

Patients with atopic dermatitis have attenuated and distinct contact hypersensitivity responses to common allergens in skin

Joel Correa da Rosa, PhD,^{a,*} Dana Malajian, BA,^{b,c,*} Avner Shemer, MD,^{d,*} Mariya Rozenblit, BA,^{a,b,e} Nikhil Dhingra, MD,^{b,e,f} Tali Czarnowicki, MD,^{a,b} Saakshi Khattri, MD,^b Benjamin Ungar, BA,^{a,b,e} Robert Finney, MD,^{b,g} Hui Xu, MSc,^a Xiuzhong Zheng, MSc,^a Yeriel D. Estrada, BS,^a Xiangyu Peng, MSc,^{a,e} Mayte Suárez-Fariñas, PhD,^{a,b} James G. Krueger, MD, PhD,^{a,b} and Emma Guttman-Yassky, MD, PhD^{b,e} *New York and Manhasset, NY, Tel-Aviv, Israel, and Philadelphia, Pa*

Background: Atopic dermatitis (AD) is the most common inflammatory disease. The prevalence of allergic contact dermatitis to allergens (eg, fragrance) is higher in patients with AD, despite a trend toward weaker clinical allergic contact dermatitis reactions. The role of the AD skin phenotype in modulating allergic sensitization to common sensitizers has not been evaluated.

Objective: We sought to investigate whether patients with AD have altered tissue immune responses on allergen challenge.

Methods: Gene expression and immunohistochemistry studies were performed on biopsy specimens from 10 patients with AD and 14 patients without AD patch tested with common contact allergens (nickel, fragrance, and rubber).

Results: Although 1085 differentially expressed genes (DEGs) were commonly modulated in patch-tested skin from patients with AD and patients without AD versus control skin, 1185 DEGs were uniquely altered in skin from patients without AD,

and only 246 DEGs were altered in skin from patients with AD. Although many inflammatory products (ie, matrix metalloproteinase 12/matrix metalloproteinase 1/S100A9) were upregulated in both groups, higher-magnitude changes and upregulation of interferon responses were evident only in the non-AD group. Stratification by allergen showed decreased expression of immune, T_H1-subset, and T_H2-subset genes in nickel-related AD responses, with increased T_H17/IL-23 skewing. Rubber/fragrance showed similar trends of lesser magnitude. Negative regulators showed higher expression in patients with AD.

Conclusions: Through contact sensitization, our study offers new insights into AD. Allergic immune reactions were globally attenuated and differentially polarized in patients with AD, with significant decreases in levels of T_H1 products, some increases in levels of T_H17 products, and inconsistent upregulation in levels of T_H2 products. The overall hyporesponsiveness in skin from patients with background AD might be explained by baseline immune abnormalities, such as increased T_H2, T_H17, and negative regulator levels compared with those seen in non-AD skin. (*J Allergy Clin Immunol* 2014;■■■:■■■-■■■.)

Key words: Atopic dermatitis, allergic contact dermatitis, patch testing, T-cell polarization, human skin, nickel, rubber, fragrance

From ^athe Center for Clinical and Translational Science and ^bthe Laboratory for Investigative Dermatology, Rockefeller University, New York; ^cColumbia University College of Physicians and Surgeons, New York; ^dthe Department of Dermatology, Tel-Hashomer, Tel-Aviv; ^ethe Department of Dermatology, Icahn School of Medicine at Mount Sinai, New York; ^fthe Department of Medicine, North Shore-Long Island Jewish Hospital, Manhasset; and ^gthe Department of Dermatology, Jefferson Medical College, Philadelphia.

*These authors contributed equally to this work.

J.C.R., J.G.K., M.S.-F., M.R., T.C., N.D., and B.U. were supported by grant no. 5UL1RR024143-02 from the National Center for Research Resources (NCRR), a component of the National Institutes of Health (NIH), and the NIH Roadmap for Medical Research. E.G.-Y. was supported by the Dermatology Foundation Physician Scientist Career Development Award and by a CTSA grant from Rockefeller University (UL1 TR000043). D.M. was supported by the American Dermatological Association's Medical Student Fellowship.

Disclosure of potential conflict of interest: J. G. Krueger has received grants paid to his institution and personal fees from Novartis, Pfizer, Janssen, Lilly, Merck, Kadmon, Dermira, Boehringer, BMS, and Paraxel during the conduct of the study; has received grants paid to his institution from Amgen, Innovaderm, and Kyowa; and has received personal fees from Serono, BiogenIdec, Delenex, AbbVie, Sanofi, Baxter, Xenoport, and Kineta. E. Guttman-Yassky is a board member for Sanofi-Aventis, Regeneron, Stiefel/GlaxoSmithKline, MedImmune, Celgene, Anacor, and Leo Pharma; has consultant arrangements with Regeneron, Sanofi Aventis, MedImmune, Celgene, Steifel/GlaxoSmithKline, Celcus, BMS, Amgen, and Drais; and has received research support from Regeneron, Celgene, BMS, and Janssen. The rest of the authors declare that they have no relevant conflicts of interest.

Received for publication October 9, 2014; revised November 13, 2014; accepted for publication November 17, 2014.

Corresponding author: Emma Guttman-Yassky, MD, PhD, Department of Dermatology, Icahn School of Medicine at Mount Sinai Medical Center, 5 E 98th St, New York, NY 10029. E-mail: eguttman@rockefeller.edu.

0091-6749/\$36.00

© 2014 American Academy of Allergy, Asthma & Immunology

<http://dx.doi.org/10.1016/j.jaci.2014.11.017>

Atopic dermatitis (AD) is a common inflammatory skin disease with increasing prevalence in industrialized countries.^{1,2} Allergic contact dermatitis (ACD), the most common occupational disease,³ is a type IV delayed hypersensitivity reaction to allergens/haptens contacting the skin. Although AD is characterized by T_H2/T_H22 polarization, with some contributions of T_H1 and T_H17 axes,^{4,5} ACD is increasingly recognized as a T_H17- and T_H22-polarized response in addition to a classic T_H1 response.⁶⁻⁸

Because AD is characterized by activation of inflammatory pathways and a disrupted epidermal barrier, increased permeation of allergens might lead to altered ACD reactions in a background of chronic AD.⁹ However, the relationship between AD and ACD remains unclear.

ACD was previously thought to occur either less frequently^{10,11} or with similar prevalence in patients with AD.¹²⁻¹⁴ Recent studies found increased ACD frequency in patients with AD¹⁵⁻¹⁷ and showed that both the severity and duration of AD are correlated with ACD in atopic children.¹⁸ Increased prevalences of polysensitization and contact sensitization to fragrances (ie, balsam of Peru)^{12,19} and metals were also shown in patients with AD.^{20,21}

Abbreviations used

ACD:	Allergic contact dermatitis
AD:	Atopic dermatitis
DC:	Dendritic cell
DEG:	Differentially expressed gene
DNCB:	Dinitrochlorobenzene
FCH:	Fold change
FLG:	Filaggrin
FoxP3:	Forkhead box P3
IHC:	Immunohistochemistry
IL1F10:	IL-1 family, member 10
LC:	Langerhans cell
MMP:	Matrix metalloproteinase
RT-PCR:	Real-time PCR

Several studies have reported diminished clinical responses to contact allergens in patients with AD^{22,23} and lower prevalence of ACD in patients with severe AD.²⁴ A few studies also suggest decreased responses to strong and clinically irrelevant sensitizers, such as dinitrochlorobenzene (DNCB); *in vitro* studies with PBMCs show impaired cytokine production after stimulation with contact allergens (ie, nickel).^{22,25} Newell et al²⁶ recently reported attenuated responses to DNCB and T_H2 skewing (with decreased IFN- γ and increased IL-4 levels) in normal-appearing skin from atopic compared with healthy subjects. The T_H2-polarized responses were independent of filaggrin (*FLG*) mutation status,²⁷ which is consistent with the lack of association between ACD and *FLG* mutations in most studies.^{26,28,29}

Past studies did not address the role of AD skin phenotype in influencing cutaneous allergic responses. We have shown recently that different allergens induce differential immune polarization, showing T_H2 and T_H22 skewing with fragrance,³⁰ which might explain the higher prevalence of ACD to fragrances in patients with AD.^{24,31} To investigate whether immune responses to contact allergens are altered in patients with AD, we have performed extensive molecular and cellular profiling of patch-tested skin with common clinically relevant allergens (ie, nickel, rubber, and fragrance). Our data suggest that the background AD skin immune phenotype affects both the magnitude and polarity of hypersensitivity responses to clinically relevant common allergens.

METHODS**Patients' characteristics and skin samples**

This study represents a subgroup analysis of 24 patients from previously reported data (15 women and 9 men; age range, 20–63 years; median, 39 years): 10 patients with concomitant AD and 14 patients without AD.³⁰ Patch testing was performed on patients with patch test–proved ACD to common allergens by using the 15 most common allergens of the North American Contact Dermatitis Group.³² Positive reactions were clinically graded at 72 hours as 1+, 2+, or 3+.^{33,34} None of the patients were noted to have “angry back syndrome” during the course of the study. Biopsy specimens were taken from allergen-reactive patches and control skin (not exposed to the allergen) under institutional review board–approved protocols. Overall, patients with AD had 4 positive reactions to nickel, 2 to fragrance, and 3 to rubber. Patients without AD had 6 positive reactions to nickel, 1 to fragrance, and 3 to rubber. Because other metals were previously shown to be differentially polarized compared with nickel and most of them were within the non-AD group, they were not considered in the subset analysis.³⁰ One patient was excluded because control skin was unavailable. *FLG* mutation status was only available for 9 patients, and details appear in Table E1 in this article's Online Repository

at www.jacionline.org. For additional details, see the **Methods** section in this article's Online Repository at www.jacionline.org.

Immunostaining

Immunohistochemistry (IHC) was performed on frozen tissue sections by using anti-human mAbs (see Table E2 in this article's Online Repository at www.jacionline.org). Epidermal thickness and positive cells per millimeter were quantified for IHC by using ImageJ V1.42 software (National Institutes of Health, Bethesda, Md), as previously reported.³⁰

Quantitative real-time PCR and gene arrays

RNA was extracted for real-time PCR (RT-PCR) with EZ-PCR Core Reagents (Life Technologies, Grand Island, NY), and custom primers were generated.^{4,5,35–40} Expression values were normalized to hARP U133Plus 2.0 arrays (Affymetrix, Santa Clara, Calif) and used for gene arrays, as previously described.^{4,5,35–40}

Statistical analysis

Quality control of microarrays was carried out by using standard quality control metrics and R package Quality Control software. Images were scrutinized for spatial artifacts with Harshlight. Expression values were obtained by using the Guanine Cytosine Robust Multi-Array Analysis algorithm. Probe sets with more than 1 sample, expression values of greater than 4, and SDs of greater than 0.15 were kept for analyses. Expression values were modeled by using linear mixed-effect models, with AD history as a fixed factor and a random effect for each patient.

Similar analyses were performed for subgroups of patients patch tested for nickel and rubber/fragrance. Fold changes (FCHs) for the comparisons of interest were estimated, and hypothesis testing was conducted by using contrasts with the linear models framework in limma package. *P* values from paired *t* tests were adjusted for multiple hypotheses by using the false discovery rate procedure. In nickel and rubber/fragrance subgroup analyses, adjustments were not applied because of small sample size, with unadjusted *P* values and FCHs to indicate differential expression. RT-PCR and IHC data were also analyzed by using the linear mixed-effect model. Statistical methodology is detailed in the **Methods** section in this article's Online Repository.

RESULTS

The majority of patch test reactions to common allergens (nickel, fragrance, and rubber) were 1+ or 2+ reactions in both the AD and non-AD groups, whereas 2 patients without AD had 3+ reactions (see Table E1 and Fig 2, B). Extensive genomic and molecular profiling of patch-tested skin was performed in patients with and without AD.

Increased cellular infiltrates in allergic reactions of atopic and healthy subjects

Similar epidermal thickness was observed after patch testing in skin from both patients with AD and patients without AD. Cellular infiltrates in biopsy specimens of allergic reactions to nickel, rubber, or fragrance from patients with AD and patients without AD were evaluated by using IHC. Cell counts and representative images are presented in Fig 1 and Fig E1 in this article's Online Repository at www.jacionline.org, respectively. Both groups showed increased infiltrates in patch tested versus control skin. With nickel, increases in CD3⁺ T-cell numbers and CD11c⁺ and DC-LAMP⁺ dendritic cell (DC) numbers were similar between patients with AD and control subjects, although decreased numbers of CD1a⁺ and langerin-positive

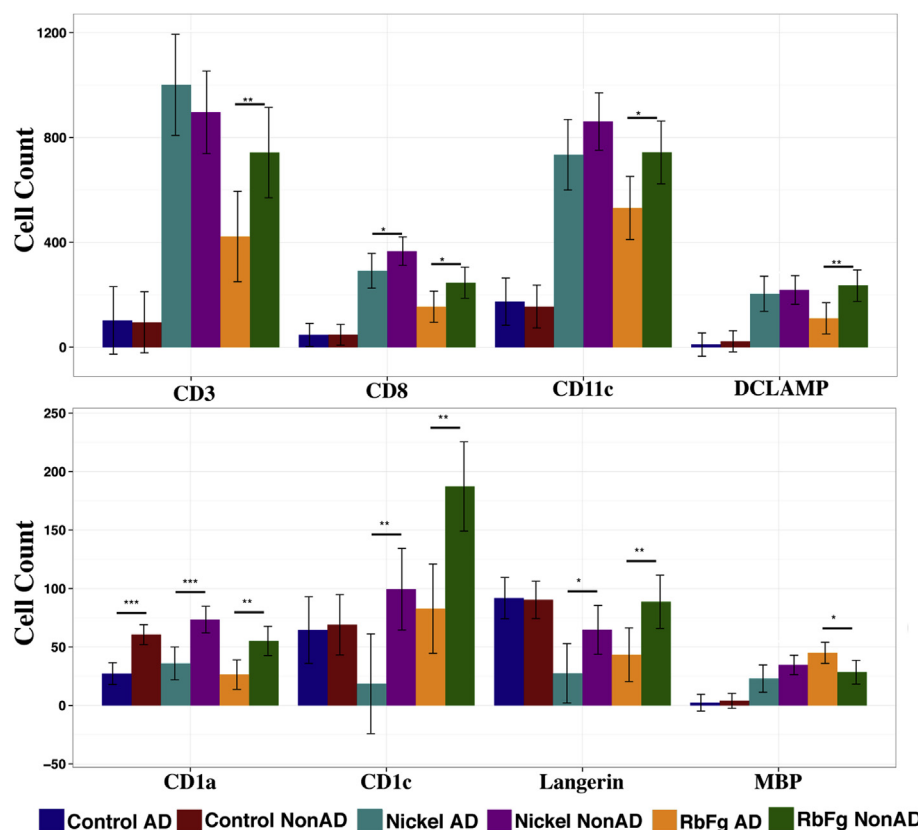


FIG 1. Cell Counts for CD3, CD8, CD11c, DC-LAMP, CD1a, CD1c, langerin, and major basic protein (MBP) in each allergen group were compared between control (not patch tested), AD, and non-AD skin. In general, non-AD skin showed increased infiltrates compared with AD skin after allergen challenge. Values are means $\pm$ SEMs. * $P < .1$, ** $P < .05$, and *** $P < .01$.

Langerhans cells (LCs), as well as resident/CD1c⁺ DCs, were seen in patients with AD. For rubber/fragrance reactions, patients with AD demonstrated significantly decreased numbers of T cells (CD3⁺ and CD8⁺) and various DC subsets compared with healthy subjects (Fig 1 and see Fig E1).

Genomic profiling identifies altered responses to contact allergens in atopic patients

Gene arrays were performed to define differentially expressed genes (DEGs; criteria of FCH > 2 and false discovery rate < 0.05) in biopsy specimens from positive reactions in patients with AD versus patients without AD, as represented in the Fig 2, A, Venn diagram. Many DEGs were common to both groups (1085 genes and 1854 probes), including T-cell (granzyme B [GZMB]), T_H1 (CXCL10), T_H17 (CXCL1), and other inflammatory (matrix metalloproteinase [MMP] 12, S100A9, and MMP1) genes, with much higher fold changes observed in reactions of patients without AD (see Table E3, A, in this article's Online Repository at www.jacionline.org).

A large group of unique DEGs was identified in patients without AD (1185 genes and 1636 probes), with fewer unique DEGs in the AD subset (246 genes and 320 probes; Fig 2, A, and see Table E3, B and C). A broad interferon signature was detected solely among the non-AD group (IFN- γ , 2'-5'-oligoadenylate synthetase 2 [OAS2], CXCR3, CXCL11, and T-box 21 [TBX21]; see Table E3, B). Increased magnitude of upregulated

gene expression in the skin of patients without AD is reflected by a darker red color within the ACD transcriptome heat map and stratified by AD status, allergen (nickel vs rubber/fragrance), and clinical scoring (Fig 2, B).

Although the AD group showed lower-magnitude responses, 246 genes were uniquely dysregulated in patients with AD (Fig 2, A), including many immune genes (ie, CCR6, CCL20, secreted phosphoprotein 1 [SPP1], and mitogen-activated protein kinase 1 [MAPK1]). Thus we performed a more detailed characterization of immune-related genes.

Lower expression of many cytokines, chemokines, and specific immune genes in patch reactions in patients with AD

Because we recently showed that common allergens (nickel, rubber, and fragrance) induce distinct tissue immune responses (T_H1/T_H17 for nickel and T_H2/T_H22 for rubber/fragrance),³⁰ responses in patients with AD versus those in patients without AD were further analyzed and stratified by these allergens. Overall, highly significant stronger expression of immune, T_H1, and T_H2 genes³⁰ was detected in nickel reactions among patients without AD, as shown in the box plots in Fig 2, C.

A detailed analysis of the immune gene subset is presented as a heat map in Fig 3 (see Tables E4, A-C, and E5, A-C, in this article's Online Repository at www.jacionline.org). Despite similar levels of cellular infiltrates, the nickel response in patients with

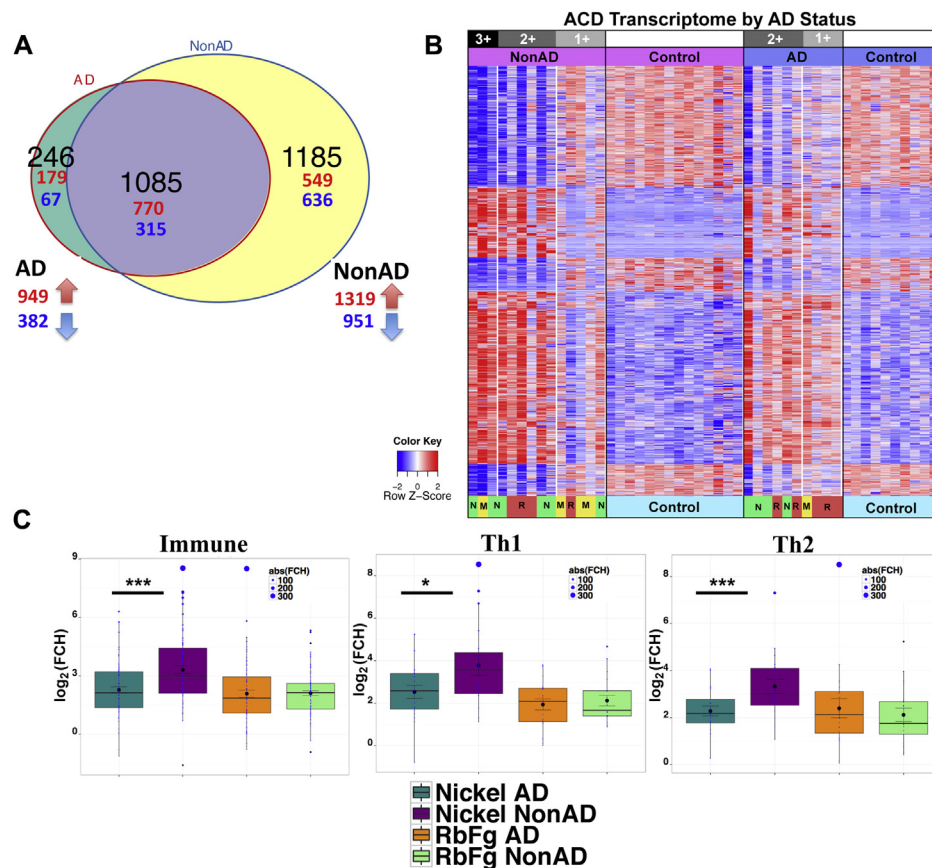


FIG 2. Common and unique DEGs for skin from patients with AD and patients without AD compared with control skin, with direction of dysregulation shown (red, upregulated; blue, downregulated). **A** and **B**, A core set of 1085 DEGs (intersection in purple; Fig 2, A) was common and defines the ACD transcriptome (depicted as a heat map in Fig 2, B). Samples were arranged by AD status, clinical scoring, and allergen group. R, Rubber/fragrance; N, nickel; M, other metals/purified protein derivative (PPD). **C**, Box plots of the allergen responses across major immune pathways represented in gene arrays. Values are means $\pm$ SEMs. * $P < .1$, ** $P < .05$, and *** $P < .01$.

AD was characterized by higher activation of IL-17–related genes (CCL20 and lipocalin 2 [LCN2]) and strong reductions in markers of general inflammation (MMP12), T cells (CD2 and CD28), DCs (integrin α X [ITGAX] and CD1B), innate immunity (IL-8), T_H1/T_H2 chemokines (CXCL9, CXCL10, CCL18, and CCL22), and T_H9 (IL-9) versus skin from patients without AD (Fig 3). Rubber/fragrance showed higher CCL22, CCL2, and IL-32 (an IL-22–induced gene)⁴¹ induction, which is consistent with higher T_H2/T_H22 polarization to rubber/fragrance.³⁰ Of note, even in patients with AD, T_H1 responses remained stronger than T_H2 responses (see Table E6 in this article's Online Repository at www.jacionline.org).

RT-PCR confirms attenuated immune responses in patch-tested AD skin

RT-PCR data adjusted for levels of immune activation in control skin confirmed decreased immune responses in patch-tested AD skin (Fig 4). Similar to array data, exposure to nickel resulted in less induction of T-cell trafficking markers (CCR7), interferon-related genes (IFN- α , IFN- γ , CXCL9, and CXCL11) and T_H2 -related (CCL18, CCL22, CCL26, IL-4, IL-5, and IL-13), T_H22 -related (IL-22), and T_H9 -related (IL-9) markers in skin of patients with AD versus patients without AD (see Table E4, A-C).

IL-17–related genes (LCN2, CCL20, and IL-17A) showed increased induction in reactions in patients with AD compared with that seen in reactions in patients without AD in nickel-exposed skin (Fig 4), which is consistent with gene array data (Fig 3 and see Table E4, C). IHC for neutrophils was performed by using neutrophil elastase to rule out possible infection as an inducer of IL-17/ T_H17 pathway genes. No significant differences were found in neutrophil elastase–positive cell counts between control AD and control non-AD skin (see Fig E2 in this article's Online Repository at www.jacionline.org).

Overall, a similar trend of lower expression in patients with AD, with smaller magnitude of differences compared with nickel, was seen with rubber/fragrance. However, several cytokines (IFN- α , IFN- γ , IL-5, IL-13, IL-22, and IL-9) showed increased, but not significant, mRNA expression in patients with AD (Fig 4). Accounting for the intensity of the reaction did not change our results. Means and P values for IHC and RT-PCR data are available in Tables E7 and E8 in this article's Online Repository at www.jacionline.org.

AD skin expresses higher induction of regulatory gene products

Because several factors might drive decreased immune responses in AD skin, we analyzed expression of immune markers

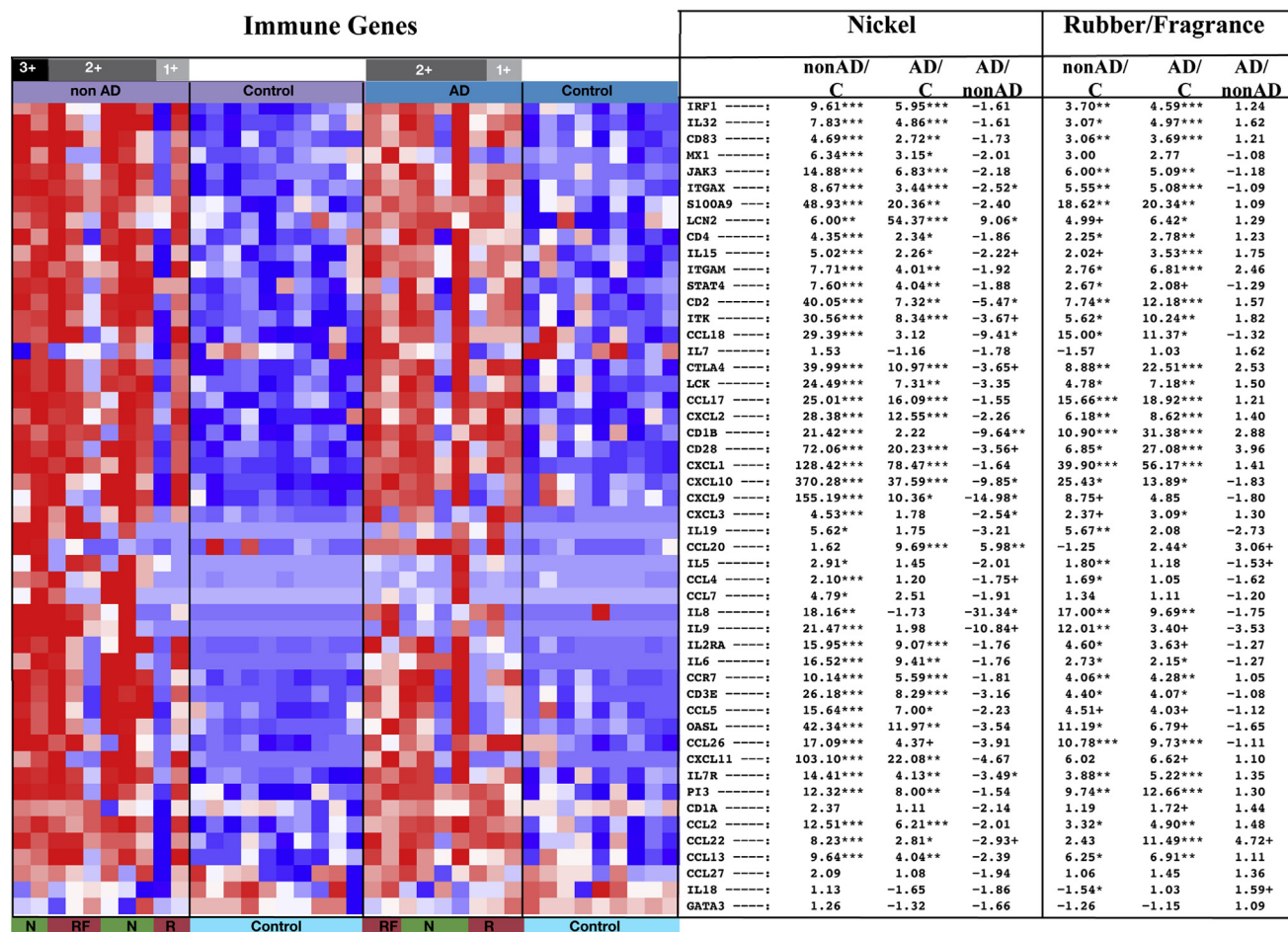


FIG 3. A subset of differentially expressed immune genes. Samples in the heat map are grouped by AD status and allergen type and arranged by clinical scoring from 1+ to 3+ (gray to black). Red, Upregulated; blue, downregulated. FCHs of allergen compared with petrolatum are shown. * $P < .1$, ** $P < .05$, and *** $P < .01$.

in control AD versus non-AD skin, as well as expression of negative regulators in both patch-tested and control skin (Fig 5). Unexposed control skin from patients with AD had highly increased expression of inflammatory products, including T-cell trafficking markers (CCR7), interferon/ T_H1 -related genes (IFN- α , IFN- γ , CXCL9, and CXCL11), and T_H17 /IL-23-related genes (IL-12/23p40 and CCL20) compared with control non-AD skin (see Fig E3 in this article's Online Repository at www.jacionline.org).

Higher induction of known negative immune regulators³⁰ was detected in both control and patch-tested AD versus non-AD skin by using a gene array (as shown in a heat map; Fig 5, A) and by means of RT-PCR (Fig 5, B). We observed significantly lower expression of IL-37, a recently reported negative regulator cytokine,⁴² in non-AD compared with AD skin exposed to rubber/fragrance (Fig 5, A); RT-PCR data confirmed this finding ($P < .001$; Fig 5, B). IL-1 family, member 10 (IL1F10), expression was also upregulated in AD versus non-AD skin (Fig 5, A). Additionally, patch-tested skin to rubber/fragrance shows increased expression of cytotoxic T-lymphocyte antigen 4 in both patients with AD and patients without AD, with a greater magnitude in AD skin (Fig 5, A, and see Table E5, A). AD skin also showed greater expression of IL-10, forkhead box P3

(FoxP3), and IL-37 mRNA in control skin and nickel patch-tested skin (Fig 5, B).

DISCUSSION

This is the first human study to extensively profile molecular and cellular responses to common and clinically relevant topical allergens (nickel, rubber, and fragrance) in AD compared with non-AD skin. Although previous studies have suggested that the ACD response might be attenuated in patients with AD,^{25,27} these studies focused on immune responses in peripheral blood, which might not be representative of skin,⁴³⁻⁴⁶ or on clinically irrelevant sensitizers, such as DNCB.²⁷ Recently, Newell et al²⁶ demonstrated an attenuated hypersensitivity reaction and T_H2 skewing in nonlesional AD versus healthy skin by using a strong hapten (DNCB), suggesting that the characteristic immune phenotype of background AD skin contributes to the distinct T_H2 polarization on challenge.²⁷ Although DNCB is a useful model to study hypersensitivity reactions, it has universal sensitization potential; alternatively, exposure to common allergens might be more significantly influenced by the genetic background that leads to barrier defects in patients with AD. Until now, the role of background AD skin phenotype in altering allergic responses has not been characterized.

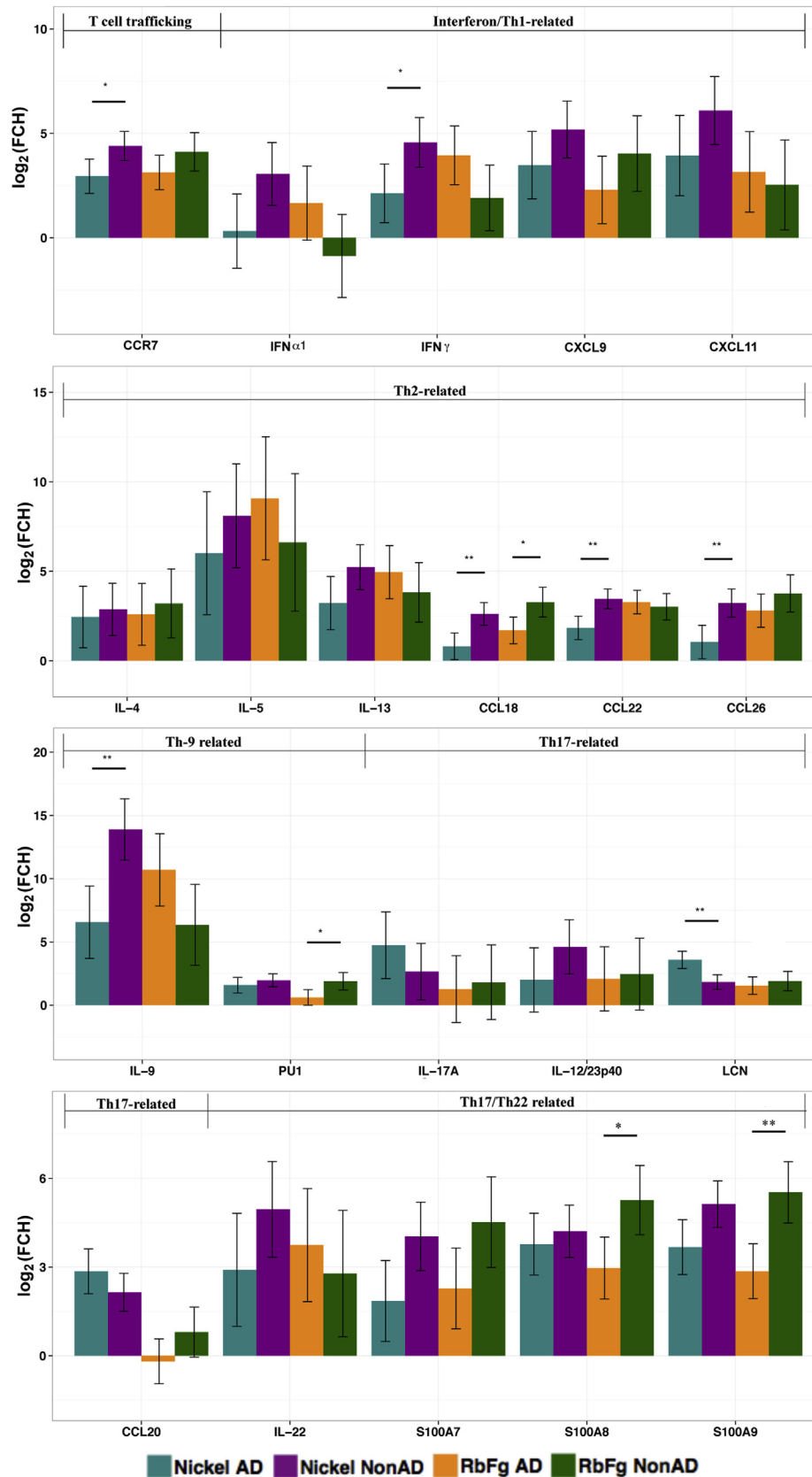


FIG 4. RT-PCR was used to evaluate the expression of major cytokines and inflammatory markers. Data were grouped according to allergen type and AD status and by the major inflammatory and regulatory pathways represented. All data were adjusted for control values. *P* values of expression in the AD versus non-AD groups are indicated, if significant. **P* < .1, ***P* < .05, and ****P* < .01.

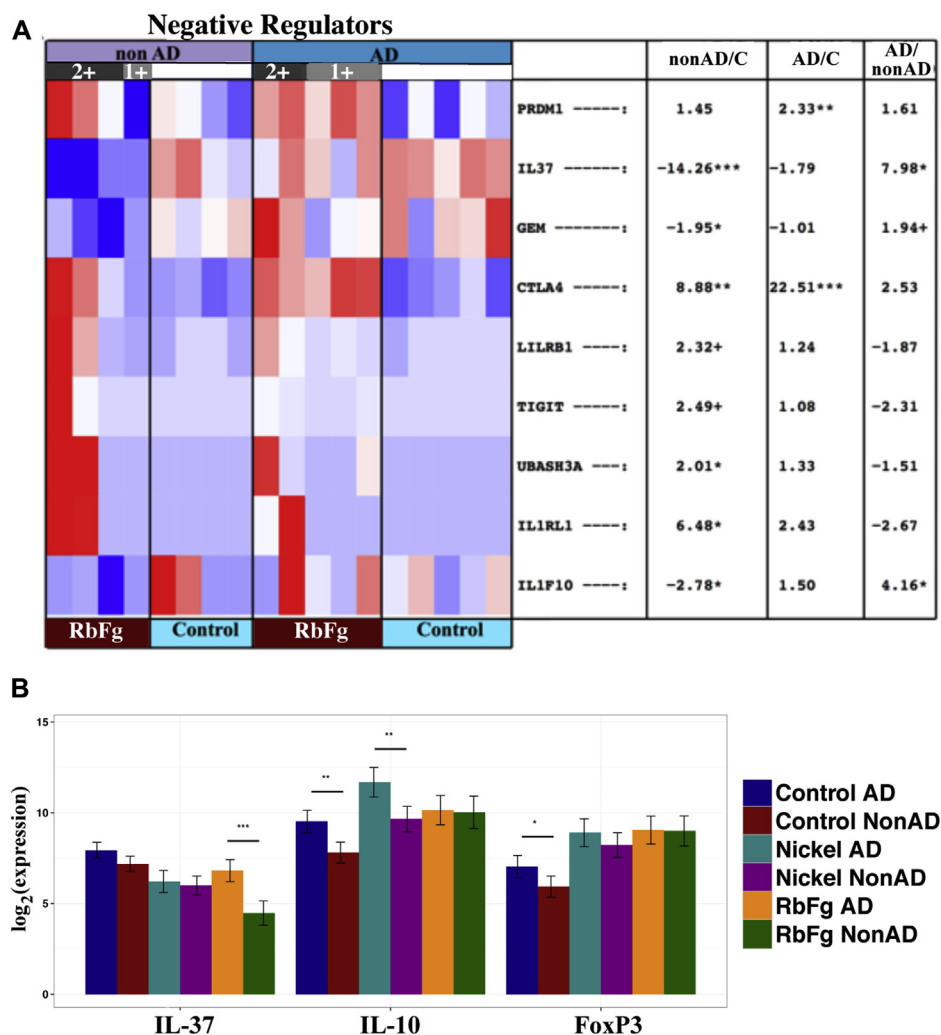


FIG 5. A subset of differentially expressed negative regulator genes. **A**, Samples in the heat map are grouped by AD status and allergen type and arranged by clinical scoring. **B**, RT-PCR was used to evaluate expression of the major negative regulators IL-37, IL-10, and FoxP3 grouped according to allergen and AD status. *P* values of expression in the AD versus non-AD groups are indicated, if significant. **P* < .1, ***P* < .05, and ****P* < .01.

Our data show that tissue responses to clinically relevant cutaneous allergens are both attenuated and differentially polarized in patients with AD compared with patients without AD. On a background of a T_H2 -polarizing disease, such as AD, one would expect perpetuated T_H2 responses after exposure to sensitizers, as observed with DNCB.²⁷ However, not only did our data not support this finding in patients with AD, it also showed mainly decreased T_H2 responses to the most common allergen, nickel. Even for antigens known for T_H2 skewing (rubber and fragrance),³⁰ we observed inconsistent and mostly nonsignificant modulation of T_H2 -related chemokines. Surprisingly, an AD background seems to predispose to T_H17 /IL-23 skewing in response to nickel. To the extent that there might be cross-regulation of T_H1 and T_H17 pathways,⁴⁷⁻⁴⁹ the decreased T_H1 responses seen with nickel might allow an increased magnitude of T_H17 response in these patients.

One possible explanation for the attenuated contact hypersensitivity response to common contact allergens in patients with AD is altered antigen-presenting responses (decreased LCs and some

DC subsets) on a background of increased antigen penetration, ultimately leading to impaired T-cell priming.⁵⁰ This hypothesis is supported by our IHC data showing significantly decreased counts of CD1a⁺ DCs, CD1C⁺ DCs, and langerin-positive LCs in patients with AD exposed to common allergens.

Another explanation for the altered responses in patients with AD is the differential expression of inflammatory pathways responsible for programming allergic sensitization in background AD skin. We have shown that baseline AD skin has increased expression of inflammatory pathways, particularly T_H17 , with significantly increased levels of IL-12/23p40 and CCL20 and increases in expression of T_H2 chemokines and cytokines (CCL22, CCL26, and IL-13; Fig 4). Thus increased levels of T_H17 and T_H2 axes at baseline might attenuate the T_H1 component of ACD, possibly resulting in decreased intensity and differential polarity in patients with AD.

Our study presents a few limitations. First, all samples were taken at 72 hours, and therefore we cannot evaluate cytokine mRNA kinetics in the ACD response. Additionally, because of the

small sample size, we used less stringent cutoffs for subgroup analyses. However, the results are consistent in showing lower-magnitude immune responses in patients with AD.

Attenuated immune responses might also stem from overexpression of recognized regulatory products in AD skin compared with non-AD skin, which was observed at baseline or after application of contact allergens. These negative regulators include FoxP3, a transcription factor necessary for regulatory T cell development⁵¹; IL-10, a known inhibitor of T_H1 responses⁵²; cytotoxic T-lymphocyte antigen 4, an inhibitory molecule found on the surfaces of T cells⁵³; and 2 novel regulatory cytokines, IL-37⁵⁴ and IL1F10.⁵⁵

IL-37 is a new member of the IL-1 family, which has been shown to be a fundamental inhibitor of innate immunity.⁵⁶ IL-37 upregulation is correlated with disease severity in patients with AD and is known to inhibit proinflammatory cytokines, such as IL-1 α , IL-1 β , TNF, and IL-6,^{42,57} and suppresses production of CXCL8, IL-6, and S100A7.^{58,59} Our data also show increased IL-37 expression in the rubber/fragrance group, possibly explaining the overall decreased induction of inflammatory pathways in this group compared with nickel.

IL1F10, also known as IL-38, is a novel member of the IL-1 family and shares homology with the IL-1 receptor antagonist. A recent study showed that IL-38 reduces the production of IL-17A, IL-22, and IL-8 through IL-36 receptor α binding.⁵⁵ Because IL-17A and IL-22 are important in the elicitation phase of contact hypersensitivity^{6-8,60,61} upregulation of these negative regulators in patients with AD might also contribute to the decreased intensity of ACD reactions.

In conclusion, immune responses to defined antigens might be altered on a background of AD, affecting both the magnitude and polarity of the responses. Our findings have implications beyond allergic sensitization; decreased vaccination responses observed in patients with AD might be mechanistically linked to attenuated ACD responses by the increased regulatory tone observed in this study.⁶² Although decreased ACD responses in patients with AD might be desirable, the decreased T_H1 component, if present in response to infectious antigens, might be undesirable for vaccinations or control of cutaneous infections. Strategies to boost T_H1 responses in AD skin might thus need to be considered to increase the effectiveness of clinically relevant immune responses.

Clinical implications: Altered reactivity to vaccinations and cutaneous infections in patients with AD might be mechanistically linked to attenuated allergic responses, and strategies to boost T_H1 responses might be necessary to increase the effectiveness of protective immunologic responses.

REFERENCES

- Guttman-Yassky E, Nograles KE, Krueger JG. Contrasting pathogenesis of atopic dermatitis and psoriasis—part I: clinical and pathologic concepts. *J Allergy Clin Immunol* 2011;127:1110-8.
- Shaw TE, Currie GP, Koudelka CW, Simpson EL. Eczema prevalence in the United States: data from the 2003 National Survey of Children's Health. *J Invest Dermatol* 2011;131:67-73.
- Thyssen JP, Linneberg A, Menné T, Johansen JD. The epidemiology of contact allergy in the general population—prevalence and main findings. *Contact Dermatitis* 2007;57:287-99.
- Suárez-Fariñas M, Dhingra N, Gittler J, Shemer A, Cardinale I, de Guzman Strong C, et al. Intrinsic atopic dermatitis shows similar T_H2 and higher T_H17 immune activation compared with extrinsic atopic dermatitis. *J Allergy Clin Immunol* 2013;132:361-70.
- Gittler J, Shemer A, Suárez-Fariñas M, Fuentes-Duculan J, Gulewicz KJ, Wang CQF, et al. Progressive activation of T(H)2/T(H)22 cytokines and selective epidermal proteins characterizes acute and chronic atopic dermatitis. *J Allergy Clin Immunol* 2012;130:1344-54.
- Pennino D, Eyerich K, Scarponi C, Carbone T, Eyerich S, Nasorri F, et al. IL-17 amplifies human contact hypersensitivity by licensing hapten nonspecific Th1 cells to kill autologous keratinocytes. *J Immunol* 2010;184:4880-8.
- Larsen JM, Bonefeld CM, Poulsen SS, Geisler C, Skov L. IL-23 and T(H)17-mediated inflammation in human allergic contact dermatitis. *J Allergy Clin Immunol* 2009;123:486-92.
- Ricciardi L, Minciullo PL, Saitta S, Trombetta D, Saija A, Gangemi S. Increased serum levels of IL-22 in patients with nickel contact dermatitis. *Contact Dermatitis* 2009;60:57-8.
- Gittler JK, Krueger JG, Guttman-Yassky E. Atopic dermatitis results in intrinsic barrier and immune abnormalities: implications for contact dermatitis. *J Allergy Clin Immunol* 2013;131:300-13.
- Jones HE, Lewis CW, McMarlin SL. Allergic contact sensitivity in atopic dermatitis. *Arch Dermatol* 1973;107:217-22.
- de Groot AC. The frequency of contact allergy in atopic patients with dermatitis. *Contact Dermatitis* 1990;22:273-7.
- Clemmensen KKB, Thomsen SF, Jemec GBE, Agner T. Pattern of contact sensitization in patients with and without atopic dermatitis in a hospital-based clinical database. *Contact Dermatitis* 2014;71:75-81.
- Spiewak R. Atopy and contact hypersensitivity: a reassessment of the relationship using objective measures. *Ann Allergy Asthma Immunol* 2005;95:61-5.
- Landeck L, Schalock P, Baden L, González E. Contact sensitization pattern in 172 atopic subjects. *Int J Dermatol* 2014;50:806-10.
- Shaughnessy CN, Malajian D, Belsito DV. Cutaneous delayed-type hypersensitivity in patients with atopic dermatitis: reactivity to topical preservatives. *J Am Dermatol* 2014;70:102-7.
- Kot M, Bogaczewicz J, Krecisz B, Wozniacka A. Contact hypersensitivity to haptens of the European standard series and corticosteroid series in the population of adolescents and adults with atopic dermatitis. *Dermatitis* 2014;25:72-6.
- Schena D, Papagrigoriaki A, Tessari G, Peroni A, Sabbadini C, Girolomoni G. Allergic contact dermatitis in children with and without atopic dermatitis. *Dermatitis* 2014;23:275-80.
- Herro EM, Matiz C, Sullivan K, Hamann C, Jacob SE. Frequency of contact allergens in pediatric patients with atopic dermatitis. *J Clin Aesthet Dermatol* 2011;4:39-41.
- Darlenski R, Kazandjieva J, Tsankov N, Fluhr JW. Acute irritant threshold correlates with barrier function, skin hydration and contact hypersensitivity in atopic dermatitis and rosacea. *Exp Dermatol* 2013;22:752-3.
- Malajian D, Belsito DV. Cutaneous delayed-type hypersensitivity in patients with atopic dermatitis. *J Am Dermatol* 2013;69:232-7.
- Yamaguchi H, Kabashima-Kubo R, Bito T, Sakabe J, Shimauchi T, Ito T, et al. High frequencies of positive nickel/cobalt patch tests and high sweat nickel concentration in patients with intrinsic atopic dermatitis. *J Dermatol Sci* 2013;72:240-5.
- Visser MJ, Verberk MM, Campbell LE, McLean WHI, Calkoen F, Bakker JG, et al. Filaggrin loss-of-function mutations and atopic dermatitis as risk factors for hand eczema in apprentice nurses: part II of a prospective cohort study. *Contact Dermatitis* 2013;70:139-50.
- Thyssen JP, Linneberg A, Ross-Hansen K, Carlsen BC, Meldgaard M, Szecsi PB, et al. Filaggrin mutations are strongly associated with contact sensitization in individuals with dermatitis. *Contact Dermatitis* 2013;68:273-6.
- Landeck L, Visser M, Skudlik C, Brans R, Kezic S, John SM. No remarkable differences in rates of sensitization to common type I and IV allergens between FLG loss-of-function mutation carriers and wild-type subjects. *Contact Dermatitis* 2014;70:27-34.
- Ross-Hansen K, Menné T, Johansen JD, Carlsen BC, Linneberg A, Nielsen NH, et al. Nickel reactivity and filaggrin null mutations—evaluation of the filaggrin bypass theory in a general population. *Contact Dermatitis* 2011;64:24-31.
- Newell L, Polak ME, Perera J, Owen C, Boyd P, Pickard C, et al. Sensitization via healthy skin programs Th2 responses in individuals with atopic dermatitis. *J Invest Dermatol* 2013;133:2372-80.
- Rees J, Friedmann PS, Matthews JN. Contact sensitivity to dinitrochlorobenzene is impaired in atopic subjects. Controversy revisited. *Arch Dermatol* 1990;126:1173-5.
- Thyssen JP, Linneberg A, Engkilde K, Menné T, Johansen JD. Contact sensitization to common haptens is associated with atopic dermatitis: new insight. *Br J Dermatol* 2012;166:1255-61.
- Forsbeck M, Hovmark A, Skog E. Patch testing, tuberculin testing and sensitization with dinitrochlorobenzene and nitrosodimethylaniline of patients with atopic dermatitis. *Acta Derm Venereol* 1976;56:135-8.

30. Dhingra N, Shemer A, Correa da Rosa J, Rozenblit M, Suárez-Fariñas M, Fuentes-Duculan J, et al. Molecular profiling of contact dermatitis skin identifies allergen-dependent differences in immune response. *J Allergy Clin Immunol* 2014;134:362-72.
31. Lammintausta K, Kalimo K, Fagerlund VL. Patch test reactions in atopic patients. *Contact Dermatitis* 1992;26:234-40.
32. Zug KA, Warshaw EM, Fowler JF, Maibach HI, Belsito DL, Pratt MD, et al. Patch-test results of the North American Contact Dermatitis Group 2005-2006. *Dermatitis* 2009;20:149-60.
33. Marks JG, Belsito DV, DeLeo VA, Fowler JF, Fransway AF, Maibach HI, et al. North American Contact Dermatitis Group patch test results for the detection of delayed-type hypersensitivity to topical allergens. *J Am Dermatol* 1998;38:911-8.
34. Pratt MD, Belsito DV, DeLeo VA, Fowler JF, Fransway AF, Maibach HI, et al. North American Contact Dermatitis Group patch-test results, 2001-2002 study period. *Dermatitis* 2004;15:176-83.
35. Guttman-Yassky E, Suárez-Fariñas M, Chiricozzi A, Nograles KE, Shemer A, Fuentes-Duculan J, et al. Broad defects in epidermal cornification in atopic dermatitis identified through genomic analysis. *J Allergy Clin Immunol* 2009;124:1235-58.
36. Nograles KE, Zaba LC, Shemer A, Fuentes-Duculan J, Cardinale I, Kikuchi T. IL-22-producing "T22" T cells account for upregulated IL-22 in atopic dermatitis despite reduced IL-17-producing Th17 T cells. *J Allergy Clin Immunol* 2009;123:1244-52.e2.
37. Guttman-Yassky E, Lowes MA, Fuentes-Duculan J, Zaba LC, Cardinale I, Nograles KE, et al. Low expression of the IL-23/Th17 pathway in atopic dermatitis compared to psoriasis. *J Immunol* 2008;181:7420-7.
38. Guttman-Yassky E, Lowes MA, Fuentes-Duculan J, Whynot J, Novitskaya I, Cardinale I, et al. Major differences in inflammatory dendritic cells and their products distinguish atopic dermatitis from psoriasis. *J Allergy Clin Immunol* 2007;119:1210-7.
39. Suárez-Fariñas M, Tintle S, Shemer A, Chiricozzi A, Nograles K, Cardinale I, et al. Nonlesional atopic dermatitis skin is characterized by broad terminal differentiation defects and variable immune abnormalities. *J Allergy Clin Immunol* 2011;127:954-64, e1-4.
40. Tintle S, Shemer A, Suarez-Farinas M, Fujita H, Gilleaudaud P, Sullivan-Whalen M, et al. Reversal of atopic dermatitis with narrow-band UVB phototherapy and biomarkers for therapeutic response. *J Allergy Clin Immunol* 2011;128:583-4.
41. Eyerich S, Eyerich K, Pennino D, Carbone T, Nasorri F, Pallotta S, et al. Th22 cells represent a distinct human T cell subset involved in epidermal immunity and remodeling. *J Clin Invest* 2009;119:3573-85.
42. Nold MF, Nold-Petry CA, Zepp JA, Palmer BE, Bufler P, Dinarello CA. IL-37 is a fundamental inhibitor of innate immunity. *Nat Immunol* 2010;11:1014-22.
43. Werfel T, Hentschel M, Renz H, Kapp A. Analysis of the phenotype and cytokine pattern of blood- and skin-derived nickel specific T cells in allergic contact dermatitis. *Int Arch Allergy Immunol* 1997;113:384-6.
44. Lindemann M, Böhmer J, Zabel M, Grosse-Wilde H. ELISpot: a new tool for the detection of nickel sensitization. *Clin Exp Allergy* 2003;33:992-8.
45. Traidl-Hoffmann C, Ring J. Is there an in vitro test for type IV allergy discriminating between sensitization and allergic disease? *Clin Exp Allergy* 2008;38:1412-5.
46. Moed H, Blomberg von M, Bruynzeel DP, Scheper R, Gibbs S, Rustemeyer T. Improved detection of allergen-specific T-cell responses in allergic contact dermatitis through the addition of 'cytokine cocktails'. *Exp Dermatol* 2005;14:634-40.
47. Wilke CM, Bishop K, Fox D, Zou W. Deciphering the role of Th17 cells in human disease. *Trends Immunol* 2011;32:603-11.
48. Kryczek I, Bruce AT, Gudjonsson JE, Johnston A, Aphale A, Vatan L, et al. Induction of IL-17+ T cell trafficking and development by IFN-gamma: mechanism and pathological relevance in psoriasis. *J Immunol* 2008;181:4733-41.
49. Cua DJ, Sherlock J, Chen Y, Murphy CA, Joyce B, Seymour B, et al. Interleukin-23 rather than interleukin-12 is the critical cytokine for autoimmune inflammation of the brain. *Nature* 2003;421:744-8.
50. Dhingra N, Gulati N, Guttman-Yassky E. Mechanisms of contact sensitization offer insights into the role of barrier defects vs. intrinsic immune abnormalities as drivers of atopic dermatitis. *J Invest Dermatol* 2013;133:2311-4.
51. Milanez-Almeida P, Klawonn F, Meyer-Hermann M, Huehn J. Differential control of immune cell homeostasis by Foxp3(+) regulatory T cells in murine peripheral lymph nodes and spleen. *Eur J Microbiol Immunol (Bp)* 2014;4:147-55.
52. Lindenberg JJ, Oosterhoff D, Sombroek CC, Loughseed SM, Hooijberg E, Stam AG, et al. IL-10 conditioning of human skin affects the distribution of migratory dendritic cell subsets and functional T cell differentiation. *PLoS One* 2013;8:e70237.
53. Wulfig C, Tunbridge HM, Wraith DC. New inhibitory signaling by CTLA-4. *Nat Immunol* 2014;15:408-9.
54. Li Y, Wang Z, Yu T, Chen B, Zhang J, Huang K, Huang Z. Increased expression of IL-37 in patients with Graves' disease and its contribution to suppression of proinflammatory cytokines production in peripheral blood mononuclear cells. *PLoS One* 2014;9:e107183.
55. van de Veerdonk FL, Stoeckman AK, Wu G, Boeckermann AN, Azam T, Netea MG, et al. IL-38 binds to the IL-36 receptor and has biological effects on immune cells similar to IL-36 receptor antagonist. *Proc Natl Acad Sci U S A* 2012;109:3001-5.
56. Ye L, Ji L, Wen Z, Zhou Y, Hu D, Li Y, et al. IL-37 inhibits the production of inflammatory cytokines in peripheral blood mononuclear cells of patients with systemic lupus erythematosus: its correlation with disease activity. *J Transl Med* 2014;12:69.
57. Sharma S, Kulk N, Nold MF, Gräf R, Kim SH, Reinhardt D, et al. The IL-1 family member 7b translocates to the nucleus and down-regulates proinflammatory cytokines. *J Immunol* 2008;180:5477-82.
58. Teng X, Hu Z, Wei X, Wang Z, Guan T, Liu N, et al. IL-37 ameliorates the inflammatory process in psoriasis by suppressing proinflammatory cytokine production. *J Immunol* 2014;192:1815-23.
59. Fujita H, Inoue Y, Seto K, Komitsu N, Aihara M. Interleukin-37 is elevated in subjects with atopic dermatitis. *J Dermatol Sci* 2013;69:173-5.
60. Nakae S, Komiyama Y, Nambu A, Sudo K, Iwase M, Homma I, et al. Antigen-specific T cell sensitization is impaired in IL-17-deficient mice, causing suppression of allergic cellular and humoral responses. *Immunity* 2002;17:375-87.
61. Peiser M. Role of Th17 cells in skin inflammation of allergic contact dermatitis. *Clin Dev Immunol* 2013;2013:261037.
62. Slifka MK, Leung DY, Hammarlund E, Raue HP, Simpson EL, Tofte S, et al. Transcutaneous yellow fever vaccination of subjects with or without atopic dermatitis. *J Allergy Clin Immunol* 2014;133:439-47.

1 **Methods**

2 **Patients' Characteristics and skin samples**

3 Patch testing was performed using the North American Contact Dermatitis
4 Group Standard (NACDG) for 15 common contact allergens and at 72 hours the
5 patch test site was evaluated for positive reactions. A reaction score of 1+, 2+, or
6 3+ was assigned to any positive reactions, assessed via established patch test
7 guidelines.^{E1,E2} Biopsies were taken from allergen-reactive patches and control
8 skin (not exposed to the allergen) under institutional review board-approved
9 protocols. Overall, AD patients had 4 positive reactions to nickel, 2 to fragrance,
10 and 3 to rubber. Patients without AD had 6 positive reactions to nickel, 1 to
11 fragrance, and 3 to rubber. Four patients with reactions to other metals were
12 excluded from the analysis due to the small sample size and previous data
13 showing that other metals and nickel polarize differently and should not be
14 grouped together.⁵⁵ One patient was excluded since control skin was
15 unavailable. Serum IgE levels were elevated in 10/219 patients (range 28.6-
16 5000, mean 560, reference range 0-114 kU/L).

17 **Immunohistochemistry**

18 Biopsy specimens were frozen in O.C.T. medium and cryostat tissue
19 sections of all patients were stained with hematoxylin (Fisher, Fair Lawn, NJ) and
20 eosin (Shandon, Pittsburgh, PA). Immunohistochemistry (IHC) was performed by
21 using purified anti-human monoclonal antibodies. For IHC, biotin-labeled horse
22 anti-mouse secondary antibodies (Vector Laboratories) were used to detect the
23 primary antibodies, and the staining signal was developed with chromogen 3-

amino-9-ethylcarbazole (AEC, Sigma-Aldrich). Epidermal thickness and positive cells per millimeter were manually quantified by using computer-assisted image analysis software (ImageJ 1.42; National Institutes of Health, Bethesda, MD).

Quantitative real-time (RT) PCR and gene-array analysis

RNA was extracted and Affymetrix Human U133Plus 2.0 arrays (Affymetrix, Santa Clara, CA) were used as previously described.^{E3} Briefly, we used human HGU133Plus2.0 GeneChip probe arrays (Affymetrix Inc, Santa Clara, Calif) containing approximately 54,000 probe sets to assess expression of more than 47,000 transcripts, including approximately 38,500 genes and unigenes. Total RNA was extracted from tissues frozen in liquid nitrogen by the Qiagen RNeasy Mini Kit (Valencia, Calif). DNA was removed with on-column DNase digestion by the Qiagen RNase-free DNase Set. Total RNA (50 ng) was reverse transcribed and amplified with Ovation Whole Blood Solution from NuGen (San Carlos, Calif). The labeled target was fragmented and hybridized to probe arrays, using Encore Biotin Module from NuGen (San Carlos, Calif). The chips were washed, stained with streptavidin-phycoerythrin, and scanned (HP GeneArray Scanner; Hewlett-Packard Company, Palo Alto, Calif). On each chip, the human housekeeping genes β -actin and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) served as controls. Suite 5.0 software normalized the expression level values using these controls.

47 RT-PCR Analysis

48 The TaqMan RT-PCR reaction was performed with EZ-PCR Core Reagents
 49 (Life Technologies), according to the manufacturer's directions. The primers and
 50 probes for CCL13 (Hs00234646_m1), CCL17 (Hs00171074_m1), CCL18
 51 (Hs00268113_m1), CCL19 (Hs00171149_m1), CCL20 (Hs01011368_m1),
 52 CCL21 (Hs00989654_g1), CCL22 (Hs01574247_m1), CCL26
 53 (Hs00171146_m1), CCL5 (Hs00174575_m1), CCR7 (Hs01013469_m1),
 54 CXCL10 (Hs01124251_g1), CXCL11 (Hs04187682_g1), CXCL9
 55 (Hs00171065_m1), Foxp3 (Hs01085834_m1), GATA3 (Hs00231122_m1), IFNA1
 56 (Hs00256882_s1), IFNG (Hs00989291_m1), IL10 (Hs00961622_m1), IL13
 57 (Hs00174379_m1), IL17A (Hs00174383_m1), IL17F (Hs00369400_m1), IL18
 58 (Hs01038788_m1), IL1B (Hs01555410_m1), IL2 (Hs00174114_m1), IL22
 59 (Hs01574154_m1), IL12p19 (Hs00900828_g1), IL23p40 (Hs01011518_m1),
 60 IL2RA (Hs00907779_m1), IL31 (Hs01098710_m1), IL32 (Hs00992441_m1), IL33
 61 (Hs00369211_m1), IL37 (Hs00367201_m1), IL4 (Hs00174122_m1), IL5
 62 (Hs01548712_g1), IL6 (Hs00985639_m1), IL7 (Hs00174202_m1), IL7R
 63 (Hs00902334_m1), IL8 (Hs00174103_m1), IL9 (Hs00914237_m1), LCN2
 64 (Hs01008571_m1), MX1 (Hs00895608_m1), OX40 (Hs00937194_g1), OX40L
 65 (Hs00182411_m1), PI3 (Hs00160066_m1), PU1 (Hs02786711_m1), RORG
 66 (Hs01076122_m1), S100A7 (Hs01923188_u1), S100A8 (Hs00374264_g1),
 67 S100A9 (Hs00610058_m1), TBET (Hs00203436_m1), TGFB (Hs00998133_m1),
 68 TNFa (Hs01113624_g1), TSLP (Hs00263639_m1), TSLPR (Hs00845692_m1)
 69 were designed by Life Technologies. The human acidic ribosomal protein (hARP)

gene, a housekeeping gene, was used to normalize each sample and each gene. The primer sequences used were generated with the Primer Express algorithm (Applied Biosystems, Foster City, Calif), version 1.0, by using published genetic sequences (National Center for Biotechnology Information–PubMed), as follows: hARP forward CGCTGCTGAACATGCTCAA, hARP reverse TGTCGAACACCTGCTGGATG, and hARP probe 6-FAM-TCCCCCTTCTCCTTTGGGCTGG-TAMRA (Gene Bank accession no. NM-001002). The data were analyzed and quantified by using the software provided with the Applied Biosystems PRISM 7700 (Sequence Detection Systems, version 1.7).

Statistical Analysis

Statistical methods are initially used in this paper to gather evidence that gene expression and population cell counts differ between two groups: subjects with atopic dermatitis personal history (AD) and the ones without it (non-AD), controlling by their control skin. Microarray, RT-PCR gene expression and immunohistochemistry cell counts were obtained from an initial sample of n=24 subjects. A subgroup of 19 patients patch tested for nickel, rubber and fragrance allergen was separated for further analysis. The comparisons carried for the whole data set were replicated for nickel and rubber/fragrance subgroups.

The comparisons are planned to identify genes that are differentially expressed in patch-tested AD and non-AD subjects when compared to their

control skin. When subgroup analysis was performed, due to small sample size, cut-offs determined for decisions about significance were less restrictive (one-sided tests and p-values below 0.10). Quality control of microarrays was carried out using standard QC metrics and R package Quality Control and Harshlight.^{E4} One patient (#17) was classified as an outlier and removed from the analysis.

Expression measures were obtained using GCRMA algorithm.^{E5} Probe sets with at least 1 sample with expression values larger than 4 and standard deviation (SD)>0.15 were kept for further analyses. To follow normality assumptions required by most of the statistical methods used in this paper, the expressions were log2-transformed. Although data from immunohistochemistry are measured as counts, we assumed normal distribution for statistical modeling purposes.

Genomics Statistical Analysis:

In Figure 2B, a heatmap for gene expression was used to illustrate differences between AD, non-AD groups and control skin. The criteria for gene selection were: fold-change higher than 2 and FDR (False-Discovery Rate) lower than 0.05. The distance matrix was built based on Euclidean distances and McQuitty algorithm was used for gene clustering, The same parameters for clustering were applied to the heatmaps in Figure 3 and 5A that included immune and negative regulators genes, respectively.

For microarray and RT-PCR data, log2-transformed expression values were modeled using linear mixed-effects models with AD status and control skin

as fixed effects and a random effect for each patient. Comparisons of interest were assessed using linear contrasts via restricted log-likelihood maximization (REML). The contrasts of interest were designed to perform the following comparisons: AD vs. Control, non-AD vs. Control and AD vs. non-AD. The later contrast was carried with and without adjusting expressions by background skin. Analysis for arrays was conducted under the general framework of *limma* package. P-values from moderated (paired) t-test were adjusted for multiple hypotheses across genes using Benjamini-Hochberg procedure. LME, as implemented in *limma*'s R package from Bioconductor, provides tools that can handle high-dimensionality data with the use of moderated t-statistics and selection of significant genes using principles from Bayesian Inference (e.g. empirical Bayes).

All differentially expressed probes were divided into three groups: AD, Common and non-AD, according to the significance found in the contrasts. Probes that are simultaneously differentially expressed in the contrasts: AD vs. Control and non-AD vs. Control are classified in the Common group.

Pathway Analysis:

For Figure 2C, box-plots were created using the distribution of the $\log_2(\text{FCH})$ of immune, Th1 and Th2 genes within each allergen (Nickel or Rubber/Fragrance) vs. control. This highlights major pathway differences between groups. The size of the individual dots (representing distinct genes) is scaled to their FCH for each allergen.

Figures 4 and 5B show bar plots of RT-PCR $\log_2(\text{FCH})$ of each allergen and with mean $\pm$ SEM displayed in a bar plot. Stars directly above the error bars indicate the significance in one-sided tests for comparisons against control ($p < 0.1$ (*), $p < 0.05$ (**), $p < 0.01$ (***)). Comparisons between AD and non-AD groups are also indicated, here with lines that link the two allergens being compared, again with stars to indicate significance.

Immunohistochemistry Statistical Analysis:

Biomarkers for IHC analysis were analyzed with linear mixed effect models as implemented in *lme* function that is part of *nlme* package in R. The high magnitude of the cell counts and inspection of the data distribution with histograms contributed to assumption of Gaussian distribution for the responses. Skin (background or patch-tested for an allergen) and AD personal history status were treated as fixed effects and a random intercept for each patient was included. Estimates and significance of linear contrasts representing differences between groups and their controls are represented in the bar plots displayed in Figure 1.

References

- E1. Marks JG, Belsito DV, DeLeo VA, Fowler JF, Jr., Fransway AF, Maibach HI, et al. North American Contact Dermatitis Group patch test results for the detection of delayed-type hypersensitivity to topical allergens. *J Am Acad Dermatol* 1998; 38:911-8.
- E2. Pratt MD, Belsito DV, DeLeo VA, Fowler JF, Jr., Fransway AF, Maibach HI, et al. North American Contact Dermatitis Group patch-test results, 2001-2002 study period. *Dermatitis* 2004; 15:176-83.
- E3. Tintle S, Shemer A, Suarez-Farinas M, Fujita H, Gilleaudeau P, Sullivan-Whalen M, et al. Reversal of atopic dermatitis with narrow-band UVB phototherapy and biomarkers for therapeutic response. *J Allergy Clin Immunol* 2011 Sep;128(3):583-93 e1-4.
- E4. Suarez-Farinas M, Pellegrino M, Wittkowski KM, Magnasco MO. Harshlight: a "corrective make-up" program for microarray chips. *BMC bioinformatics*. 2005;6:294.
- E5. Wu Z, Irizarry R, Gentleman R, Murillo F, Spencer F. A model-based background adjustment for oligonucleotide expression arrays. *J Am Stat Assoc* 2004;99:909-17.
- E6. Storrs FJ, White CR, Jr. False-positive "poral" cobalt patch test reactions reside in the eccrine acrosyringium. *Cutis* 2000; 65:49-53.

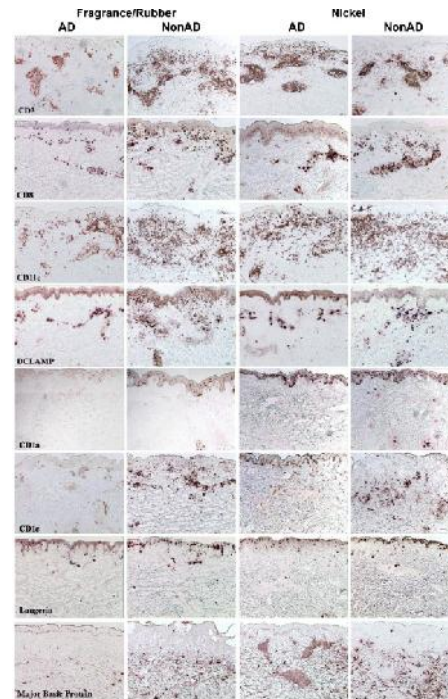


Fig E1

Joel Correa da Rosa , Dana Malajian , Avner Shemer , Mariya Rozenblit , Nikhil Dhingra , Tali Czarnowicki , Saaksh...

Patients with atopic dermatitis have attenuated and distinct contact hypersensitivity responses to common allergens in skin

Journal of Allergy and Clinical Immunology, 2015

<http://dx.doi.org/10.1016/j.jaci.2014.11.017>

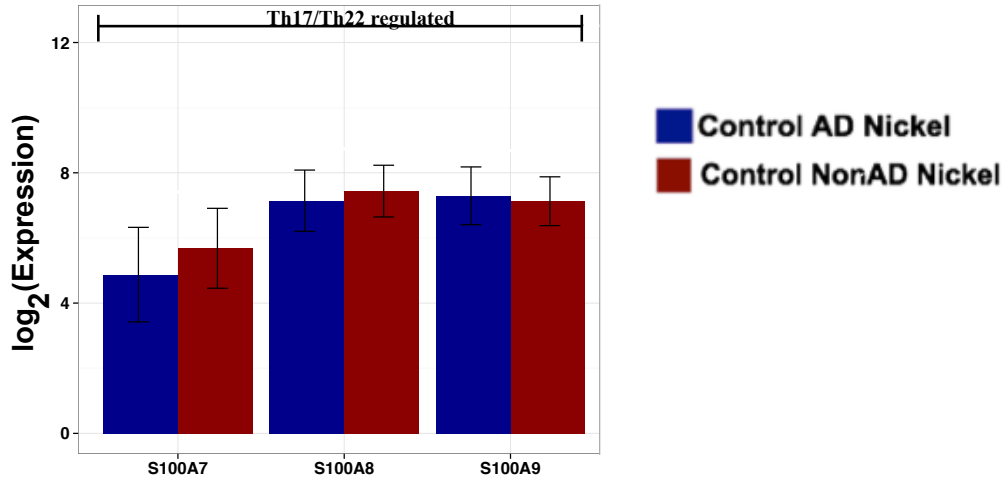
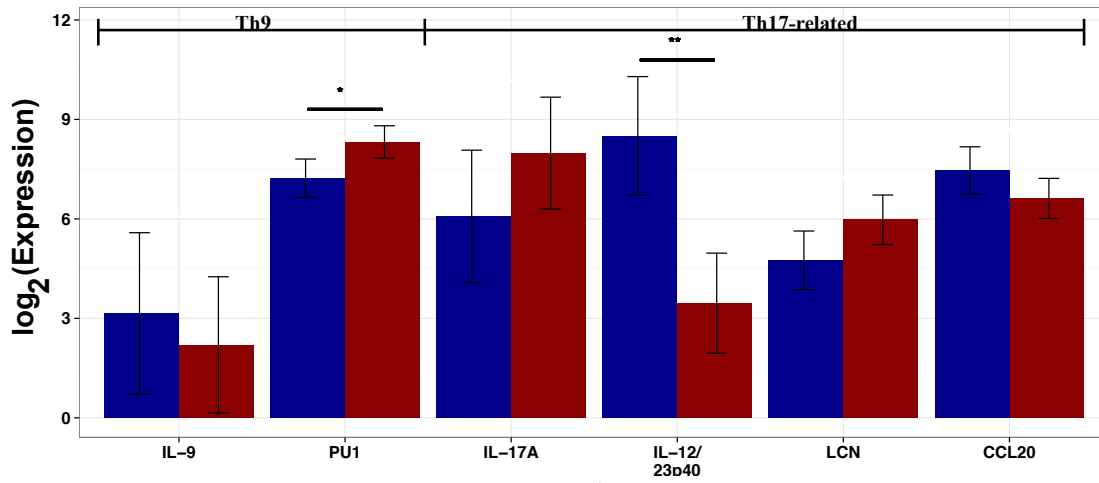
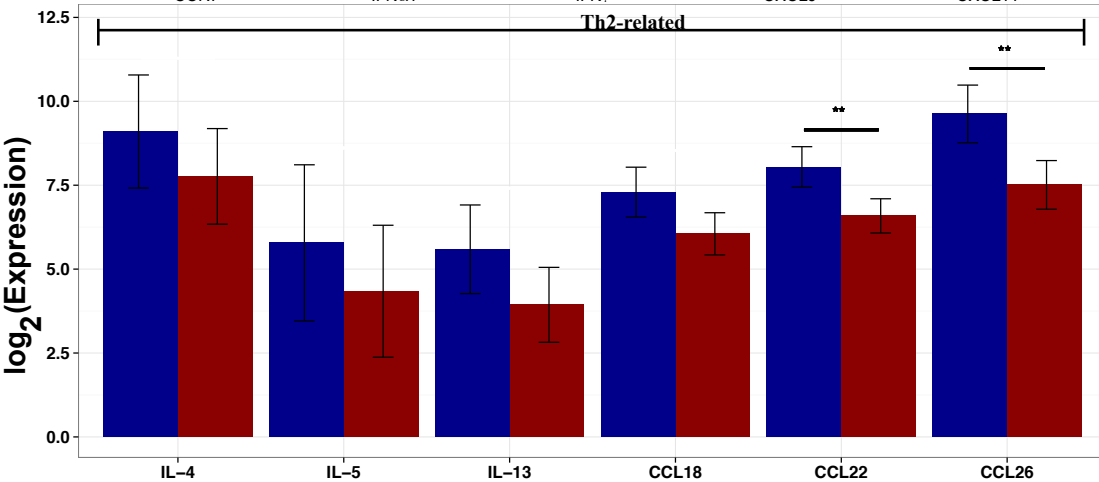
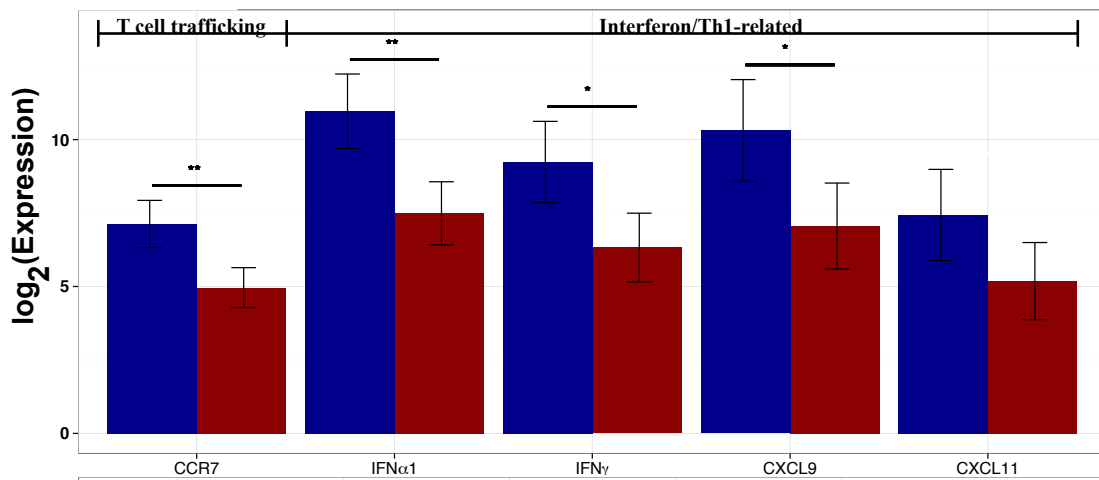


Table E1. Characteristics of Study Patients

Pt #	Gender	Age	Allergen Group^a	Reaction^b	AD History	Serum IgE (kU/L)	FLG mutation	Other Positive Patch-Test Reactions
1	F	37	Nickel	+3	N	225	Negative	Cobalt +2 Phenoxyethanol +1 Phenylenediamine +1 BND +1 Potassium Dichromate +1 Dust Mite +1
2	F	28	Nickel	+3	N	99.4	n/a	Dust Mite +1
3	M	39	Rubber	+2	N	94	Negative	Thiuram +2 Carba Mix +2 Cobalt +2 Peanut +1 Dust Mite +1
4	M	20	Fragrance	+2	N	98.7	n/a	Dust Mite +1
5	M	63	Rubber	+2	N	134	n/a	Balsam of Peru +1 Cobalt +1 Peanut 1 Soybean +1 Lentils +1 Wheat +1 Oat +1 Codfish +1 Apple +1
6	F	55	Nickel	+2	N	36.6	Negative	Dust Mite +1
7	F	57	Nickel	+2	N	28.6	Negative	Dust Mite +1 Codfish +1
8	F	51	Nickel	+2	N	39.8	n/a	Cobalt +1
9	M	23	Rubber	+1	N	59	n/a	Dust Mite +2
10	F	54	Nickel	+1	N	264	Negative	Quaternum-15 +1 Carrot +1 Celery +1
11	F	50	Fragrance	+2	Y	1414	R501Xneg 2282del4- hetero	Codfish +1
12	F	55	Rubber	+2	Y	83.8	Negative	None
13	F	52	Nickel	+2	Y	528	n/a	Dust Mite +2 Lentils +1 Sesame +1
14	F	48	Nickel	+2	Y	5000	Negative	Cobalt +2
15	F	45	Nickel	+2	Y	39.5	n/a	None
16	F	33	Nickel	+2	Y	1205	n/a	Dust Mite +1 Carba +2 BOP +1
17	M	31	Rubber	+1	Y	143	n/a	None
18	M	34	Rubber	+1	Y	827	Negative	None
19	M	42	Fragrance	+1	Y	322	n/a	Carba Mix +1 Soybean +1 Barley +1
20	M	28	PPD	+3	N	n/a	n/a	Dust Mite +2 Lentils +1 Wheat +1 Cow Milk +1 Egg White +1
21	M	36	Cobalt	+1	N	n/a	n/a	Dust Mite +1 Peanut +1
22	F	32	Cobalt	+1	Y	783	n/a	Dust Mite +1

23	F	33	Potassium Dichromate	+1	N	n/a	n/a	Lentils +1
24	F	39	Cobalt	+1	N	n/a	n/a	Dust Mite +1

a: Allergens were grouped into two categories: Nickel (10 patients) and Rubber/Fragrance (2 BOP, 1 Fragrance mix, 5 Carba Mix, 1 Thiuram mix patients), Cobalt (3 patients), PPD (1 patient), Potassium Dichromate (1 patient)

b: Positive-patch tests were graded according to established guidelines (See Methods and Supplementary Methods)

Table E2. Antibodies used for Immunohistochemistry

Antibody	Vendor	Clone	Isotype	Dilution
CD3	BD Biosciences	SK7	IgG1	1:100
CD8	Abcam	144B	IgG1	1:25
CD11c	BD Biosciences	B-ly6	IgG1	1:100
DC-LAMP	Fisher Scientific	104.G4	IgG1	1:50
Langerin	Immunotech	DCGM4	IgG1	1:100
CD1c	Miltenyi Biotec	AD5-8E7	IgG2a	1:100
MBP	Thermo Scientific	BMK13	IgG1	1:15

Table E6. Summary of log2-Fold Changes relative to background skin in Th1- and Th2-related genes within specific allergen subsets.

Allergen / Group	Th1			Th2			p-value*
	Mean	SD	N	Mean	SD	N	
Nickel non-AD	3.07	2.28	25	2.49	1.94	14	0.41
Nickel AD	2.20	1.46	25	1.41	1.26	14	0.08
RbFG non-AD	1.60	1.14	25	1.75	1.63	14	0.76
RbFG AD	1.48	1.07	25	2.00	1.59	14	0.28

*P-values are calculated for the two-sided t-test that compares average log2-FCH in Th1 genes vs. Th2 genes. Significant p-values were observed for Th1 vs Th2 fold-changes in Nickel AD.

Table E7. Descriptive Statistics for Cell Counts from Immunohistochemistry

	CD3			CD8		
Group	Mean	SD	N	Mean	SD	N
Control non-AD	94.91	43.74	11	47.27	24.58	11
Control AD	102.22	68.44	9	46.67	17.85	9
Nickel non-AD	895.83	458.97	6	366.00	179.01	6
Nickel AD	1001.75	890.89	4	291.00	218.08	4
RbFg non-AD	742.40	614.56	5	245.80	244.59	5
RbFg AD	421.40	123.96	5	154.80	108.42	5
	CD11			DCLAMP		
Group	Mean	SD	N	Mean	SD	N
Control non-AD	47.27	24.58	11	22.55	29.62	11
Control AD	46.67	17.85	9	10.11	14.32	9
Nickel non-AD	366.00	179.01	6	218.17	229.90	6
Nickel AD	291.00	218.08	4	203.75	211.74	4
RbFg non-AD	245.80	244.59	5	234.80	205.80	5
RbFg AD	154.80	108.42	5	110.40	83.49	5
	CD1a			CD1c		
Group	Mean	SD	N	Mean	SD	N
Control non-AD	60.64	23.88	11	69.09	40.54	11
Control AD	27.33	15.22	9	64.44	36.66	9
Nickel non-AD	73.50	38.62	6	101.33	79.43	6
Nickel AD	36.00	22.46	4	17.00	10.89	4
RbFg non-AD	55.20	49.92	5	185.00	209.46	5
RbFg AD	26.40	8.26	5	84.00	61.16	5
	LANGERIN			MBP		
Group	Mean	SD	N	Mean	SD	N
Control non-AD	90.36	67.74	11	4.00	3.62	11
Control AD	91.89	64.76	9	2.38	4.03	9
Nickel non-AD	64.67	33.29	6	34.67	26.90	6
Nickel AD	26.25	14.61	4	23.00	7.00	4
RbFg non-AD	88.80	41.76	5	28.50	12.12	5
RbFg AD	44.40	28.59	5	45.00	44.40	5

Table E8. Summary Statistics for log2-Expressions for RT-PCR in allergen subsets

	CCR7			IFNa1		
Group	Mean	SD	N	Mean	SD	N
Control non-AD	5.23	1.16	10	7.59	3.45	10
Control AD	6.54	1.30	9	9.94	3.15	9
Nickel non-AD	9.36	1.78	6	10.55	2.58	6
Nickel AD	10.08	2.79	4	11.28	2.39	4
RbFg non-AD	9.82	2.71	4	6.90	5.06	4
RbFg AD	9.08	1.11	5	10.58	1.26	5
	IFNg			CXCL9		
Group	Mean	SD	N	Mean	SD	N
Control non-AD	6.92	2.76	10	7.02	2.83	10
Control AD	8.14	2.78	9	9.96	2.20	9
Nickel non-AD	10.89	3.15	6	12.24	3.81	6
Nickel AD	11.36	3.78	4	13.79	5.19	4
RbFg non-AD	9.87	5.26	4	10.99	5.66	4
RbFg AD	10.99	1.67	5	11.91	2.86	5
	CXCL11			IL-4		
Group	Mean	SD	N	Mean	SD	N
Control non-AD	5.66	1.88	10	7.43	1.95	10
Control AD	7.13	1.77	9	7.97	2.44	9
Nickel non-AD	11.28	3.76	6	10.63	3.94	6
Nickel AD	11.37	5.41	4	11.55	5.34	4
RbFg non-AD	9.03	5.28	4	10.04	3.71	4
RbFg AD	9.98	3.19	5	9.43	3.57	5
	IL-5			IL-13		
Group	Mean	SD	N	Mean	SD	N
Control non-AD	4.73	4.88	10	4.61	2.90	10
Control AD	6.11	4.71	9	5.66	2.64	9
Nickel non-AD	12.44	5.45	6	9.17	1.79	6
Nickel AD	11.79	6.03	4	8.81	3.96	4
RbFg non-AD	12.03	7.42	4	9.62	2.65	4
RbFg AD	15.50	1.10	5	10.67	0.60	5
	CCL18			CCL22		
Group	Mean	SD	N	Mean	SD	N
Control non-AD	5.66	1.07	10	6.83	1.11	10
Control AD	6.57	2.07	9	7.39	1.09	9
Nickel non-AD	8.67	1.15	6	10.04	1.45	6
Nickel AD	8.10	2.16	4	9.88	1.78	4
RbFg non-AD	8.25	3.60	4	10.27	2.47	4

RbFg AD	7.54	0.93	5	10.02	1.37	5
	CCL26			IL-9		
Group	Mean	SD	N	Mean	SD	N
Control non-AD	7.59	1.34	10	3.52	4.34	10
Control AD	9.37	1.40	9	2.82	3.32	9
Nickel non-AD	10.73	2.95	6	16.10	5.91	6
Nickel AD	10.67	1.55	4	9.71	8.19	4
RbFg non-AD	11.48	1.92	4	12.18	7.99	4
RbFg AD	11.92	1.60	5	13.20	3.54	5
	PU1			IL-17A		
Group	Mean	SD	N	Mean	SD	N
Control non-AD	8.10	1.25	10	8.17	3.69	10
Control AD	7.70	1.19	9	6.91	3.77	9
Nickel non-AD	10.29	1.48	6	10.64	4.34	6
Nickel AD	8.81	1.23	4	10.82	5.74	4
RbFg non-AD	9.60	1.14	4	10.30	4.24	4
RbFg AD	8.79	1.05	5	9.00	4.10	5
	IL-12/23p40			LCN		
Group	Mean	SD	N	Mean	SD	N
Control non-AD	4.29	3.98	10	6.11	2.20	10
Control AD	8.94	3.73	9	5.45	1.73	9
Nickel non-AD	8.06	3.43	6	7.80	1.74	6
Nickel AD	10.50	2.90	4	8.33	1.39	4
RbFg non-AD	8.21	5.33	4	8.26	1.22	4
RbFg AD	11.45	2.15	5	7.69	1.36	5
	CCL20			IL-22		
Group	Mean	SD	N	Mean	SD	N
Control non-AD	6.90	2.30	10	7.12	1.97	10
Control AD	8.21	1.09	9	8.61	3.36	9
Nickel non-AD	8.76	0.79	6	11.65	3.64	6
Nickel AD	10.31	0.94	4	11.39	5.08	4
RbFg non-AD	8.19	1.01	4	10.63	4.92	4
RbFg AD	8.77	0.79	5	12.48	1.20	5
	S100A7			S100A8		
Group	Mean	SD	N	Mean	SD	N
Control non-AD	5.89	3.01	10	7.67	2.65	10
Control AD	5.79	2.45	9	7.64	1.90	9
Nickel non-AD	9.72	2.34	6	11.64	1.91	6
Nickel AD	6.72	4.85	4	10.92	0.80	4
RbFg non-AD	10.77	0.95	4	13.35	1.21	4
RbFg AD	8.98	1.58	5	11.09	0.88	5

	S100A9			IL-37		
Group	Mean	SD	N	Mean	SD	N
Control non-AD	7.41	2.66	10	7.18	1.77	10
Control AD	7.59	2.13	9	7.93	0.57	9
Nickel non-AD	12.26	1.64	6	5.93	1.62	6
Nickel AD	10.97	1.28	4	6.19	1.11	4
RbFg non-AD	13.42	1.07	4	4.59	2.17	4
RbFg AD	10.75	1.50	5	6.83	0.70	5
	IL-10			FOXP3		
Group	Mean	SD	N	Mean	SD	N
Control non-AD	7.80	2.06	10	5.93	1.94	10
Control AD	9.53	1.26	9	7.03	1.57	9
Nickel non-AD	9.41	2.36	6	7.89	2.26	6
Nickel AD	11.95	2.35	4	8.90	2.93	4
RbFg non-AD	10.45	2.19	4	9.57	1.25	4
RbFg AD	9.87	1.17	5	9.05	1.40	5

Supplementary Figure Legends

Fig E1. Staining for CD3, CD8, CD11c, CDLAMP, CD1a, CD1c, Langerin, and Major Basic Protein in AD and NonAD patients patch tested with Fragrance/Rubber and Nickel, demonstrating increased infiltrates of T-cells, DCs, and Langerhans cells in non-AD skin compared to AD patch-tested skin (histology magnification, X10).

Fig E2. Cell counts for neutrophil elastase was compared between control (not patch-tested) AD and non-AD skin. There was no statistical difference in neutrophil elastase+ infiltrates in the AD and non-AD samples.

Fig E3. Real-time PCR was used to evaluate expression of major cytokines and inflammatory markers in skin from healthy controls who were matched against nickel patch-tested skin. Data were grouped according to AD status and by the major inflammatory pathways represented. *P* values of AD vs non-AD expression are indicated if significant. **P* < .05, ***P* < .01, and ****P* < .001.