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Dust mite induces multiple polar T-cell axes in human skin

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Abbreviations

ACD: allergic contact dermatitis

AD: atopic dermatitis

APT: atopy patch test

DEG: differentially expressed gene

DNCB: dinitrochlorobenzene

DPCP: diphenylcyclopropanone

DTH: delayed type hypersensitivity

FCH: fold change

FDR: false discovery rate

GCRMA: GC Robust multi-array average

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hARP: human acidic ribosomal protein

HDM: house dust mite

IDEC: inflammatory dendritic epidermal cell

IFN: interferon

IgE-RAST: IgE radioallergosorbent test

IHC: immunohistochemistry

LC: Langerhans cell

MAD-3: meta-analysis derived transcriptome of psoriasis

mDC: myeloid dendritic cell

PAR-2: protease activated receptor-2

PBMC: peripheral blood monocyte

PCA: principal component analysis

POSTN: periostin

RPTN: repetin

RT-PCR: real-time polymerase chain reaction

TEWL: transepidermal water loss

TSLP: thymic stromal lymphopoietin

ABSTRACT

Background: House dust mite/HDM atopy patch test/APT elicits positive reactions in a high fraction of atopic dermatitis/AD and healthy individuals. Experimental systems for new-onset/chronic AD are needed to support rapid therapeutic development, particularly since animal models representing human AD are lacking. While HDM APT has been considered to simulate AD, its suitability to model AD's emerging Th2/Th22 phenotype with Th1 and Th17 components is unknown.

Objective: To assess whether HDM APT reproduces AD

Methods: Positive HDM APTs (n=15) from patients with and without AD were evaluated, using genomic and immunohistochemistry studies, against intrapersonal control skin.

Results: APT lesions showed higher T-cell and dendritic cell infiltrates vs. controls. 743 up- and 326 downregulated genes were differentially expressed in HDM APT (fold-change>2 and false-discovery rate<0.05), with increased expression of Th2, Th9, Th17/Th22 polar cytokines (i.e. IL-5, IL-13, IL-9, IL-17, IL-22).

Conclusion: While HDM caused significant Th2 skewing, it also illustrated differences in Th2 induction and barrier defects, thus HDM APT does not fully simulate AD. Given its widespread availability and sensitization rates, HDM may potentially be a useful tool that represents select aspects of AD, psoriasis, or contact dermatitis.

INTRODUCTION

Atopic dermatitis (AD) is characterized by immune and barrier abnormalities. It is primarily Th2/Th22-skewed, with Th1 and Th17 contributions.¹ AD nonlesional skin already shows alterations, with further increases in acute, and chronic lesions.^{2,3} Although several mouse models (NC/Nga, flaky-tail, *Flg*-mutated, Oxa-challenged) exhibit selected immune and/or barrier features of human AD, a single model accurately representing the global disease phenotype, including progression from nonlesional to chronic lesions, is unavailable.^{4,5} Thus, experimental systems for new-onset and chronic AD are needed. Furthermore, an IL-23-injected mouse previously reported to best represent psoriasis, another polar skin disease, seems to better mimic AD, emphasizing the lack of optimal experimental models for human inflammatory skin diseases.^{6,7}

Atopy patch testing/APT involves epicutaneous application of aeroallergens that elicit delayed-type hypersensitivity/DTH reactions.⁸ *D.pteronyssius/farinae*, are ubiquitous organisms sensitizing up to 40% of the population and up to 70% of AD patients, and their antigens are the most commonly used in patch-tests.⁹⁻¹¹

The historic understanding of AD as a biphasic disease with Th2-skewing in acute and Th2-to-Th1 switch in chronic-phase was largely based on HDM studies prior to discovery of newer Th17/Th22 subsets.¹²⁻¹⁴ In these studies, HDM lesions at 24h after APT showed increased Th2/IL-4 markers compared to controls, while Th1/IFN- γ markers were elevated after 48h. HDM-specific T-cell clones from skin and blood of AD patients also showed a dichotomous pattern, with increases in IL-4-secreting/Th2 and IFN- γ -secreting/Th1 T-cells.¹⁵ HDM APT induced large infiltrates of eosinophils, mast cells, and Langerhans cells/LCs, with increased transepidermal water loss/TEWL after 48h and 72h, implicating barrier disruption.^{16, 17} Comparable clinical¹⁸⁻²⁰, skin and blood^{21, 22} HDM reactions were documented in both AD and non-AD populations.

Recent studies have assessed select immune markers, primarily in experimental systems. In animal models, HDM showed early Th2/Th22 skewing and upregulation of S100A8/9/12 at 6hrs post-application, with parallel barrier changes (epidermal hyperplasia, decreased differentiation, increased TEWL).²³⁻²⁸ In ex-vivo cultures, HDM induced both Th2 (IL-4, CCL17, TSLP) and Th17/Th22 markers, while in human keratinocytes co-treated with IL-17A, HDM showed robust increases in S100A8/9 expression.²⁹⁻³¹ In AD patients, HDM upregulated inflammatory dendritic epidermal cells/IDECs 72h after sensitization.³² A global characterization of positive HDM responses in AD and non-AD individuals is lacking.

With accelerated therapeutic development, there is a large need for experimental models that mimic human AD.^{1, 33} To better understand whether HDM simulates immune and barrier features of AD,^{34, 35} we evaluated HDM-induced tissue changes in individuals with and without AD, vs. intrapersonal control skin. This is the first global skin profiling of human HDM reactions, potentially providing a model to study polar inflammatory reactions across skin diseases. HDM elicited Th2,

Th9, and Th17/Th22 activation vs. controls, with AD presence conferring no overall differences in response. While the HDM profile showed some similarities to AD lesions, HDM had greater resemblance to psoriasis and contact responses to nickel.³⁶⁻³⁸

METHODS

Patients' characteristics and samples

Patch-testing was performed with HDM [*D.Pteronyssinus/Farinae* 50/50 mix antigen in 30% petrolatum (Chemotechnique Diagnostics, Vellinge, Sweden)] on clinically normal upper back of 30 individuals per Institutional Review Board (IRB)-approved protocols, as reported.³⁷ Irritant reactions to HDM APT were excluded and eczematous lesions were considered positive and clinically graded after 72hrs. 15/30 patients (11 healthy, 4 meeting diagnostic AD criteria; mean age 38.6yrs; 11 with IgE <200, 4 with IgE>200) showed positive HDM responses³⁹. Nonlesional skin ≥ 2 cm from positive reactions was included.

Positive reactions to nickel from our prior report on contact allergens using the same cohort were included for comparison. Previously reported acute, chronic (>72 hours) and nonlesional AD biopsies,³⁶ and psoriasis lesions were also included.³⁸ See Supplementary Methods and Table S1 for demographics.

Histology and RT-PCR analyses were performed on all samples. One HDM sample was unavailable for gene-arrays. Data are deposited in Gene Expression Omnibus repository (accession no. GSE95759).

Immunohistochemistry/IHC

IHC was performed using antibodies listed in Table S2, as described. Cell counts were quantified using ImageJ V1.42 (National Institutes of Health, Bethesda, Md).³⁷

Gene expression analyses

RNA was extracted for RT-PCR with EZ-PCR Core Reagents (Life Technologies, Grand Island, NY) as described. Expression values were normalized to hARP. Primers are in Table S3.

HGU133Plus2.0 microarrays (Affymetrix, Santa Clara, CA) were used for gene-arrays, as described.⁴⁰

See supplementary methods.

The Icahn School of Medicine at Mount Sinai Program for the Protection of Human Subjects and The Rockefeller University Institutional Review Board approved this study (approval nos. HS# 12-00023 & EGU-0757, respectively). Written informed consent was obtained using IRB approved consent forms.

Statistical Analysis

Statistical analysis was performed using R-language (www.R-project.org) and packages available through bioconductor (www.bioconductor.org).

For gene-arrays, quality control was performed as described.⁴¹ Expression values were obtained using the GCRMA algorithm. Batch effects were adjusted using *ComBat*.⁴² Probe-sets with ≥ 1 sample with expression values of > 3 were kept for analyses. Data was log2-transformed prior to analysis with linear mixed-effect model, with HDM and tissue (lesional and control) as fixed factors and a random effect for each patient. FCHs between comparisons of interest were estimated and hypothesis testing was conducted using contrasts under general framework for linear models in R's *limma* package. P-values from the moderated (paired) t-test were adjusted for multiple hypotheses using the Benjamini–Hochberg procedure. Genes with $FCH > 2$ and $FDR < 0.05$ were considered differentially expressed, and constituted the HDM transcriptome. Hierarchical clustering of samples/conditions used a McQuitty agglomeration algorithm. Gene-set over-representation analysis was performed using XGR software.⁴³ RT-PCR and IHC data were analyzed using the linear mixed effect model. See supplementary methods.

RESULTS

To investigate the immune and barrier profile of HDM reactions and their simulation of human AD, we performed patch tests with HDM on normal-appearing upper back of 30 individuals, as reported.³⁷ 15/30 individuals showed positivity to HDM [12 (1+) and 3 (2+) reactions] after 72h; 4/15 HDM-positive individuals had coexistent AD; 4/15 patients had elevated IgEs (>200). Healthy control skin ≥ 2 cm from positive reactions was also included. Comparisons were also made to positive nickel reactions from a prior report using the same patient cohort, as well as to published data on acute and chronic (>72h) phase AD lesions,³⁶ and psoriasis lesions³⁸ (Table S1) . See Methods, Supplementary Methods, and Table S1.

HDM induces robust infiltrates of dendritic cells, T-cells, and eosinophils

Clinically, HDM reactions showed elevated, erythematous, vesicular patches vs. control skin (Fig. 1A, B; Table S1). Hematoxylin and eosin (H&E) staining of tissue sections from HDM lesions demonstrated a preserved granular layer, spongiosis, parakeratosis with retained nuclei in the stratum corneum, and slight acanthosis vs. control skin, similar to the slight increase in hyperplasia already observed with acute AD and nickel, and much less than the hyperplasia in chronic AD (Fig. 1C, D, O; Fig. S1; Table S4). Through immunostaining, HDM demonstrated significant increases in CD3⁺ T-cells and CD11c⁺ myeloid dendritic cells/mDCs vs. controls ($p < 0.01$; Fig. 1E-H, P,Q). CD3⁺ T-cells and CD11c⁺ mDC counts were higher in HDM than in acute and chronic AD lesions ($p < 0.05$ for all), and HDM lesions showed greatest similarity to nickel-induced responses which had highest CD3⁺ and CD11c⁺ infiltrates (Fig. S1). DC-LAMP, a marker of mature DCs, was significantly elevated in HDM lesions as compared to controls ($p < 0.05$; Fig. 1 I,J,R). Major basic protein/MBP⁺ eosinophils showed higher increases in HDM lesions vs. controls compared to chronic AD tissues, but less than in nickel, which had the greatest infiltrates (Fig. 1K, L, S; Fig. S1). Similarly, CD1a⁺ Langerhans cells/LCs were increased in HDM tissues vs. controls, more similar to nickel responses which had highest LC infiltrates, than to AD lesions (Fig. 1M, N, T; Fig. S1).

The HDM transcriptome is characterized by Th2-, Th9-, Th17-, and Th22-polarization

Using criteria of fold change/FCH>2 and false-discovery rate/FDR<0.05, we defined differentially expressed genes/DEGs in HDM positive reactions vs. control skin, referred to as the HDM transcriptome. A total of 743 up- and 326 downregulated DEGs were induced in the HDM transcriptome, which was characterized by robust inflammation/immune activation (MMP12, GZMB, CD28, CCR7), as well as Th2 (CCL17, CCL18), Th17 (DEFB4A, CXCL1, PI3, LCN2, CCL20, VNN1, KYNU, IL36G), Th22/Th17 (S100A8/9), and Th9 (IL9, CISH, SOCS3) induction (Fig. 2; Table S5). Downregulated DEGs included only select barrier (LCE1E) and lipid genes (LPL, LEPR). Many terminal differentiation (FLG, LOR), tight junction (CLDN's), and lipid (ELOVL, FADS, FABP, GAL, LPIN1) genes that are characteristically downregulated in AD,⁴⁴ were not decreased in HDM tissues. The cornified envelope gene repetin/RPTN that was shown to be upregulated in AD,⁴⁵ was downregulated in the HDM transcriptome, and periostin/POSTN, a matricellular protein typically increased in AD⁴⁶, was decreased in HDM tissues.

The HDM transcriptome revealed higher magnitudes of genomic inductions compared to acute/chronic phase AD lesions and lower compared to responses induced by nickel (Fig. S2,3; Table S6). HDM showed greater and more significant inflammation, including higher upregulation of markers related to general inflammation (MMP12), Th9 (IL-9), Th17/22 (IL-22, LCN2, IL-19, PI3) vs. AD. While both HDM and AD lesions showed significant upregulation of Th2 markers (CCL17, CCL18, CCL22), some Th2 products were not as highly upregulated by HDM (CCL13, CCL26) compared to chronic AD lesions (Table S6). Some Th1 markers also showed greater upregulation in AD (CXCL9, CXCL10, OASL). Accounting for reaction intensity did not change our results.

HDM activation of multiple polarized immune axes is validated by real-time (RT)-PCR

To evaluate polar cytokines and key markers that are often below detection levels on microarrays, we performed RT-PCR for a large panel of inflammatory genes (Fig. 3; Fig. S4).⁴⁷

Overall, HDM did not show significant Th1-activation vs. control skin (except CXCL10), with stronger induction in AD and nickel responses vs. respective nonlesional/control skin (Fig. S5).

The Th2 axis, which is established as pathogenic in AD,⁴⁸ showed significant induction in HDM tissues for several Th2 markers (IL-5, IL-13, CCL13/CCL17/CCL18/CCL22/CCL26; $p < 0.05$; Fig. 3; Fig. S4) compared to healthy skin, but not all Th2 markers. For example, IL-4, IL-10, IL-31, IL-33, CCL5, TSLPR, and OX40L were not significantly upregulated in HDM APT responses vs. healthy skin, whereas the majority of these markers were significantly upregulated in AD lesions (Fig. S4-6).^{49, 50}

HDM produced a robust and significant induction of IL-9, a cytokine characteristically increased by contact allergens ($p < 0.01$; Fig. 3).^{37, 51, 52} Th17- related markers (IL-8, IL-23p40, PI3, CCL20, LCN2) were strongly upregulated in HMD tissues vs. controls ($P < 0.05$; Fig. 3), showing greater similarity to nickel responses rather than to AD lesions, which showed lesser Th-17 skewing (Fig. S5,6). IL-22, and the IL-17/IL-22-induced S100As were significantly ($p < 0.001$) elevated in HDM tissues vs. controls, similar to AD lesions and nickel responses.

The HDM transcriptome shows robust correlations in patients with or without concomitant AD

To examine whether AD presence impacted the HDM signature, we performed a subset analysis using gene arrays comparing HDM responses in AD and non-AD individuals. Overall, HDM tissues showed comparable genomic inductions, with patients with concomitant AD displaying more robust inflammatory responses to HDM as compared to healthy individuals (Fig. 4A, S7). In AD patients, HDM showed greater immune activation across all inflammatory axes (Th2/CCL17, CCL26; Th9/IL-9; Th17-related/S100A9/12, PI3, LCN2, CCL20; and Th22/IL-22). Similar trends were seen in both groups and no significant differences were observed between AD and non-AD individuals for most genes. In fact, the HDM transcriptome showed an impressive correlation in both groups ($r = 0.96$, $p = 2.2 \times 10^{-16}$; Fig. 4B). A similar picture was also seen using RT-PCR, with AD patients showing a trend of greater expression of Th2 (CCL18, CCL26), Th9 (IL-9), Th17 (LCN2), and Th22 (IL-22)-

markers (vs. controls) as compared to non-AD individuals (Fig. 5A, Fig. S8). However, several markers including key Th2 products (IL-13, CCL22) and the Treg product FOXP3 showed more significant upregulation in healthy individuals, vs. respective controls. Histopathological comparison of HDM APT responses in individuals with or without AD, as compared to their respective controls, also showed no statistically significant differences between the groups, with similar differences in epidermal thickness and cellular infiltrate levels (T-cells, DCs, eosinophils, and LCs) observed between HDM lesions and uninvolved skin in AD or healthy individuals (Fig. S9). Additionally, a sensitivity analysis showed no differences in HDM-responses between patients with elevated (>200) or normal IgE levels (data not shown).

HDM shows strongest Th17-skewed inflammation, but captures aspects of various polar inflammatory skin diseases

Given the strong Th17 induction by HDM, we hypothesized that this model might simulate several inflammatory skin conditions, particularly psoriasis, a Th17-skewed disease.⁵³ We thus compared the HDM transcriptome to psoriasis lesions,³⁸ with inclusion of acute and chronic phase AD lesions, and nickel responses as a representative contact allergen (Fig. 6) Of total DEGs dysregulated in acute AD lesions, 14/117 (12%) were shared with the HDM fingerprint; 220/718 (30.6%) of total DEGs in chronic AD lesions were shared with HDM; 962/1080 (89%) of total DEGs dysregulated in nickel were shared with HDM, and 544/4223 (17.7%) of total DEGs dysregulated in psoriasis lesions were common to HDM. Interestingly, when all diseases, including AD, psoriasis, and nickel-responses, were included in an unsupervised clustering of microarray data, HDM grouped closest to psoriasis, followed by nickel, chronic AD, and then acute AD lesions (Fig. 6E).

Pathway enrichment analysis of the HDM transcriptome, conducted using functional-based pathway databases (KEGG, REACTOME, Biocarta, Pathway Interaction Database, MSigDB; Table S8; Supplementary Methods), revealed many significantly upregulated pathways in the HDM signature (FDR<0.05). Among these, some were common to AD and psoriasis [(T-cell receptor,

NOD and toll-like-receptor signaling, JAK-STAT signaling, interferon gamma signaling), Th22 (IL-22)], specific to psoriasis [neutrophil, Th17(IL-17, IL-12/23), tryptophan catabolism], or specific to AD [Th2(IL-4), FCεRI signaling].^{44, 54, 55} We also evaluated how the HDM profile enriched with available signatures of other inflammatory diseases.⁵⁶ The HDM transcriptome showed similarity to the signature of psoriasis vulgaris, other Th17-polarized diseases (allergic contact dermatitis, acne, Crohn's disease)^{37, 57, 58}, as well as Th2-centered conditions (eczema, ulcerative colitis, asthma)⁵⁹ (Fig. 6F; Table S9).

DISCUSSION

HDM is ubiquitous in the environment, and many individuals are sensitized to its associated protein antigens der p1/2.⁶⁰ The ability of HDM antigens to elicit APT reactions in a high frequency of individuals makes it a valuable tool to study cutaneous hypersensitivity.¹⁰ In our study ~55% of individuals without AD had positive HDM-responses and ~40% of individuals with AD had positive HDM-reactions, similar to prior reports.^{61, 62}

While allergic sensitivity to HDM is highly based on elevated HDM-specific IgE levels, many studies have not found associations between IgE levels and APT reactivity in atopic individuals.^{10, 61} We also did not find differences in APT responses based on total IgE levels, albeit HDM-specific IgEs were not determined for our cohort. Based on past data on HDM APT response, including its strong association with T-cell infiltration and cytokine increases,^{27, 30} and our global skin profiling of HDM tissue reactions that shows induction of multiple, polar T-cell axes, HDM tissue reactions are best classified as T-cell mediated DTH responses.

Given that T-cell and inflammation-related gene responses are robust in HDM APT reactions, the major question we addressed is whether HDM can model AD or other inflammatory skin disease at the molecular level, and whether this is affected by AD presence. AD and psoriasis are classified as T-cell mediated diseases in which resident skin cells undergo complex activation programs in response to cytokines produced by “polar” T-cell subsets. Psoriasis is driven mainly by Th17 cytokines (IL-17, IL-26, IL-29), with Th22 and Th1 components.^{63, 64} AD is driven mainly by Th2

(IL-4, IL-13) and Th22 (IL-22) cytokines, but with background Th1 activation and some Th17 induction.^{1, 65} Some AD subsets, e.g. Asians, intrinsic, or early pediatric AD, have higher IL-17 activation, overlapping with psoriasis, while sharing the characteristic Th2/Th22 skewing of AD.^{66, 67} Hence, any inflammatory reaction taken as a model for AD should have strong Th2/Th22 components, while a psoriasis model should have high IL-17 production with little, if any, Th2 activation. Disease-related classification of the HDM reaction should revolve around these differing axes.

This is the first comprehensive molecular profiling of positive HDM APT responses in human skin. Our data characterizes the HDM transcriptome with Th2-, Th9-, and Th22- activation. While demonstrating a strong Th2 skewing, HDM lesions illustrated some qualitative differences in Th2 induction and epidermal abnormalities as compared to AD tissues. For example, HDM induced several key Th2 cytokines (IL-5, IL-13, CCL13/CCL17/CCL18/CCL22/CCL26), but lacked significant expression of other Th2 markers that may have a role in AD (IL-4, IL-10, IL-31, IL-33, CCL5, TSLPR, OX40L, POSTN). Additionally, barrier defects integral to AD pathology (downregulation of tight junction and lipid measures) were not fully captured by the HDM profile.^{47, 68} Our data shows that HDM APT profiles are similar regardless of concurrent AD, in line with prior reports,^{19, 22} with overall greater immune activation and Th2, Th9, and Th17-inductions among AD patients. While past work has suggested that HDM is a trigger of AD,^{12-14, 69} the HDM APT skin phenotype does not fully reproduce AD at the molecular level, even in the background of nonlesional AD skin. Other work has identified autoantigens that drive T-cell activation in this disease.⁷⁰

AD and psoriasis are two inflammatory diseases that share common characteristics including hyperplasia, increased cellular infiltrates, Th1, Th22, and systemic inflammation.^{3, 71-74} The HDM model manifests some of these commonalities, such as increases in T-cells and DCs at the histological level as well as increases in IL-22-induced S100As that characterize both diseases, which may be valuable in replicating shared features across AD and psoriasis, as well as ACD, that HDM may simulate best.

HDM-positive responses showed striking induction of IL-9 and IL-5, even greater than in AD tissues, and more characteristic of hapten-induced DTH reactions.^{37, 41, 52, 75} IL-9, a Th9 cytokine, promotes proliferation and differentiation of mast- and T-cells,⁷⁶ and has a regulatory role in ACD, through direct effects on Th1.⁷⁷ HDM stimulates IL-9 production via protease-activated-receptor-2 (PAR-2), present on eosinophils.⁵¹ In mice, HDM elevates IL-5, the eosinophil-recruiting cytokine, and HDM patch-tests in AD patients increase eosinophils, with corresponding IL-5 up-regulation.^{75, 78}

The study of T-cell-induced effector immunity in human skin is critical across different immune axes that are expressed across a range of inflammatory skin diseases. An advantage of the HDM model is its expanded clinical applicability. Currently, strong haptens like diphenylcyclopropanone/DPCP or dinitrochlorobenzene/DNCB are used to study these reactions.^{79, 80} These require prior sensitization and are uncomfortable to patients, precluding use in humans.^{81, 82} While nickel can also model human ACD, the prevalence of HDM-sensitized individuals is much larger compared to nickel (sensitizes ~20% of the population), highlighting its potential as an experimental model of ACD, and beyond, as our data suggests.^{9, 37, 41}

Our study presents several limitations: 1) APT responses were evaluated at 72hrs, without evaluation of reaction kinetics, including earlier (24hrs or 48hrs) and later (7d) time points, which requires obtaining multiple repeat biopsies from patients, and is often not feasible in human subjects. Nevertheless, acute AD in humans is defined as lesions occurring within 72hr, and the molecular profiling of acute AD is based on lesional AD biopsies at 72hrs;^{36, 83} earlier time points have not been characterized. Thus, our assessments of APT responses at 72hrs can be appropriately compared with early, or acute AD lesions. Furthermore, while DTH responses to epicutaneous antigens peak at 24hrs in mice, these peak at 72hrs in humans,^{82, 84} hence our 72hr biopsy approach should also reflect data from earlier reactions (24hr) in mouse models. 2) We examined biopsies of positive HDM responses, without inclusion of non-reacting individuals/reactions. 3) HDM-specific IgEs were not evaluated. Nevertheless, positive APTs are often discordant from total or HDM-specific IgE levels.^{61, 85, 86}

Our study increases the understanding of the HDM molecular profile and its ability to induce various polar T-helper responses. Positive HDM tissue reactions provide a highly inflammatory model with robust Th2, Th9, and Th17/Th22 activation, providing potential experimental versatility to encompass multiple inflammatory skin disorders. Given its widespread sensitization and availability, HDM may provide a tool that represents select aspects of AD, psoriasis, or ACD phenotypes.^{5, 84, 87} In an era of rapid therapeutic development and preclinical testing, we propose that HDM APT can be utilized for the testing/screening of pathway-specific drugs in early phase trials, regardless of AD status.

Conflicts of Interest: The authors have no relevant conflicts of interest.

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Figure Legends

Figure 1. Clinical and histopathologic responses to dust mite atopy patch test.

Representative patch reactions to dust mite (HDM) vs. control skin (**A-B**). Hematoxylin and Eosin (H&E) staining of HDM vs. control skin (10x magnification) (**C-D**). Staining of CD3 (**E-F**), CD11c (**G-H**), DC-LAMP (**I-J**), major basic protein (MBP) (**K-L**), and CD1a (**M-N**) in HDM vs. control skin. Epidermal thickness (**O**), CD3 (**P**), CD11c (**Q**), DC-LAMP (**R**), MBP (**S**), and CD1a (**T**) cell counts in HDM vs. controls. Stars above horizontal lines denote significance in change between two groups. Means $\pm$ SEMs. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Figure 2. Dust mite transcriptome.

Differentially expressed genes (DEGs) for dust mite compared to control skin, with direction of dysregulation (red: upregulated; blue: downregulated), by individual patient.

Figure 3. Dust mite induced cytokines and inflammatory markers as evaluated with real time-PCR.

Dust mite (HDM) induced mRNA expressions of cytokines and inflammatory markers compared to controls. Data were grouped by major inflammatory/regulatory pathways. Black dots represent individual samples. Blue circles represent means $\pm$ SEMs. Lines within boxes represent median values. Stars above horizontal lines denote significance among groups. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Figure 4. The dust mite transcriptome by atopic dermatitis presence.

Heatmap of top dysregulated immune genes in dust mite (DM) patch-tested lesions (LS) vs. control skin, stratified by the presence of atopic dermatitis (AD) (**A**). Red: upregulated; blue: downregulated.

Values adjacent to heatmap are fold-changes for the comparisons indicated. Pearson correlation of the the DM transcriptome in patch-tested patients with AD vs. healthy individuals **(B)**. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Figure 5. Dust mite induced cytokines and inflammatory markers by atopic dermatitis presence, as evaluated with real time-PCR.

Dust mite (HDM) induced mRNA expressions of cytokines and inflammatory markers (groups by major inflammatory/regulatory pathways) compared to control skin, in patients with concomitant atopic dermatitis (AD) vs. healthy individuals, summarized as a heatmap. Red: upregulated; blue: downregulated. Values adjacent to heatmap are fold-changes for the comparisons indicated. ⁺ $p < 0.1$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Figure 6. Comparison of the dust mite transcriptome with inflammatory skin diseases.

Overlaps of the dust mite transcriptome with acute **(A)** and chronic **(B)** phase atopic dermatitis (AD) lesions, with nickel responses as a representative contact allergen. **(C)** The dust mite transcriptome compared with the acute lesional AD and lesional psoriasis signatures and **(D)** with chronic lesional AD and lesional psoriasis. Red: upregulated; blue: downregulated **(E)** Euclidean distance based phylogenetic tree of fold-changes between lesional vs. nonlesional skin for dust mite, acute/chronic phase AD, psoriasis, and nickel. **(F)** Disease signature enrichment analysis of upregulated genes in the dust mite transcriptome. X-axis indicates significance based on log value of FDR, e.g. eczema has an FDR of 5×10^{-10} .

Figure 1

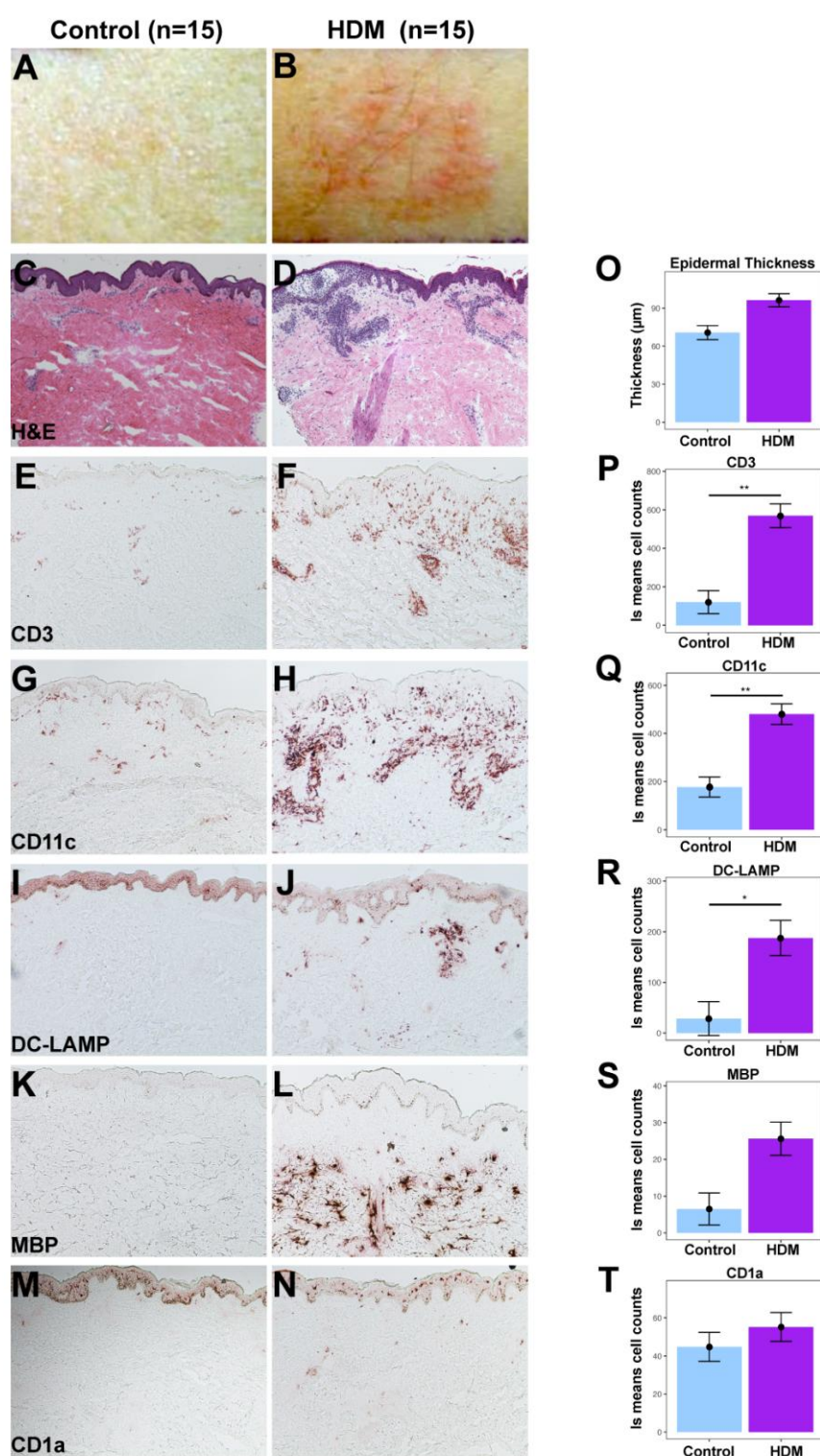


Figure 2

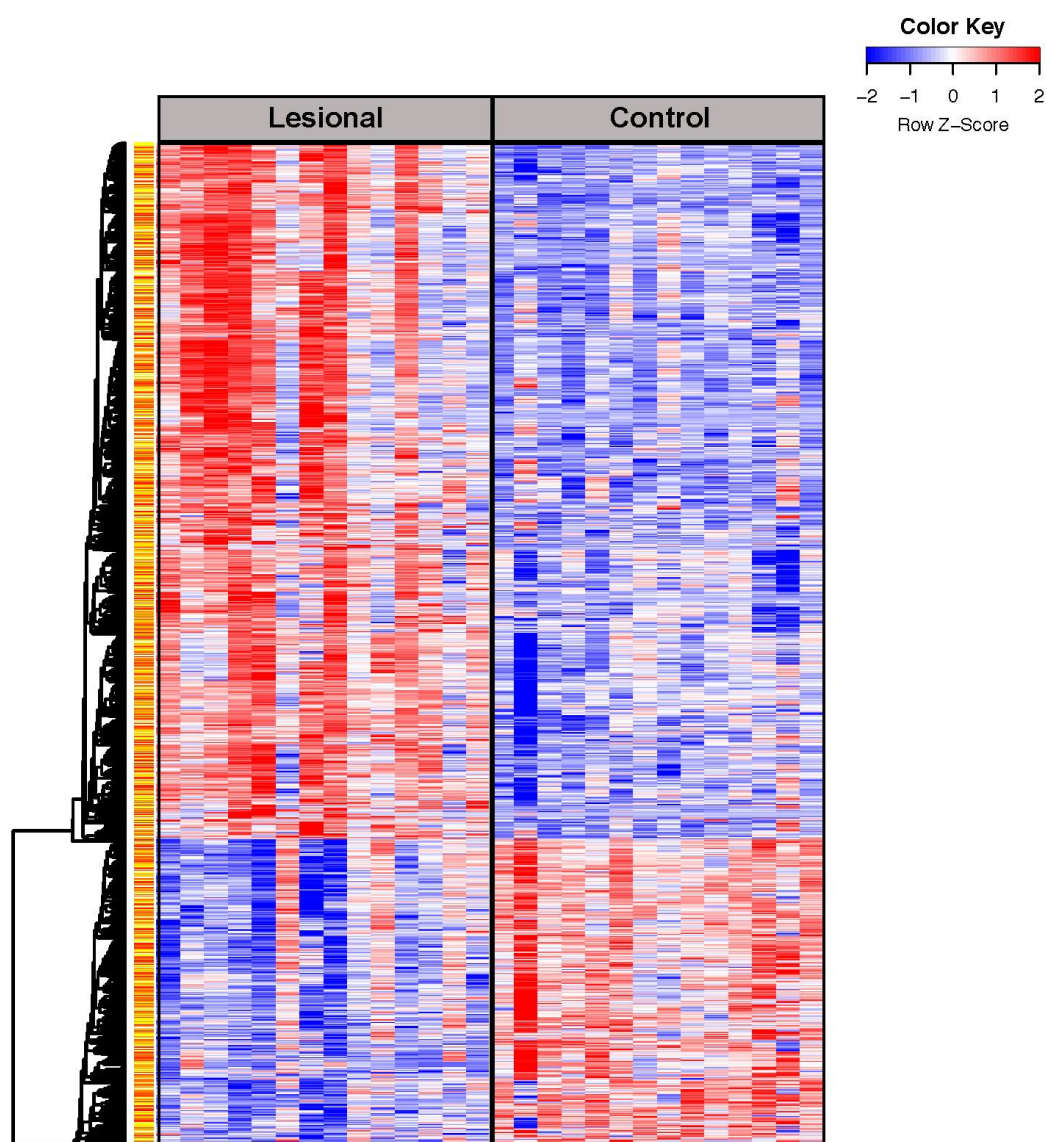


Figure 3

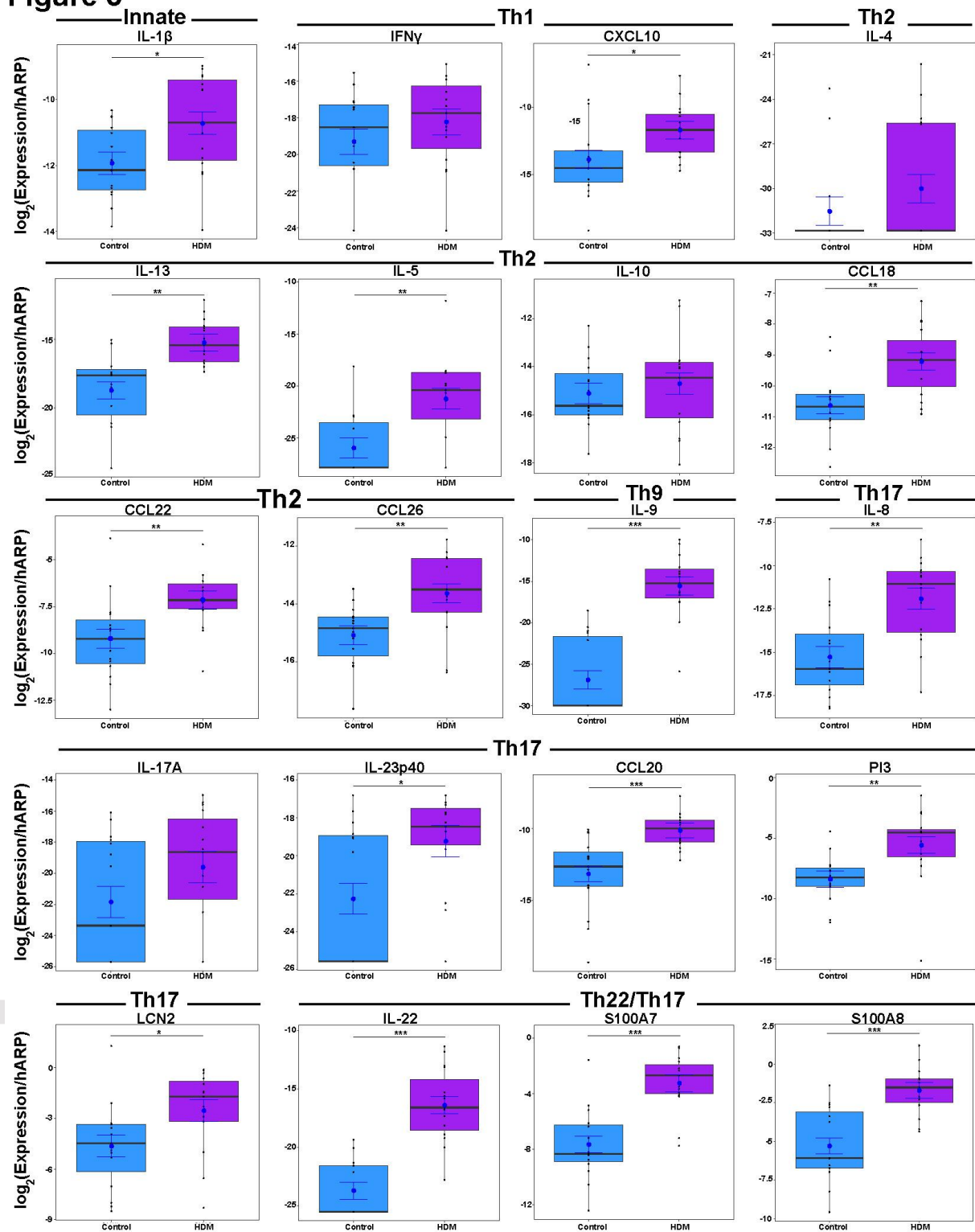
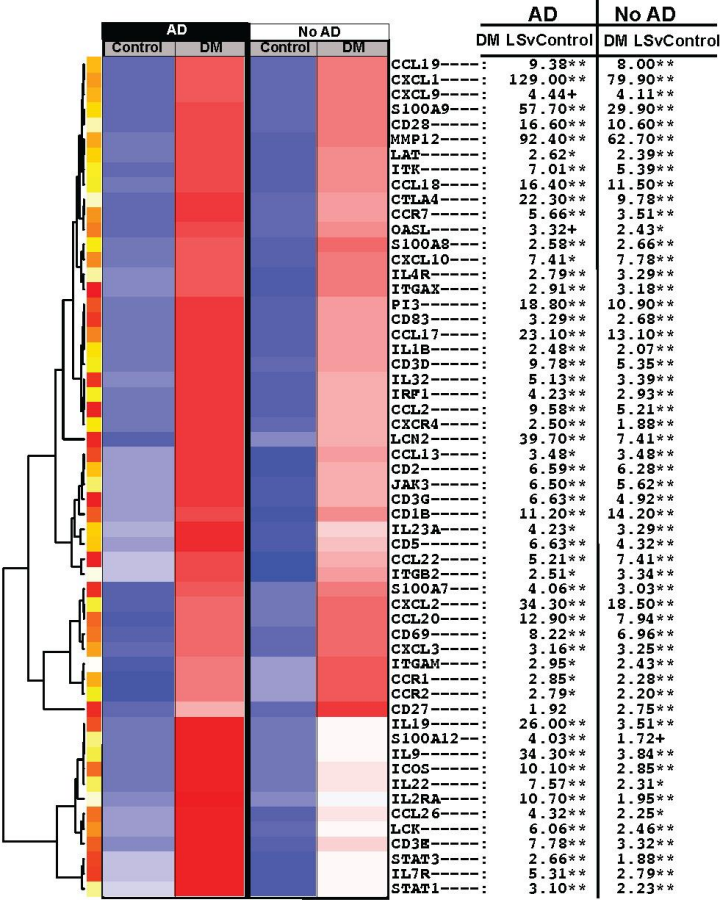


Figure 4

Microarray

A



B

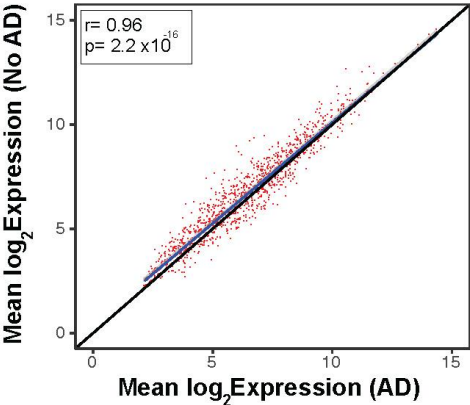


Figure 5

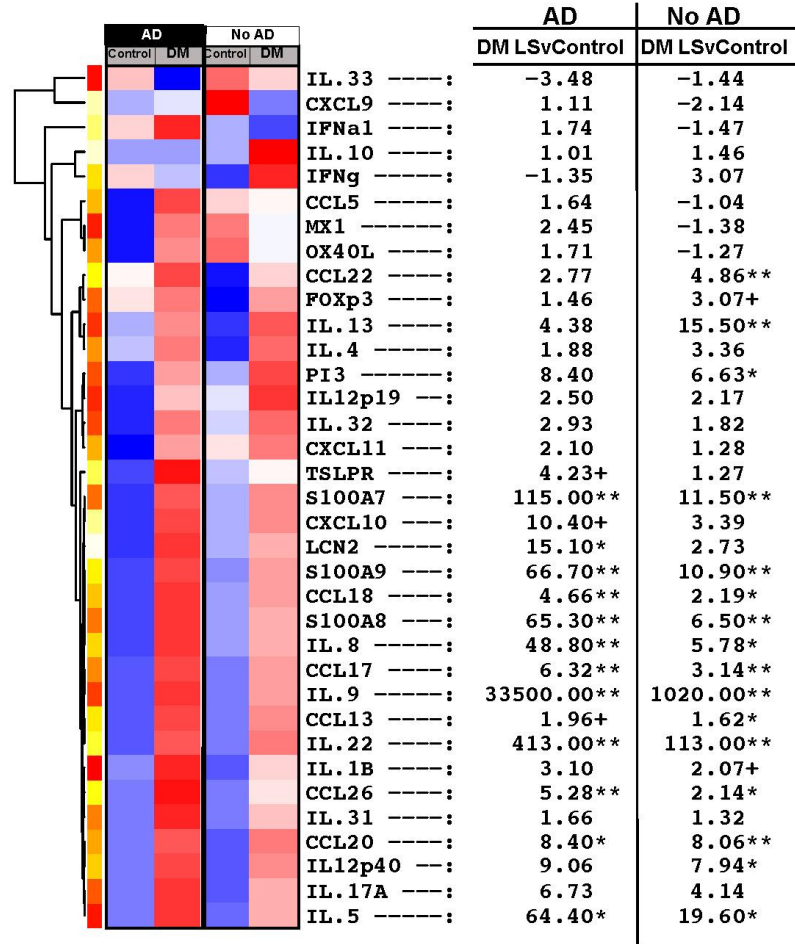


Figure 6

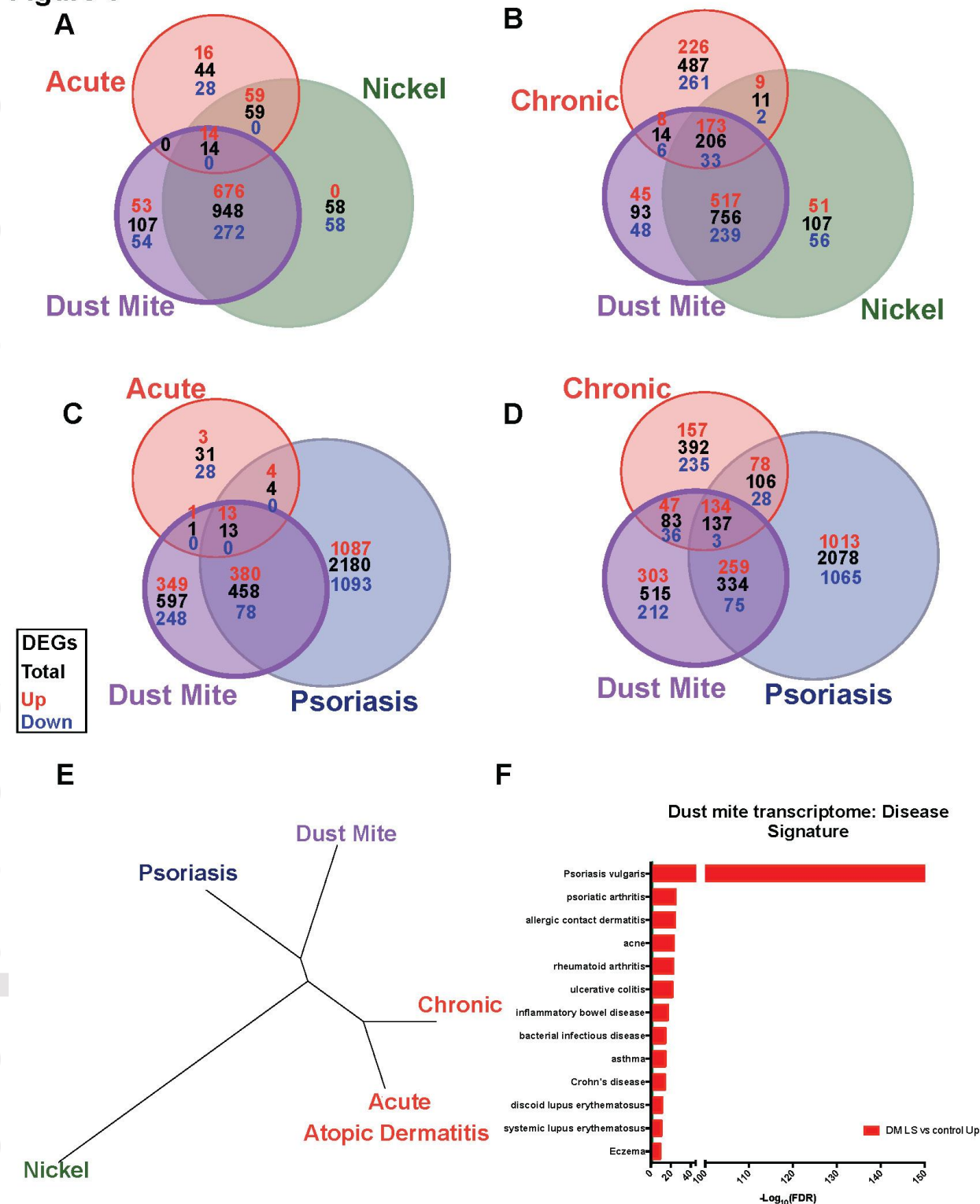
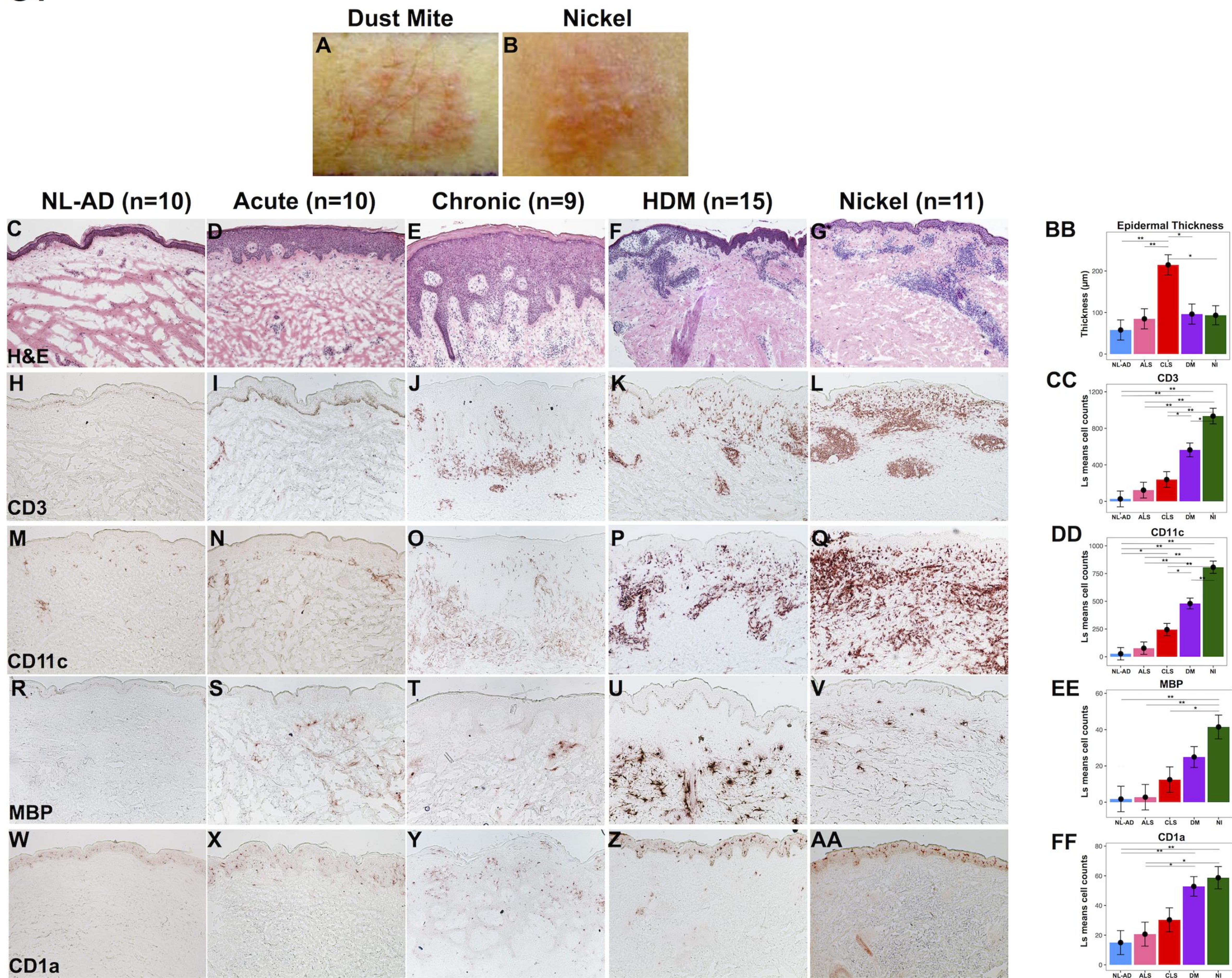


Figure S1



Supplementary Tables

Table S1. Patient demographics

Pt #	Gender	Age	Allergen Group	Reaction^a	Presence of AD (SCORAD)	Serum IgE (IU/mL)
1	M	37	Dust mite	1+	AD (39)	140
2	F	37	Dust mite	1+	N	225
3	F	28	Dust mite	1+	N	45
4	F	57	Dust mite	1+	N	28.6
5	F	29	Dust mite	2+	AD (33)	3309
6	F	39	Dust mite	1+	N	37.4
7	F	28	Dust mite	1+	N	99.4
8	F	52	Dust mite	2+	AD (26)	528
9	M	20	Dust mite	1+	N	98.7
10	M	28	Dust mite	2+	N	73.7
11	F	32	Dust mite	1+	AD (18)	783
12	M	62	Dust mite	1+	N	31.2
13	M	39	Dust mite	1+	N	94
14	F	55	Dust mite	1+	N	36.6
15	M	36	Dust mite	1+	N	15.6

^aPositive patch tests were graded according to established guidelines (See Methods and Supplementary Methods)

Table S2. Antibodies used for immunohistochemical analyses

Antigen	Manufacturer	Clone ^a	Isotype	Dilution
CD3	BD Biosciences	SK7	IgG1	1:100
CD11c	BD Biosciences	B-Iy6	IgG1	1:100
DC-LAMP	Fisher Scientific	104.G4	IgG1	1:50
MBP	Thermo Scientific	BMK13	IgG1	1:15
CD1a	Abcam	CD1a007	IgG1	1:25

^aAntibodies used in this experiment were mouse monoclonal unless stated otherwise.

Table S3. Primers and probes used for real-time/RT- PCR

Marker	Probe
CCL11	Hs04187682_g1
CCL13	Hs00234646_m1
CCL17	Hs00171074_m1
CCL18	Hs00268113_m1
CCL19	Hs00171149_m1
CCL2	Hs00234140_m1
CCL20	Hs01011368_m1
CCL21	Hs00989654_g1
CCL22	Hs01574247_m1
CCL26	Hs00171146_m1
CCL5	Hs00174575_m1
CCR7	Hs01013469_m1
CDSN	Hs00169911_m1
CX3CL1	Hs00171086_m1
CXCL10	Hs01124251_g1
CXCL11	Hs04187682_g1
CXCL9	Hs00171065_m1
FLG	Hs00856927_g1
FOXP3	Hs01085834_m1
GATA3	Hs00231122_m1
HBD2	Hs00175474_m1
IFNa1	Hs00256882_s1
IFNg	Hs00989291_m1
IL-10	Hs00961622_m1
IL-13	Hs00174379_m1
IL-17A	Hs00174383_m1
IL-17F	Hs00369400_m1
IL-18	Hs01038788_m1
IL-19	Hs00604657_m1
IL-1B	Hs01555410_m1
IL-2	Hs00174114_m1
IL-20	Hs00218888_m1
IL-21	Hs00222327_m1
IL-22	Hs01574154_m1
IL-31	Hs01098710_m1
IL-32	Hs00992441_m1
IL-33	HS00369211_m1
IL-4	Hs00174122_m1
IL-5	Hs00174200_m1

Marker	Probe
IL-6	Hs00985639_m1
IL-7	Hs00174202_m1
IL-7R	Hs00902334_m1
IL-8	Hs00174103_m1
IL-9	Hs00914237_m1
IL12p19	Hs00900828_g1
IL12p40	Hs01011518_m1
IL2RA	Hs00907779_m1
IL37	Hs00367201_m1
K16	Hs00955082_g1
LCN	Hs01008571_m1
LOR	Hs01894962_s1
MMP12	Hs00899662_m1
MX1	Hs00895608_m1
OX40	Hs00937194_g1
OX40L	Hs00182411_m1
PDE6A	Hs00166495_m1
PI3	Hs00160066_m1
PPL	Hs00160312_m1
PU.1	Hs02786711_m1
RORG	Hs01076122_m1
S100A12	Hs00942835_g1
S100A7	Hs01923188_u1
S100A8	Hs01923188_u1
S100A9	Hs00374264_g1
TBET	Hs00203436_m1
TGFB	Hs00998133_m1
TGM1	Hs00165929_m1
TGM3	Hs00162752_m1
TNFa	Hs01113624_g1
TRAIL	Hs00366278_m1
TSLPR	Hs00845692_m1

Table S4. Histopathological characterization of house dust mite induced skin reactions.

Parameter	<i>Dust Mite</i>	<i>Acute AD</i>	<i>Chronic AD</i>	<i>Nickel</i>
Parakeratosis	Present	Minimal	Minimal	Present
Granular layer	Present	Decreased	Decreased	Present
Acanthosis	Slightly increased	Slightly increased	Present	Slightly increased
Spongiosis	Present	Present	Present	Present
Dermal T-cell infiltrate	Present	Slightly increased	Present	Present
Dendritic cells	Present	Slightly increased	Present	Present
Langerhans cells	Present	Present	Present	Present
Eosinophils	Present	Slightly increased	Present	Present

AD: Atopic dermatitis