the construction of heat maps and determination of signaling pathways). Principal component analysis was performed using Transcriptome Analysis Console software and also Volcano plot and differential expression. Data were then analyzed with the use of QIAGEN Ingenuity Pathway Analysis software (Krämer et al, 2014; <https://digitalinsights.qiagen.com/IPA>). Canonical pathways and disease and biofunction enrichment analysis were generated with the species set to Human and the confidence level set to experimentally observed; ranking was based on statistical significance ($P < .05$).

Quantitative PCR

Real Time PCR was performed on triplicate samples in a Roche LightCycler 480 using Roche supermix for PCR (Roche). Primer sequences are described in [Supplementary Table S2](#). The PCR conditions were 95°C for 10 minutes, followed by 40 cycles of denaturation at 95°C for 15 seconds and annealing at 60°C for 20 seconds. We used 3 genes for normalization by geometric averaging determined by a method previously described (Vandesompele et al, 2002). The relative expression levels of mRNA were determined using the dCT formula, and fold changes were calculated using the $2^{-\Delta\Delta\text{Ct}}$ ratio according to the previously described method (Vandesompele et al, 2002). All PCR products were checked by sequencing, and the primer efficiencies were examined using the Roche software analysis program.

Protein analysis

CCL20 quantification by ELISA was performed following the manufacturer's instructions (Bio-Techne SAS). Protein extraction was performed by agitating samples on ice for 30 minutes in a solution of 350 mM sodium chloride, 0.5% NP40 in 50 mM Tris-hydrogen chloride, pH 7.4, with a cocktail of protease inhibitors (Complete mini, Roche Diagnostics). Protein assay was performed with the BCA kit (Sigma-Aldrich) for data normalization. The absorbance was determined instantly using an ASYS 340 microplate reader (Biochrom) at wavelengths of 450 and 540 nm. The concentration of each chemokine was calculated by subtracting the absorbance at 540 nm from the absorbance at 450 nm before normalizing against blanks. All assays were performed in technical duplicates that were averaged. A standard curve was created with a quadratic polynomial fitting curve in Microsoft Excel for personal computer, version 2013. The standard curve was considered as best fitted if the r^2 value of the fitting line was >0.98 .

Statistical analysis

Means were used for continuous variables, and numbers or percentages were used for qualitative variables. Comparisons of means and unpaired data were performed using Student *t*-test, and comparisons of percentages were performed using chi-square analysis or chi-square corrected if needed. *P*-values under .05 were considered significant. Analyses were performed using the R foundation for statistical computing software. For transcriptomic analysis, DEGs were validated when an increase was >2 -fold or a decrease was <2 -fold, with a $P \leq .05$. GraphPad Prism (9.4.1) was used to perform the following tests: nonparametric Mann–Whitney test for RT-PCR data and Kruskal–Wallis test for CCL20 protein data. $P < .05$ was considered statistically significant for both tests.

DATA AVAILABILITY STATEMENT

All the data are available upon reasonable request. Raw data are available in the Mendeley Data nonprofit repository (<https://data.mendeley.com/>), with the accession number <https://doi.org/10.17632/222mnygnjz.1> and the following link: <https://data.mendeley.com/datasets/222mnygnjz/1>.

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CONFLICT OF INTEREST

The authors state no conflict of interest.

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AUTHOR CONTRIBUTIONS

Conceptualization: MC, SG, PF, MT, CP, MS; Data Curation: MT, MC, CP, PF, MW, BV, SG; Funding Acquisition, SG, MC, MS; Investigation: MT, MC, CP, PF, MW, BV, SG; Resources: MT, MC, CP, PF, MW, BV, SG; Validation: SG, GK, EC, MS, MC; Writing – Original Draft Preparation: SG, MS; Writing – Review and Editing: MS, MT, SG, GK, CP, MC, PF, BV, MW, SB, EC

Disclaimer

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SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at www.jidonline.org, and at <https://doi.org/10.1016/j.jid.2024.08.024>.

REFERENCES

- Achten RE, Van Luijk C, Van der Rijst L, Bakker D, Spekhorst L, Zuithoff N, et al. Identification of risk factors for dupilumab-associated ocular surface disease in patients with atopic dermatitis. *Acta Derm Venereol* 2022;102:adv00666.
- Agache I, Rocha C, Beltran J, Song Y, Posso M, Solà I, et al. Efficacy and safety of treatment with Biologics (Benralizumab, dupilumab and omalizumab) for severe allergic asthma: A systematic review for the EAACI Guidelines - recommendations on the use of Biologics in severe asthma. *Allergy* 2020;75:1043–57.
- Akinlade B, Guttman-Yassky E, de Bruin-Weller M, Simpson EL, Blauvelt A, Cork MJ, et al. Conjunctivitis in dupilumab clinical trials. *Br J Dermatol* 2019;181:459–73.
- Bakker DS, Ariens LFM, van Luijk C, van der Schaft J, Thijs JL, Schuttelaar MLA, et al. Goblet cell scarcity and conjunctival inflammation during treatment with dupilumab in patients with atopic dermatitis. *Br J Dermatol* 2019;180:1248–9.
- Barnett BP, Afshari NA. Dupilumab-associated mucin deficiency (DAMD) [published correction appears in *Transl Vis Sci Technol* 2020;9:21]. *Transl Vis Sci Technol* 2020;9:29.
- Beck KM, Seitzman GD, Yang EJ, Sanchez IM, Liao W. Ocular co-morbidities of atopic dermatitis. Part I: Associated ocular diseases. *Am J Clin Dermatol* 2019;20:797–805.
- Blauvelt A, de Bruin-Weller M, Gooderham M, Cather JC, Weisman J, Pariser D, et al. Long-term management of moderate-to-severe atopic dermatitis with dupilumab and concomitant topical corticosteroids (Liberty AD Chronos): a 1-year, randomised, double-blinded, placebo-controlled, phase 3 trial. *Lancet* 2017;389:2287–303.
- Böhner A, Jargosch M, Müller NS, Garzorz-Stark N, Pilz C, Lauffer F, et al. The neglected twin: nummular eczema is a variant of atopic dermatitis with codominant TH2/TH17 immune response. *J Allergy Clin Immunol* 2023;152:408–19.
- Boyle JV, Lam K, Han JK. Dupilumab in the treatment of chronic rhinosinusitis with nasal polyposis. *Immunotherapy* 2020;12:111–21.
- Bridgwood C, Wittmann M, Macleod T, Watad A, Newton D, Bhan K, et al. T helper 2 IL-4/IL-13 dual blockade with dupilumab is linked to some emergent T helper 17-type diseases, including seronegative arthritis and enthesitis/enthesopathy, but not to humoral autoimmune diseases. *J Invest Dermatol* 2022;142:2660–7.
- Bron AJ, Evans VE, Smith JA. Grading of corneal and conjunctival staining in the context of other dry eye tests. *Cornea* 2003;22:640–50.
- Castro M, Coren J, Pavord ID, Maspero J, Wenzel S, Rabe KF, et al. Dupilumab efficacy and safety in moderate-to-severe uncontrolled asthma. *N Engl J Med* 2018;378:2486–96.
- Cejková J, Ardan T, Simonová Z, Cejka C, Malec J, Jirsová K, et al. Nitric oxide synthase induction and cytotoxic nitrogen-related oxidant formation in conjunctival epithelium of dry eye (Sjogren's syndrome). *Nitric Oxide* 2007;17:10–7.
- Chen X, Liu M, Wu S, Huang Z, Li X, Lai X, et al. Treatment-emergent adverse events in dupilumab-treated patients with allergic diseases: a meta-analysis. *Allergy* 2021;76:593–6.
- Costedoat I, Wallaert M, Gaultier A, Vasseur R, Vanhaecke C, Viguier M, et al. Multicenter prospective observational study of dupilumab-induced ocular events in atopic dermatitis patients. *J Eur Acad Dermatol Venereol* 2023;37:1056–63.
- de Paiva CS, Trujillo-Vargas CM, Schaefer L, Yu Z, Britton RA, Pflugfelder SC. Differentially expressed gene pathways in the conjunctiva of Sjogren syndrome keratoconjunctivitis sicca. *Front Immunol* 2021;12:702755.
- Dohman TH, Chauhan SK, Kodati S, Hua J, Chen Y, Omoto M, et al. The CCR6/CCL20 axis mediates Th17 cell migration to the ocular surface in dry eye disease. *Invest Ophthalmol Vis Sci* 2013;54:4081–91.
- Emmert H, Fonfara M, Rodriguez E, Weidinger S. NADPH oxidase inhibition rescues keratinocytes from elevated oxidative stress in a 2D atopic dermatitis and psoriasis model. *Exp Dermatol* 2020;29:749–58.
- Fusconi M, Candelori F, Weiss L, Riccio A, Priori R, Businaro R, et al. Qualitative mucin disorders in patients with primary Sjogren's syndrome: a literature review. *Med Oral Patol Oral Cir Bucal* 2021;26:e71–7.
- Ghosh S, Ahearn CP, Isabella CR, Marando VM, Dodge GJ, Bartlett H, et al. Human oral lectin ZG16B acts as a cell wall polysaccharide probe to decode host-microbe interactions with oral commensals. *Proc Natl Acad Sci U S A* 2023;120:e2216304120.
- Gontier J, Fisch U. Schirmer's test: its normal values and clinical significance. *ORL J Otorhinolaryngol Relat Spec* 1976;38:1–10.
- Haider AS, Lowes MA, Suárez-Fariñas M, Zaba LC, Cardinale I, Khatcherian A, et al. Identification of cellular pathways of "type 1," Th17 T cells, and TNF- and inducible nitric oxide synthase-producing dendritic cells in autoimmune inflammation through pharmacogenomic study of cyclosporine A in psoriasis. *J Immunol* 2008;180:1913–20.
- Hirano I, Dellon ES, Hamilton JD, Collins MH, Peterson K, Chehade M, et al. Efficacy of dupilumab in a Phase 2 randomized trial of adults with active eosinophilic esophagitis. *Gastroenterology* 2020;158:111–22.e10.
- Jaulent L, Staumont-Sallé D, Tauber M, Paul C, Aubert H, Marchetti A, et al. De novo psoriasis in atopic dermatitis patients treated with dupilumab: a retrospective cohort. *J Eur Acad Dermatol Venereol* 2021;35:e296–7.
- Jumblatt MM, McKenzie RW, Steele PS, Emberts CG, Jumblatt JE. MUC7 expression in the human lacrimal gland and conjunctiva. *Cornea* 2003;22:41–5.
- Kagami S, Rizzo HL, Lee JJ, Koguchi Y, Blauvelt A. Circulating Th17, Th22, and Th1 cells are increased in psoriasis. *J Invest Dermatol* 2010;130:1373–83.
- Kim HS, Yeung J. Psoriasis appearing after dupilumab therapy in atopic dermatitis: a case report. *SAGE Open Med Case Rep* 2020;8:2050313X20940458.
- Krämer A, Green J, Pollard J Jr, Tugendreich S. Causal analysis approaches in Ingenuity Pathway Analysis. *Bioinformatics* 2014;30:523–30.
- Kychygina A, Cassagne M, Tauber M, Galiacy S, Paul C, Fournié P, et al. Dupilumab-associated adverse events during treatment of allergic diseases. *Clin Rev Allergy Immunol* 2022;62:519–33.
- Langan SM, Irvine AD, Weidinger S. Atopic dermatitis [published correction appears in *Lancet* 2020;396:758]. *Lancet* 2020;396:345–60.
- Maudinet A, Law-Koune S, Duret C, Lasek A, Modiano P, Tran THC. Ocular surface diseases induced by dupilumab in severe atopic dermatitis. *Ophthalmol Ther* 2019;8:485–90.
- McMonnies CW, Chapman-Davies A. Assessment of conjunctival hyperemia in contact lens wearers. Part I. *Am J Optom Physiol Opt* 1987;64:246–50.
- Oka T, Sugaya M, Takahashi N, Nakajima R, Otobe S, Kabasawa M, et al. Increased interleukin-19 expression in cutaneous T-cell lymphoma and atopic dermatitis. *Acta Derm Venereol* 2017;97:1172–7.
- Patra V, Woltsche N, Cerpas U, Bokanovic D, Repelnig M, Joshi A, et al. Persistent neutrophil infiltration and unique ocular surface microbiome typify dupilumab-associated conjunctivitis in patients with atopic dermatitis. *Ophthalmol Sci* 2024;4:100340.
- Rabe KF, Nair P, Brusselle G, Maspero JF, Castro M, Sher L, et al. Efficacy and safety of dupilumab in Glucocorticoid-Dependent Severe Asthma. *N Engl J Med* 2018;378:2475–85.

- Richard MA, Corgibet F, Beylot-Barry M, Barbaud A, Bodemer C, Chaussade V, et al. Sex- and age-adjusted prevalence estimates of five chronic inflammatory skin diseases in France: results of the « OBJECTIFS PEAU » study. *J Eur Acad Dermatol Venereol* 2018;32:1967–71.
- Simpson EL, Bieber T, Guttman-Yassky E, Beck LA, Blauvelt A, Cork MJ, et al. Two Phase 3 Trials of Dupilumab versus Placebo in Atopic Dermatitis. *N Engl J Med* 2016;375:2335–48.
- Singh SP, Zhang HH, Foley JF, Hedrick MN, Farber JM. Human T cells that are able to produce IL-17 express the chemokine receptor CCR6. *J Immunol* 2008;180:214–21.
- Sweeney DF, Millar TJ, Raju SR. Tear film stability: a review. *Exp Eye Res* 2013;117:28–38.
- Thormann K, Lüthi AS, Deniau F, Heider A, Cazzaniga S, Radonjic-Hoesli S, et al. Dupilumab-associated ocular surface disease is characterized by a shift from Th2/Th17 toward Th1/Th17 inflammation. *Allergy* 2024;79:937–48.
- Touhouche AT, Cassagne M, Bérard E, Giordano-Labadie F, Didier A, Fournié P, et al. Incidence and risk factors for dupilumab associated ocular adverse events: a real-life prospective study. *J Eur Acad Dermatol Venereol* 2021;35:172–9.
- Treister AD, Kraff-Cooper C, Lio PA. Risk factors for dupilumab-associated conjunctivitis in patients with atopic dermatitis. *JAMA Dermatol* 2018;154:1208–11.
- Vandesompele J, De Preter K, Pattyn F, Poppe B, Van Roy N, De Paepe A, et al. Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. *Genome Biol* 2002;3:RESEARCH0034.
- Varma A, Levitt J. Dupilumab-induced phenotype switching from atopic dermatitis to psoriasis. *JAAD Case Rep* 2020;6:217–8.
- Wenzel S, Castro M, Corren J, Maspero J, Wang L, Zhang B, et al. Dupilumab efficacy and safety in adults with uncontrolled persistent asthma despite use of medium-to-high-dose inhaled corticosteroids plus a long-acting β_2 agonist: a randomised double-blind placebo-controlled pivotal phase 2b dose-ranging trial. *Lancet* 2016;388:31–44.
- Wollenberg A, Ariens L, Thurau S, van Luijk C, Seegräber M, de Bruin-Weller M. Conjunctivitis occurring in atopic dermatitis patients treated with dupilumab-clinical characteristics and treatment. *J Allergy Clin Immunol Pract* 2018;6:1778–17780.e1.
- Wollenberg A, Beck LA, Blauvelt A, Simpson EL, Chen Z, Chen Q, et al. Laboratory safety of dupilumab in moderate-to-severe atopic dermatitis: results from three phase III trials (Liberty AD SOLO 1, Liberty AD SOLO 2, Liberty AD Chronos). *Br J Dermatol* 2020;182:1120–35.
- Yang D, Chen Q, Hoover DM, Staley P, Tucker KD, Lubkowski J, et al. Many chemokines including CCL20/MIP-3alpha display antimicrobial activity. *J Leukoc Biol* 2003;74:448–55.
- You IC, Coursey TG, Bian F, Barbosa FL, de Paiva CS, Pflugfelder SC. Macrophage phenotype in the ocular surface of experimental murine dry eye disease. *Arch Immunol Ther Exp (Warsz)* 2015;63:299–304.



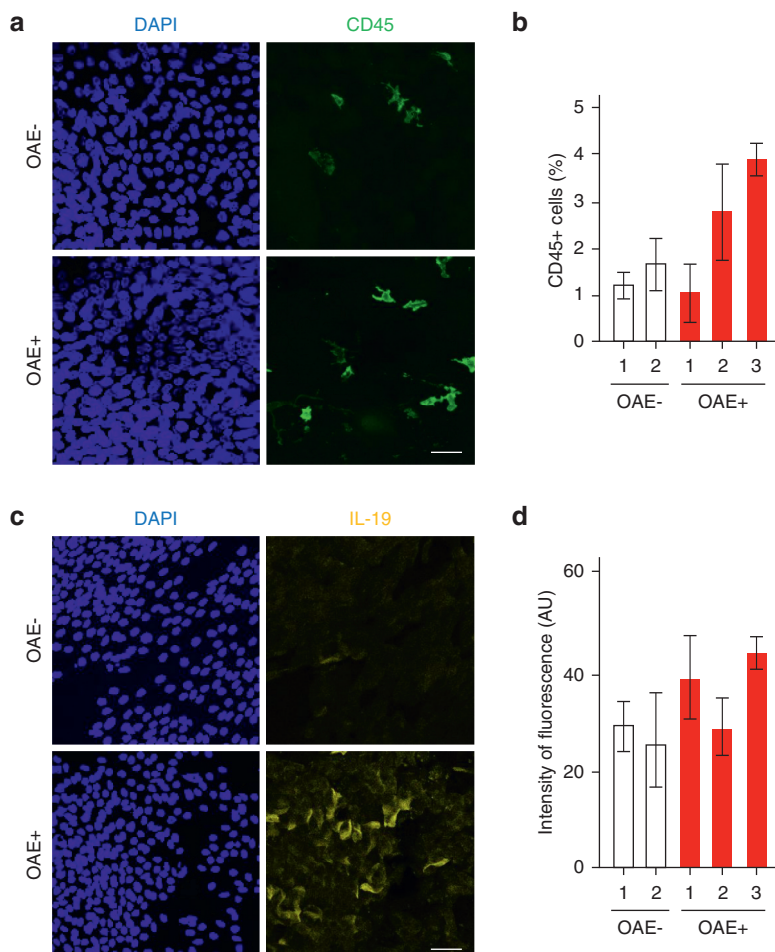
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SUPPLEMENTARY MATERIALS AND METHODS

Indirect immunofluorescence

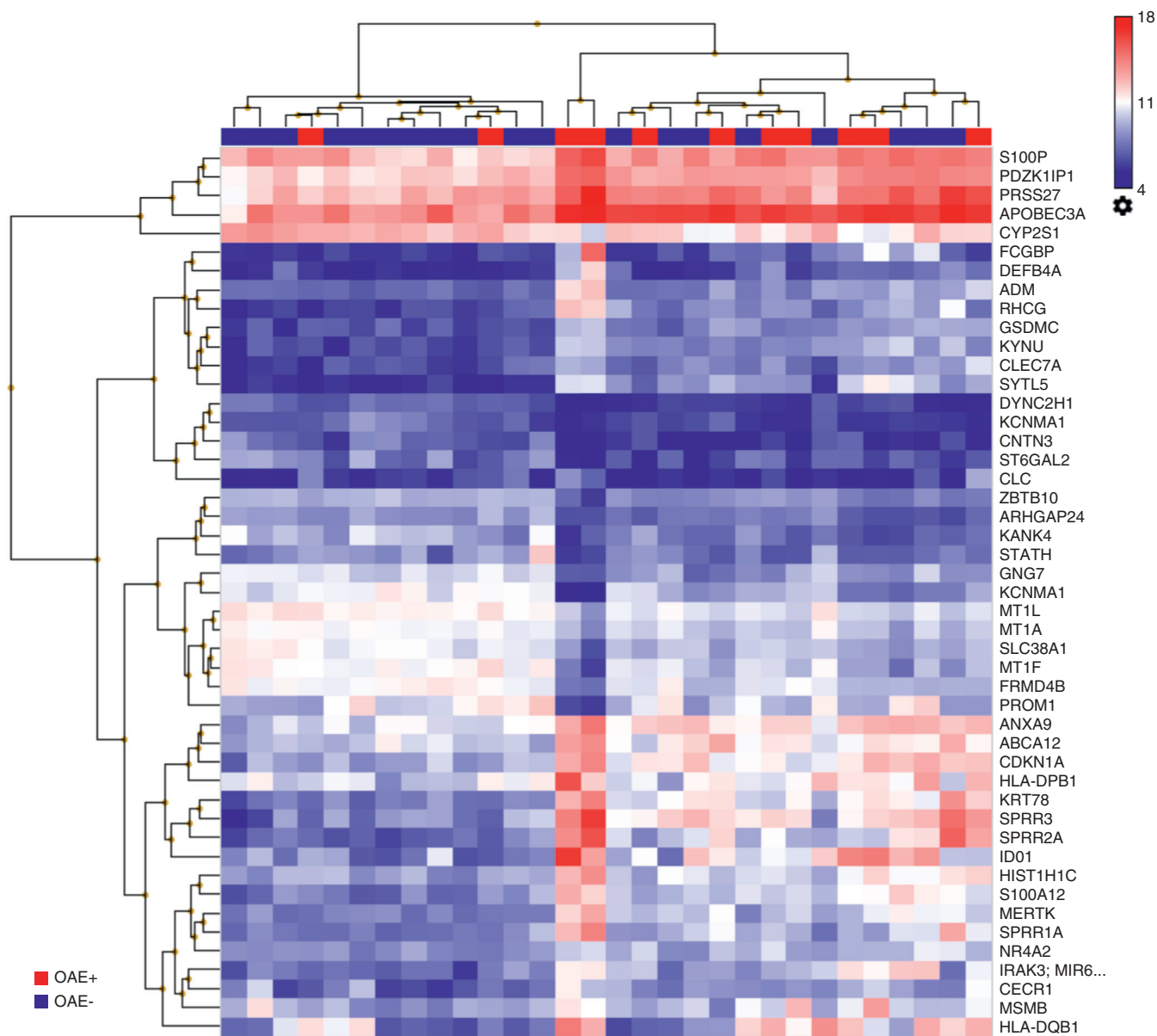
Cells were fixed on the impression membranes with 4% paraformaldehyde solution in PBS for 10 minutes. Then, they were permeabilized, and nonspecific sites were blocked with a PBS solution containing 0.5% BSA and 0.3% Triton for 30 minutes. Rat anti-human IL-19 (Mab2915, Bio-Techne) was diluted at 0.5 $\mu\text{g}/\text{ml}$ in PBS containing 0.5% BSA and 0.1%

saponin and incubated overnight at 4 °C. The secondary antibody was incubated at room temperature for 1 hour. Then, Alexa 488-coupled mouse anti-human CD45 (clone H130, number 304019, BioLegend) diluted in the same solution at 0.2 $\mu\text{g}/\text{ml}$ was incubated for 2 hours at room temperature. Finally, nuclei were labeled with DAPI. After mounting in Mowiol 4-88 solution, samples were observed with a SP8 Leica confocal microscope.



Supplementary Figure S1. Less than 4% of the cells captured on the impression cytology membranes were immune cells.

Impression membranes, 4 months after dupilumab treatment beginning, from 3 patients with OAEs (OAE+ patient 1 to OAE+ patient 3) and 2 patients without (OAE- patient 1 and OAE- patient 2) were analyzed by indirect immunofluorescence and confocal microscopy with either a CD45-specific antibody (green) or an anti-IL-19 antibody (yellow). Nuclei were stained with DAPI (blue). **(a, c)** Representative images. Bar = 25 μm . **(b)** CD45+ cells were quantified and expressed as a percentage of total cells counted (between 3172 and 5261). **(d)** IL-19 immunodetection intensity was quantified and expressed in AU, arbitrary unit; OAE, ocular adverse event.



Supplementary Figure S2. Transcriptomic analysis of superficial conjunctiva samples of patients with AD before dupilumab treatment (M0), some patients with OAEs (OAE+), and some without (OAE-) 4 months after start of treatment. Heat map of DEGs between OAE+/M0 and OAE-/M0 samples. Each column corresponds to 1 sample, and each row corresponds to a DEG. DEG expression color system is from dark blue (low signal) to dark red (strong signal). AD, atopic dermatitis; DEG, differentially expressed gene; OAE, ocular adverse event.

Supplementary Table S1. Full List of the DEGs ($0.5 \geq \text{Fold Change} \geq 2$, $P < .05$)

Gene Symbol	Fold Change	P-Value	FDR	OAE+ /M4 (Avg)	OAE+ /M0 (Avg)
OAE+ /M4 vs OAE+ /M0					
PMP22	2.24	.0373	0.9996	7.58	6.42
NOX1	2.04	.0363	0.9996	8.57	7.54
				OAE+ /M0 (Avg)	OAE- /M0 (Avg)
OAE+ /M0 vs OAE- /M0					
HLA-DQB1	17.83	.0171	0.4717	11.83	7.67
SYTL5	11.45	.0087	0.4574	9.09	5.57
K78	5.83	.0132	0.4605	11.21	8.66
IDO1	5.75	.0102	0.4574	11.42	8.9
SPRR3	5.39	.0337	0.4954	11.95	9.52
IRAK3	4.69	.0159	0.4717	9.95	7.73
SPRR2A	4.33	.0404	0.4968	10.33	8.22
APOBEC3A	2.99	.0202	0.4736	16.67	15.09
DEFB4A	2.93	.0306	0.4954	6.99	5.44
MERTK	2.91	.011	0.4574	9.87	8.33
CDKN1A	2.74	.0399	0.4965	11.46	10.01
FCGBP	2.66	.0226	0.4824	7.3	5.89
S100A12	2.63	.0234	0.4824	10.13	8.73
RHCG	2.51	.0317	0.4954	8.16	6.83
ADGRF1	2.39	.0482	0.5053	14.73	13.48
KYNU	2.36	.0328	0.4954	8.26	7.02
GSDMC	2.34	.0149	0.4657	8.27	7.04
PRSS27	2.34	.0239	0.4834	14.71	13.48
ADM	2.29	.009	0.4574	8.54	7.35
ANXA9	2.26	.0175	0.4722	12.26	11.09
CLEC7A	2.25	.0358	0.4954	8.15	6.98
SPRR1A	2.24	.0379	0.4957	9.41	8.24
ABCA12	2.24	.0236	0.4824	11.53	10.36
S100P	2.21	.0131	0.4604	14.4	13.26
HIST1H1C	2.21	.0105	0.4574	10.65	9.51
MSMB	2.11	.0347	0.4954	10.45	9.38
NR4A2	2.07	.0238	0.4834	9.48	8.43
CDRT1	2.06	.0462	0.5025	10.67	9.63
CECR1	2.04	.0175	0.4722	8.76	7.73
HLA-DPB1	2.03	.0152	0.4694	11.43	10.41
PDZK1IP1	2.01	.0259	0.4841	13.71	12.71
CLC	2.01	.0097	0.4574	6.3	5.29
CYP2S1	0.49	.0051	0.4455	11.7	12.71
MT1A	0.49	.0281	0.4932	9.72	10.73
CNTN3	0.49	.0181	0.4722	5.53	6.56
KCNMA1	0.49	.0151	0.4682	5.51	6.54
ANXA8L1	0.49	.0488	0.5055	10.54	11.58
DYNC2H1	0.49	.0075	0.4574	5.66	6.7
SLC38A1	0.49	.0079	0.4574	9.32	10.36
MT1L	0.49	.0126	0.4578	10.17	11.22
ARHGAP24	0.48	.037	0.4954	7.16	8.21
ATP6V1B1	0.48	.047	0.5038	11.5	12.55
ZBTB10	0.48	.004	0.4419	8.1	9.16
FRMD4B	0.48	.0076	0.4574	9.67	10.73
ST6GAL2	0.44	.0109	0.4574	6.18	7.34
ACSF2	0.40	.0496	0.5071	8.65	9.98
MT1F	0.39	.0164	0.4717	9.5	10.86
STATH	0.38	.0149	0.4653	6.68	8.09

(continued)

Supplementary Table S1. Continued

				OAE+ /M0 (Avg)	OAE- /M0 (Avg)
PROM1	0.37	.005	0.4455	9.16	10.59
GNG7	0.35	.0087	0.4574	8.37	9.87
KANK4	0.34	.0064	0.4574	7.06	8.62
KCNMA1	0.33	.0249	0.4834	8.8	10.4
				OAE+ /M4 (Avg)	OAE- /M0 (Avg)
OAE+/M4 versus OAE-/M4					
SLC26A4	20.62	.0029	0.18	10.42	6.05
IDO1	17.37	.0161	0.2563	13.33	9.21
SPRR2A	13.7	.0496	0.3731	11.74	7.97
SYTL5	12.31	.0051	0.1945	9.68	6.06
DUOXA2	12.26	.0137	0.2468	11.36	7.74
HLA-DQB1	11.43	.0051	0.1945	11.45	7.93
K78	6.19	.0371	0.3362	11.64	9
IL19	5.33	.029	0.3111	11.12	8.71
RND1	4.92	.0227	0.2874	9.35	7.05
DEFB4A	4.61	.0015	0.1549	7.97	5.77
S100A12	4.53	.0072	0.2062	11.03	8.85
KDR	4.28	.0154	0.2527	8.69	6.6
STEAP1	4.25	.0479	0.3679	9.45	7.36
KYNU	4.25	.0069	0.2062	9.21	7.12
RHCG	4.15	.014	0.2473	8.96	6.91
IRAK3	4.05	.0205	0.2822	10.17	8.16
TRIM31	3.81	.0092	0.2177	10.27	8.34
FPR3	3.54	.0004	0.1205	8.18	6.35
DEFB4B	3.51	.0025	0.1737	7.53	5.72
WBSCR17	3.51	.0149	0.2514	8.27	6.46
CRYM	3.4	.0336	0.3246	15.15	13.39
MERTK	3.38	.0208	0.2839	10.54	8.78
SPDEF	3.32	.0355	0.3314	8.52	6.79
AIF1	3.29	.0003	0.1205	10.04	8.33
BCL2A1	3.19	.0087	0.2126	8.25	6.58
HLA-DPB1	3.05	.0008	0.1405	11.94	10.33
SRPX2	2.97	.0326	0.3219	9.68	8.11
ADM	2.97	.0034	0.1861	9.1	7.53
PI3	2.94	.0108	0.2265	11.58	10.03
TNFAIP2	2.85	.0252	0.2973	12.28	10.76
IRAK2	2.83	.0146	0.2495	10.42	8.92
DHRS9	2.76	.0188	0.2713	13.11	11.65
EMP3	2.75	.0094	0.219	11.62	10.16
ZC3H12A	2.73	.0054	0.1948	12.08	10.63
GLIPR1	2.72	.0000557	0.0775	6.61	5.17
CECR1	2.71	.0018	0.1635	8.99	7.55
GDA	2.7	.008	0.2099	6.22	4.79
NOS2	2.67	.0021	0.1665	10.74	9.32
SECTM1	2.62	.0347	0.3291	12.26	10.86
ARSF	2.62	.0009	0.1405	7.48	6.09
GSDMC	2.61	.0491	0.3708	8.55	7.17
HLA-DQB2	2.61	.0000797	0.0949	9.6	8.22
BCAP29	2.6	.0029	0.18	11.31	9.93
FCER1G	2.58	.0019	0.165	8.25	6.88
SRGN	2.57	.01	0.2242	12.09	10.73
NTN4	2.57	.0107	0.2254	9.81	8.45
HIST1H1C	2.56	.0304	0.3131	10.89	9.53
VSIG4	2.56	.0003	0.1205	7.22	5.86
ANGPTL2	2.55	.0464	0.3631	9.84	8.49

(continued)

Supplementary Table S1. Continued

				OAE+ /M4 (Avg)	OAE- /M0 (Avg)
IL4R	2.55	.018	0.2675	10.71	9.36
RDH10	2.53	.0075	0.2064	12.14	10.8
PLEKHF1	2.51	.01	0.2242	10.75	9.42
LAPTM5	2.5	.0015	0.1549	7.74	6.42
FCCGBP	2.48	.0118	0.2346	6.57	5.26
FCCGBP	2.47	.0063	0.2048	7.29	5.98
HLA-DPA1	2.46	.0175	0.2655	11.49	10.2
GALNT14	2.44	.0245	0.2951	9.96	8.67
ABCA12	2.43	.01	0.2242	11.98	10.7
HUNK	2.4	.0142	0.2479	7.04	5.77
FGL2	2.37	.0047	0.1924	9.56	8.32
LRMP	2.35	.0152	0.2524	8.35	7.11
ID1	2.34	.0121	0.2352	11.57	10.34
TMOD1	2.31	.0262	0.3004	8.48	7.27
FCER1A	2.25	.0199	0.2796	7.37	6.2
S100P	2.25	.0131	0.2424	14.59	13.43
SMPDL3A	2.23	.0425	0.3527	7.8	6.65
EPHX3	2.2	.0306	0.3131	13.14	12.0
PMP22	2.19	0.0057	0.1964	7.58	6.45
K39	2.18	.005	0.1931	7.57	6.45
OLR1	2.17	.0097	0.2219	8.09	6.98
CCL20	2.16	.0122	0.236	6.09	4.98
SLC45A4	2.16	.0015	0.1549	11.01	9.89
ARHGAP15	2.15	.0061	0.2033	6.13	5.03
LAIR1	2.15	.0012	0.146	6.4	5.29
HLA-DRA	2.15	.007	0.2062	12.78	11.67
GCNT3	2.15	.0213	0.2842	13.91	12.8
NOX1	2.1	.0106	0.2254	8.57	7.5
CPVL	2.09	.0002	0.1075	5.78	4.72
OLFML3	2.07	.0269	0.3024	6.36	5.31
HLA-DMA	2.07	.0154	0.2527	13.23	12.18
TMEM51	2.07	.007	0.2062	12.07	11.02
FMO3	2.06	.0055	0.1958	7.88	6.84
HIST1H2BM	2.06	.0424	0.3525	7.04	6.0
ISYNA1	2.05	.0183	0.2691	12.81	11.77
MS4A4A	2.05	.0000174	0.0186	5.72	4.68
PRC1	2.04	.0344	0.3281	9.39	8.36
DSE	2.04	.0025	0.1726	10.38	9.35
LCP1	2.04	.0045	0.1899	8.72	7.69
PDZK1IP1	2.03	.0166	0.26	13.98	12.96
GMFG	2.03	.0026	0.1752	9.74	8.72
GRAMD1B	2.03	.0221	0.2863	10.23	9.21
FER1L6	2.02	.0457	0.3608	10.35	9.33
CA1	0.5	.0157	0.2542	6.77	7.77
ZNF391	0.49	.0036	0.1861	5.88	6.89
ELOVL4	0.49	.0491	0.3708	5.25	6.28
XRCC5	0.49	.0011	0.146	7.4	8.43
SPDYE6	0.49	.0028	0.1799	7.23	8.26
ST6GAL2	0.48	.045	0.3588	6.36	7.42
FAM45A	0.47	.0004	0.1205	6.86	7.93
CYP2S1	0.46	.0096	0.2219	11.59	12.72
KHDRBS3	0.45	.0243	0.2937	6.95	8.08
AUTS2	0.44	.0009	0.1405	9.87	11.05
FRMD4B	0.43	.0278	0.3049	9.7	10.91
SPDYE5	0.43	.0105	0.2253	6.7	7.94
PIWIL4	0.41	.0121	0.2353	5.91	7.18
MLANA	0.41	.0005	0.121	6.63	7.92
ZFP42	0.40	.0233	0.2898	4.93	6.25

(continued)

Supplementary Table S1. Continued

				OAE+ /M4 (Avg)	OAE- /M0 (Avg)
<i>SOX5</i>	0.39	.0252	0.2974	8.42	9.78
<i>SYCP3</i>	0.38	.0056	0.1958	6.19	7.58
<i>LYPD1</i>	0.31	.0373	0.3364	7.99	9.68
<i>KANK4</i>	0.30	.0077	0.2087	6.38	8.1
<i>ATP6V1B1</i>	0.27	.0104	0.2251	10.89	12.79
<i>KCNMA1</i>	0.25	.0173	0.2645	8.24	10.23
<i>MUC7</i>	0.40	.0558	0.3826	5.3	6.62

Abbreviations: Avg, average dCT value; DEG, differentially expressed gene; K78, keratin 78; OAE, ocular adverse event.

Shown is a DNA array transcriptomic analysis of superficial conjunctiva samples of patients with atopic dermatitis before (M0) and 4 month after (M4) dupilumab treatment, some patients with OAEs (OAE+) and some without (OAE-).

Supplementary Table S2. List of Primers Used in qPCR

Gene Name	Forward (5'–3')	Reverse (5'–3')
<i>NOX1</i>	TTGTTTGTTAGGGCTGAATGT	GCCAATGTTGACCCAAGGATTTT
<i>FCER1A</i>	GTTCTTCGCTCCAGATGGC	TTGTGGAACCATTTGGTGGAA
<i>SPRR2D</i>	TTCAGGATTCATCAGGAGCATGAG	CAGGCAGGCCACAGGTTAAGGAG
<i>SPRR1A</i>	TGGCCACTGGATACTGAACA	CCCAAATCCATCCTCAAATG
<i>SPRR2A</i>	TATTTGGCTCACCTCGTTCC	CCAGGACTTCCTTTGCTCAG
<i>SPRR3</i>	TTCCACAACCTGGAAACACA	TTCAGGGACCTTGGTGTAGC
<i>KRT39</i>	CTCGCTTTTGTCCGAAGCC	GTTGATGCCTTCACCATACCAG
<i>SYTL5</i>	ATGATCCTGGGCGTCCTAAAG	TCCCACTTCTACGTTTTGCTTC
<i>SLC26A4</i>	GCTCCCAAATACCGAGTCAA	CACCACTGGAAAAGGTCCAAC
<i>S100A12</i>	AGCATCTGGAGGGAATTGTCA	GCAATGGCTACCAGGGATATGAA
<i>NOS2</i>	TTCAGTATCACAACTCAGCAAG	TGGACCTGCAAGTTAAAATCCC
<i>IL19</i>	ATCCAAGCTAAGGACACCTTCC	GTCACGCAGCACACATCTAAG
<i>MUC7</i>	CACCAGAAGCCGTTATTAGAA	GGGTTGACCACACTGCTATTT
<i>MLANA</i>	GCTCACTTCTATGTTACCC	GACTCCCAGGATCACTGTCAG
<i>IDO1</i>	GCCAGCTTCGAGAAAGAGTTG	ATCCCAGAAGTAGACGTGCAA
<i>CCL20</i>	TGCTGTACCAAGAGTTTGCTC	CGCACACAGACAACTTTTTCTTT
<i>TBP¹</i>	CCACTCACAGACTCTACAAC	CTGCGGTACAATCCCAGAAGT
<i>B2M¹</i>	GAGGCTATCCAGCGTACTCCA	CGGCAGGCATACTCATCTTTT
<i>COX15¹</i>	AGTCTGGCCTCTCGATGGTAG	ACCCACATTCGGTGTGAGTA

¹Genes used for the normalization.