Background: Severe atopic dermatitis (AD) has a high unmet need for effective and safe therapeutics. In early-phase trials, dupilumab, a fully human mAb targeting IL-4 receptor α, markedly improved disease activity, but the effect of IL-4/IL-13 blockade on AD at the molecular level has not been characterized.

Objectives: We sought to evaluate dupilumab modulation of the AD molecular signature.

Methods: We performed transcriptomic analyses of pretreatment and posttreatment skin biopsy specimens from patients with moderate-to-severe AD treated weekly with 150 or 300 mg of dupilumab or placebo.

Results: Exacerbation of the AD transcriptome was observed in placebo-treated patients. Dupilumab improved the AD signature in a dose-dependent manner. Expression of genes upregulated in AD lesions decreased in patients treated with dupilumab by 26% (95% CI, 21% to 32%) and 65% (95% CI, 60% to 71%) for treatment with 150 and 300 mg, respectively. Genes downregulated in AD lesions increased by 21% (95% CI, 16% to 27%) and 32% (95% CI, 26% to 37%) with dupilumab (150 and 300 mg, respectively). The molecular changes paralleled improvements in clinical scores. A dupilumab treatment signature of 821 probes (>2-fold change, P < .05) significantly modulated in the 300-mg dupilumab group at week 4 compared with baseline was identified in this sample set. Significant (P < .05) decreases in mRNA expression of genes related to hyperplasia (K16 and MKI67), T cells, and dendritic cells (CD1b and CD1c) and potent inhibition of TLR2-associated chemokines (CCL17, CCL18, CCL22, and CCL26) were noted without significant modulation of TLR1-associated genes (IFNG).

Conclusions: This is the first report showing rapid improvement of the AD molecular signature with targeted anti–IL-4 receptor α therapy. These data suggest that IL-4 and IL-13 drive a complex, TH2-centered inflammatory axis in patients with AD.

Key words: Atopic dermatitis, dupilumab, IL-4 receptor α inhibition, transcriptome, gene expression, skin, T1h2 axis

Atopic dermatitis (AD), the most common inflammatory skin disorder, has a prevalence of 10% in adults (in the United States) and up to 25% among children (worldwide); approximately 20% of patients have moderate-to-severe disease. Although AD imposes a substantial burden on patients and the health care system, the physical and psychological effects are often underestimated. These effects might be reflected by a marked increase in suicidal ideation in patients with AD.

Despite the increasing worldwide incidence of AD, treatments are limited, with only 3 approved (depending on country) systemic therapeutic options for patients with severe disease who are inadequate responders to topical agents: oral corticosteroids, oral cyclosporin A (CsA), and UVA1/narrow-band UVB (NB-UVB) phototherapy. These therapies are not uniformly effective, and their use is limited by toxicity (corticosteroids and CsA) or inconvenience (NB-UVB). Thus there is an unmet need for a safe and effective systemic treatment for this subset of patients.

It is thought that AD is fundamentally a disease of barrier dysfunction. However, active AD lesions are always accompanied by underlying immune activation. Skin lesions have been best characterized in chronic AD skin and defined as lesions persisting for more than 3 days. Features usually present in chronic lesions include increased infiltration by T cells, dendritic cells (DCs), and eosinophils; increased production of cytokines and chemokines; and reactive epidermal hyperplasia, in which
epidermal differentiation products (ie, filaggrin and loricrin) are highly suppressed.12-14 Although AD has been classified as a Th2-dominated disease, other T-cell subsets (Th22, Th17, and Th1 cells) might also contribute to pathogenesis.9-11,15

On the basis of the hypothesis that IL-4 and IL-13 are key drivers of clinical disease and that IL-4 receptor α (IL-4Rα) is a requisite receptor for signaling from both cytokines, we tested whether blocking IL-4Rα could modify molecular mechanisms of AD pathogenesis in the skin. Dupilumab, a fully human mAb to IL-4Rα that inhibits both IL-4 and IL-13 signaling, is being tested as a potential therapy for AD, asthma, and nasal polyps. We recently reported positive results in early-phase trials in both patients with AD16 and a Th22-enriched subpopulation of asthmatic patients.17 In this report we relate the efficacy observed in phase 1 studies to molecular changes in the skin.18 This is the first study to evaluate the relationship between the molecular effects of a targeted Th2 antagonist and AD pathomechanisms.

METHODS

Study subjects and skin samples

Pretreatment and posttreatment lesional skin biopsy specimens (LSs) and nonlesional skin biopsy specimens (NLs; ≥1 cm from any active lesion) were obtained from 18 adult patients with moderate-to-severe chronic AD (Table I) who participated in 2 phase 1 studies and provided additional consent for the biopsy specimens. Both studies were multicenter, randomized, double-blind, placebo-controlled trials of weekly subcutaneous injections of 150 or 300 mg of dupilumab or placebo for 4 weeks (baseline and weeks 1-3) under institutional review board–approved protocols (NCT01259323 and NCT01259324).

 både's Table I

<table>
<thead>
<tr>
<th>Study subject and skin samples</th>
<th>Treatment</th>
<th>Treatment arm</th>
<th>Baseline LSs</th>
<th>NLs</th>
<th>Pretreatment</th>
<th>Posttreatment</th>
<th>EASI-50</th>
<th>Phadiatop test</th>
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<tr>
<td></td>
<td>placebo</td>
<td></td>
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<td></td>
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<tr>
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<td>dupilumab</td>
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Results indicated sensitization to at least 1 allergen (Table I).

RESULTS

As recently reported, EASI scores improved significantly in adults treated with dupilumab compared with scores in those receiving placebo.16 The mean percentage change in EASI scores in the biopsy substudy (n = 18) was consistent with that of the parent studies (n = 67; Fig 1, A, and Table I). The pretreatment serum IgE level was greater than 150 kU/L in 14 of 18 substudy patients, and the majority of patients had positive Phadiatop test results, indicating sensitization to at least 1 allergen (Table I). EASI-50 data for each treatment arm are also listed in Table I and show that EASI-50, which represents at least 50% improvement in EASI score relative to baseline, was achieved by all but 1 patient in the substudy treated with 300 mg of dupilumab versus none in the placebo group.

Improvement of the AD transcriptome

After 4 weeks of treatment, significant dose-dependent changes from baseline in the previously defined AD transcriptome (differentially expressed genes between lesional and nonlesional AD skin) were detected in LSs by using microarrays in the dupilumab group compared with the placebo arm of the study.22 Dose-dependent changes of −26% (SEM, 2.17%) and −65% (SEM, 3.45%) in upregulated genes were observed in the patients treated with 150 and 300 mg of dupilumab, respectively, with accompanying changes of +32% (SEM, 2.44%) and +21% (SEM, 1.69%) in downregulated genes (Fig 1, B, and see Fig E1 and Table E2 in this article’s Online Repository at www.jacionline.org).

Expression profiling was performed to evaluate the effects of IL-4Rα blockade on LSs and NLs from patients with AD. RNA was extracted, followed by quantitative RT-PCR (qRT-PCR) and Affymetrix Human U133Plus 2.0 arrays (Affymetrix, Santa Clara, Calif) analyses, as previously described.18 qRT-PCR was used to assess the expression of key AD-related genes and microarray findings (primers and probes are listed in Table E1 in this article’s Online Repository at www.jacionline.org).
<table>
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<th>Characteristic</th>
<th>Biopsy substudy</th>
<th>Combined parent trials</th>
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<tr>
<td></td>
<td>Placebo (n = 4)</td>
<td>Dupilumab, 150 mg (n = 7)</td>
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<tr>
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<td></td>
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<tr>
<td>Age (y), mean (SD)</td>
<td>40.8 (26.1)</td>
<td>38.0 (10.4)</td>
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<tr>
<td>Male sex, no. (%)</td>
<td>3 (75)</td>
<td>5 (71)</td>
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<tr>
<td>Baseline EASI score, mean (SD)</td>
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<td>Percentage change in EASI score, week 4, mean (SD)</td>
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<td>EASI-50 responders at week 4 (%)</td>
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<tr>
<td>Baseline serum total IgE (kU/L), geometric mean (95% CI)</td>
<td>2,833 (269.9-29,736.1)</td>
<td>1,090.3 (226.3-5,252.8)</td>
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<tr>
<td>Percentage change in IgE level at week 4, mean (SD)</td>
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<td>-7 (24)</td>
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<tr>
<td>Phadiatop result positive at baseline, no. (%)</td>
<td>4 (100)</td>
<td>6 (86)%</td>
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*Week 4 data were only available for 2 of 4 patients in the placebo group.†Baseline Phadiatop data were only available from 6 of 7 patients.

**FIG 1.** Clinical and molecular changes with dupilumab. **A,** Clinical responses (percentage change in EASI score) in the substudy were similar to parent trials. Bar plots represent means ± SEMs. **B,** Dose-dependent changes in the AD transcriptome with dupilumab (150/300 mg) versus placebo. Bar plots represent means ± SEMs. **C,** Transcriptomic differences between lesional and nonlesional skin showed dose-dependent decreases with dupilumab. Red indicates upregulated and blue indicates downregulated genes in the AD transcriptome (lesional vs nonlesional). Week 4 NLs were not available for the placebo group. Bar plots represent means ± SDs. P values of changes from baseline or between groups are listed.
Changes were of greater magnitude than concurrent decreases in EASI scores (Fig 1, A, and Table 1). For example, there was a 74% difference between the 300-mg dupilumab group compared with the placebo arm (152% improvement in the 300-mg group vs an overall exacerbation of 21.5% in the placebo group). In contrast, only a 45.3% difference between the 300-mg dupilumab and placebo arms was observed for the percentage change in EASI scores (Fig 1, A and B). The molecular differences between lesional and nonlesional skin before treatment were minimized in a dose-dependent manner, which was most evident in the 300-mg dupilumab group (Fig 1, C).

**Differential gene expression with treatment at week 4**

Additional differences in gene expression not limited to the previously defined AD transcriptome between week 4 and baseline were determined in LSs of patients treated with dupilumab and placebo (Fig 2, A, and see Table E3 in this article’s Online Repository at www.jacionline.org). As shown in the heat map in Fig 2, A, dose-dependent changes with treatment were most evident in the 300-mg dupilumab group. Strikingly, the LS transcriptome after treatment with 300 mg of dupilumab resembles that of nonlesional skin (Fig 2, A). At week 4, 821 probes (473 upregulated and 348 downregulated) were significantly modulated with 300 mg of dupilumab versus only 275 probes with placebo (105 and 160 upregulated and downregulated, respectively; >2-fold change, P < .05; see Table E3). Markers of epidermal proliferation (MKI67), keratin 16 (K16), keratin 6B (K6B), and multiple inflammatory mediators were among the large set of genes downregulated by dupilumab (Fig 2, A, and see Table E3). Among the top genes upregulated with dupilumab were those with structural (eg, MATN4), lipid metabolism (eg, PLIN4, ADIPOQ, and PLIN1), and barrier-related (eg, CLDN8, ELN, and CLDN11) functions (see Table E3). Expression of some of these genes decreased in the placebo group (eg, PLIN4, CLDN11, and ADIPOQ). Among the most significantly downregulated immune genes were inflammatory markers (MMP12 and S100A12), T-cell markers (eg, ITK and ICOS), T-reg and eosinophil-attracting chemokines (eg, CCL13 and CCL26), DC antigens (ITGAX/CD11c, CD1b, and CD83), and keratinocyte-associated mediators (IL6, IL8, and IL7R; Figs 2 and 3 and see Tables E3 and E4 in this article’s Online Repository at www.jacionline.org). By using Gene Set Enrichment Analysis to evaluate changes in an immune gene-subset, a prominent molecular signal of inflammation was seen in lesional skin at baseline (Fig 2, B, and see Table E4). Dose-dependent decreases were observed.
with dupilumab, with lesional skin showing a more nonlesional immune phenotype with 300 mg of dupilumab. No significant changes in this immune subset were seen with placebo (Fig 2, B).

Dose-dependent mRNA suppressions of immune and epidermal responses measured by using qRT-PCR

To measure modulation of various immune axes activated in lesional skin, we performed qRT-PCR for a wide array of mRNAs shown in Figs 3 and 4 (red indicates upregulation and blue indicates downregulation). With 300 mg of dupilumab, there was strong and significant modulation of Th2-associated chemokines (CCL13, CCL17, CCL18, and CCL26) and some epidermal products, particularly the proliferation marker K16 and elafin (PI3), whereas increased immune activation was observed with placebo (Figs 3 and 4 and see Fig E2 in this article’s Online Repository at www.jacionline.org). No significant changes with treatment were observed in mRNAs of major Th2 cytokines (IL4, IL13, IL5, and IL31; see Fig E3 in this article’s Online Repository at www.jacionline.org).

Although one might hypothesize that inhibiting the Th2 pathway would upregulate Th1 activity, increases in Th1/IFN-γ-related gene expression were not observed with dupilumab treatment. In fact, small decreases in expression of these genes (IFNG, OASL, MX1, and CXCL10) were detected by using arrays and qRT-PCR for the treatment arms, whereas expression of some of these genes increased with placebo (Figs 3 and 4 and see Tables E2 and E3).

IL17A and IL22 mRNAs were not significantly reduced at week 4 with dupilumab. However, significant suppression of IL-17/IL-22–modulated genes (ie, CXCL1, CXCL2, PI3, IL-23p19/IL-23A, and S100 genes) was observed with dupilumab treatment compared with placebo. CCL20 expression significantly increased with placebo. Of the IL-17/IL-22–modulated genes, PI3 and S100A12 showed the greatest suppression with 300 mg of dupilumab (Figs 3 and 4 and see Fig E3).

The barrier defect in patients with AD is characterized by hyperplasia (as measured by the proliferation marker K16), induction of S100As, and suppression of terminal differentiation (ie, FLG and LOR). Four weeks of 300 mg of dupilumab resulted in significant suppression of K16 (−10.7-fold change, P < .001, Fig 3). We also observed significant decreases in expression of S100A genes (ie, S100A12 and S100A8) and a modest trend of increases in terminal differentiation proteins (Figs 3 and 4). Dose-dependent increases in terminal differentiation genes were observed with dupilumab after adjusting for changes in hyperplasia (as measured by K16 mRNA changes, see Fig E4 in this article’s Online Repository at www.jacionline.org).

When assessing dupilumab’s effects on gene signatures for epidermal differentiation, cytokine-treated keratinocytes, and various immune axes, dose-dependent responses from baseline were observed, with concurrent exacerbations with placebo (Fig 5 and see Table E5 in this article’s Online Repository at www.jacionline.org). Substantial suppression of the Th17 signature was observed in the 300-mg dupilumab group (with a large exacerbation in the placebo group).

Correlation with improvements in disease activity

We evaluated the association of the markers measured by using qRT-PCR with clinical improvement by determining the Pearson correlations of the change with treatment of each variable measured in lesional skin with EASI score improvements (data not shown). Reductions in CCL26 and CCL13 expression had the highest correlation with improvement in percentage change in the EASI score (CCL26: r = 0.8, P = .005; CCL13: r = 0.55, P = .1). In patients who achieved 50% or greater improvement in EASI scores, K16 showed the highest correlation with clinical improvement (r = 0.98, P = .01; data not shown).

DISCUSSION

This is the first evaluation of a targeted immune antagonist in patients with AD that correlates mechanistic changes with clinical measures of disease. Previous studies with broad
immune suppressants (ie, CsA, efalizumab, and alefacept) demonstrated clinical improvements (using EASI or SCORAD scores) but most lacked evaluations of molecular markers. We recently established a relationship between activation of cytokine pathways and epidermal alterations in skin and clinical disease activity in a CsA study. However, studies with targeted immune antagonists are needed to understand the contribution of specific cytokine pathways to the epidermal pathology in patients with AD.

In this study statistically significant, dose-dependent improvements of the AD transcriptome were observed in patients treated with 4 weeks of dupilumab compared with placebo.

Dupilumab suppressed mRNA expressions of genes related to activation of T cells, DCs, eosinophils, inflammatory pathways,
and TH2-inducing chemokines in skin lesions, with increases or insignificant decreases observed with placebo. Although we did not detect significant reductions in IL-17A or IL-22 levels, large reductions in expression of IL-17–related genes, such as elafin (PI3), IL23p19/IL23A, and S100A8, and trends for suppression (CXCL1 and S100A7) were found with 300 mg of dupilumab. Furthermore, with placebo, there was an overall exacerbation of the inflammatory AD lesional phenotype, with large increases in expression of TH17-associated genes, particularly CCL20. These results are consistent with recent findings from flaky tail mice suggesting IL-4 signaling might be regulated, at least in part, by the IL-17 pathway (IL-17A deficiency attenuated TH2 inflammation in this model).28

The treatment was associated with a dose-response reversal of the epidermal lesional phenotype. In particular, major suppressions of hyperplasia-related genes (eg, K16) and reductions in expression of S100A genes were evident with 300 mg of dupilumab by using microarrays and qRT-PCR. The reduction in K16 expression was of greater magnitude than that observed in patients with similar disease severity treated for 12 weeks with 5 mg/kg CsA.24 We also measured changes in other critical epidermal alterations in AD skin, including significant increases in claudin and lipid product levels with 300 mg of dupilumab, whereas decreases were observed in the placebo group. A trend of dose-dependent increases in expression of differentiation genes (LOR and FLG) was observed after adjusting for suppression of epidermal hyperplasia, as demonstrated by K16 reductions with dupilumab. Increases in LOR and FLG expression were also seen with placebo, but these can be attributed to the increased epidermal hyperplasia in the placebo arm and were not evident after adjusting for K16 expression. These data suggest that IL-4/IL-13 blockade might restore abnormal lipid and differentiation alterations in the skin of patients with AD.29

No significant differences were found in either clinical or tissue (molecular) responses to treatment between patients with normal or increased serum total IgE levels.16 These results suggest the hypothesized model of barrier inhibition through activation of TH2 cytokines could be operative in most patients with AD, regardless of IgE levels, and that targeting TH2 inflammation might be an efficacious treatment for patients with either intrinsic or extrinsic AD.18,30

These data are consistent with the suggested roles of IL-4 and IL-13 in patients with AD and the proposal that dual antagonism of these cytokines with a single agent has the potential to potentially inhibit TH2 effector responses. The IL-4 and IL-13 cytokines have been suggested to exert several effects in AD, including activation and survival of TH2 T cells, induction of differentiation and activation of myeloid and atopic DCs, activation of B cells, stimulation of IgE class-switching, and eosinophil recruitment.31-34 IL-4 and IL-13 also suppress lipid production and keratinocyte differentiation, ultimately disrupting the epidermal barrier, with possible feedback pathways that could induce epidermal hyperplasia.32,35-37

These data support AD as an immune-driven disease and establish IL-4 and IL-13 as pathogenic cytokines in patients with AD, driving complex TH2-centered inflammation that regulates epidermal responses. The data also provide further evidence that IL-4 is a key regulator of TH2 activity in AD skin. Because IL-4Rα is expressed on many inflammatory cells involved in AD (eg, keratinocytes, T cells, DCs, and eosinophils),13,37 blocking signaling through this receptor might exert a range of anti-inflammatory effects beyond the known TH2 axis. Modulating IL-4/IL-13 signaling through IL-4Rα inhibition might be an effective method for treating existing skin lesions and for

![FIG 5. Dose-dependent genomic changes were observed by using microarrays in previously defined immune pathways and epidermal gene subsets with dupilumab. Transcriptomic improvement refers to transition toward a noninflammatory molecular phenotype. Values represent means ± 95% CIs. Numbers in the bottom row represent the number of probes representing AD genes in each pathway. Detailed gene lists are provided in Table E5.](image-url)
limiting disease exacerbations. These results suggest that inhibition of a single target has the potential to reverse AD pathomechanisms, opening the door to a new era of targeted treatment for this common and debilitating inflammatory skin disease. Dupilumab trials to evaluate longer-term disease suppression, including cellular and molecular analyses of changes in epidermal pathology and immune abnormalities, are underway. These larger studies of longer treatment duration will not only clarify the effect of dupilumab on lesional skin but might also provide greater insight as to whether nonlesional skin is normalized. These studies might also be used to compare the long-term dupilumab data with studies using conventional treatments (ie, CsA and NB-UVB), as well as the respective molecular remnants across conventional and specific therapeutic agents.

Molecular effects of dupilumab on the AD transcriptome in larger studies should also consider recognized AD subtypes based on IgE level, ethnicity, and other variables.

We thank all patients who participated in the study; as well as Linda Williams, Usman Chaudhry, Scott J. Mellis, Steven P. Weinstein, Melissa Hager, E. Jay Bienen, Chaim “Josh” Cantor, Haobo Ren, Robert Phillips, Warren Brooks.

Clinical implications: Targeted therapeutics might open the door to a new treatment paradigm for patients with AD.

REFERENCES