

## Molecular profiling of contact dermatitis skin identifies allergen-dependent differences in immune response

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**Background:** Allergic contact dermatitis (ACD) is the most common occupational disease. Although murine contact hypersensitivity provides a framework for understanding ACD, it carries important differences from its human counterpart. Unlike the contact hypersensitivity model, which is induced by potent sensitizers (ie, dinitrofluorobenzene), human ACD is induced by weak-to-moderate sensitizers (ie, nickel), which cannot induce reactions in mice. Distinct hapten-specific immune-polarizing responses to potent inducers were suggested in mice, with unclear relevance to human ACD.

**Objective:** We explored the possibility of distinct T-cell polarization responses in skin to common clinically relevant ACD allergens.

**Methods:** Gene-expression and cellular studies were performed on common allergens (ie, nickel, fragrance, and rubber) compared with petrolatum-occluded skin, using RT-PCR, gene arrays, and immunohistochemistry.

**Results:** Despite similar clinical reactions in all allergen groups, distinct immune polarizations characterized different allergens. Although the common ACD transcriptome consisted of 149 differentially expressed genes across all allergens versus petrolatum, a much larger gene set was uniquely altered by

individual allergens. Nickel demonstrated the highest immune activation, with potent inductions of innate immunity, T<sub>H</sub>1/T<sub>H</sub>17 and a T<sub>H</sub>22 component. Fragrance, and to a lesser extent rubber, demonstrated a strong T<sub>H</sub>2 bias, some T<sub>H</sub>22 polarization, and smaller T<sub>H</sub>1/T<sub>H</sub>17 contributions.

**Conclusions:** Our study offers new insights into the pathogenesis of ACD, expanding the understanding of T-cell activation and associated cytokines in allergen-reactive tissues. It is the first study that defines the common transcriptome of clinically relevant sensitizers in human skin and identifies unique pathways preferentially activated by different allergens, suggesting that ACD cannot be considered a single entity. (*J Allergy Clin Immunol* 2014;134:362-72.)

**Key words:** Allergic contact dermatitis, patch testing, T-cell polarization, human skin, allergens, nickel, fragrance, rubber

Allergic contact dermatitis (ACD) is the most common occupational disease, with a prevalence of 15% to 25%.<sup>1-3</sup> It is characterized by intensely pruritic patches, with erythema, edema, and occasional vesicles. ACD represents a type IV delayed-type hypersensitivity response to antigens that come in contact with the skin. It is induced by allergens or haptens, which are small chemically reactive molecules (<500 Da). The most common clinically relevant sensitizers in occupational and nonoccupational exposure include metals (particularly nickel), fragrance, and rubber.<sup>1-6</sup> Two distinct phases are involved in ACD: the sensitization phase, which represents the first contact with the allergen and has no clinical manifestations, and the elicitation phase, which occurs on re-challenge, producing visible dermatitis with peak inflammatory reactions at 72 and 24 hours in human and mice, respectively.<sup>7-10</sup> Patch testing is the criterion standard procedure to diagnose ACD.<sup>11</sup> A positive patch test result induces the elicitation phase, resulting in an eczematous rash, identifying the offending sensitizer.

Immune mechanisms of ACD are incompletely understood. In murine contact hypersensitivity (CHS), a model for human ACD, reactions to strong sensitizers (ie, dinitrochlorobenzene, diphenylcyclopropenone, dinitrofluorobenzene, and trinitrochlorobenzene) have been characterized at the molecular level, but mice do not have reactions to weak sensitizers (ie, metals, fragrance, and rubber) that are clinically relevant in human ACD.<sup>2,12,13</sup> Hence, whether effector immune responses are similar between strong haptens and medically relevant antigens is unknown. Furthermore, most studies generalized ACD as a single phenomenon rather than investigating the differences among the many allergens.<sup>8,9,14-17</sup> In murine CHS

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#### Abbreviations used

ACD:	Allergic contact dermatitis
AD:	Atopic dermatitis
CCL13:	Chemokine, CC motif, ligand 13
CCL17:	Chemokine, CC motif, ligand 17
CCL18:	Chemokine, CC motif, ligand 18
CCL26:	Chemokine, CC motif, ligand 26
CHS:	Contact hypersensitivity
CTLA4:	Cytotoxic T-lymphocyte antigen 4
CXCL1:	Chemokine, CXC motif, ligand 1
CXCL2:	Chemokine, CXC motif, ligand 2
CXCL9:	Chemokine, CXC motif, ligand 9
CXCL10:	Chemokine, CXC motif, ligand 10
CXCL11:	Chemokine, CXC motif, ligand 11
DC:	Dendritic cell
DEG:	Differentially expressed gene
IHC:	Immunohistochemistry
PI3:	Peptidase inhibitor 3
RT-PCR:	Real-time PCR
S100A:	S100-Calcium binding protein

models, different potent sensitizers have been used to induce distinct polar T-cell responses,<sup>9</sup> implying the possibility that effector immune activation pathways could also be allergen specific in human ACD.

Both CD4<sup>+</sup> and CD8<sup>+</sup> T cells can act as effectors in type IV delayed-type hypersensitivity and CHS reactions (although CD4<sup>+</sup> T cells appear to dominate human effector responses).<sup>14,18</sup> Effector responses involve a complex interplay between dendritic cells (DCs), Langerhans cells, keratinocytes, T-cell activation, and loss of regulatory T cell-mediated suppression. Although it was historically considered a T<sub>H</sub>1-dominated or a mixed T<sub>H</sub>1/T<sub>H</sub>2 response, ACD is increasingly recognized as involving production of T<sub>H</sub>17 and T<sub>H</sub>22 cytokines.<sup>14-21</sup>

Most human studies that evaluated effector responses to common allergens have been performed on peripheral blood from patients with ACD using cytokine activation assays.<sup>17,22-28</sup> Very few studies were performed directly on inflamed skin, and these studies were further restricted to analyses of a limited subset of immune pathways.<sup>14,18,29-32</sup>

This study is the first to perform extensive molecular and cellular profiling for a range of clinically relevant allergens identified by patch tests to establish common denominators of ACD (irrespective of allergen) and identify potential allergen-specific differences. Our data suggest that allergens induce differential immune activation in patch-tested skin.

## METHODS

### Patients' characteristics and skin samples

Patch testing was performed on 24 patients with patch-test proven ACD to common allergens using the 15 most common allergens of the North American Contact Dermatitis Group<sup>33</sup> and petrolatum-occluded skin (as control). At 72 hours, the patch test site was evaluated for reactivity and positive reactions were clinically graded as 1+, 2+, or 3+.<sup>34,35</sup> Biopsies were taken from petrolatum and allergen-reactive patches of 24 patients (16 women/8 men, ages 20-63 years, median = 40.5 years) under institutional review board-approved protocols. Overall, 10 patients had positive reactions to nickel, 3 to fragrance, 7 to rubber, and 4 to metals other than nickel (for details, see Table E1 and the Methods section in this article's Online Repository at [www.jacionline.org](http://www.jacionline.org)).

## Histologic analysis

Immunohistochemistry (IHC) and immunofluorescence were performed on frozen tissue sections using antihuman mAbs (see Table E2 in this article's Online Repository at [www.jacionline.org](http://www.jacionline.org)). Epidermal thickness and positive cells/millimeter were quantified for IHC using ImageJ V1.42 (National Institutes of Health, Bethesda, Md) and immunofluorescence was imaged with MetaView Software (Visitron Systems, Puchheim, Germany).

## Quantitative real-time PCR and gene-array analysis

RNA was extracted for real-time (RT)-PCR with EZ-PCR Core Reagents (Life Technologies, Grand Island, NY), and custom primers were generated.<sup>36-43</sup> Expression values were normalized to hARP. Affymetrix U133Plus 2.0 arrays (Affymetrix, Santa Clara, Calif) were used for gene arrays, as previously described.<sup>36-43</sup>

## Statistical analysis

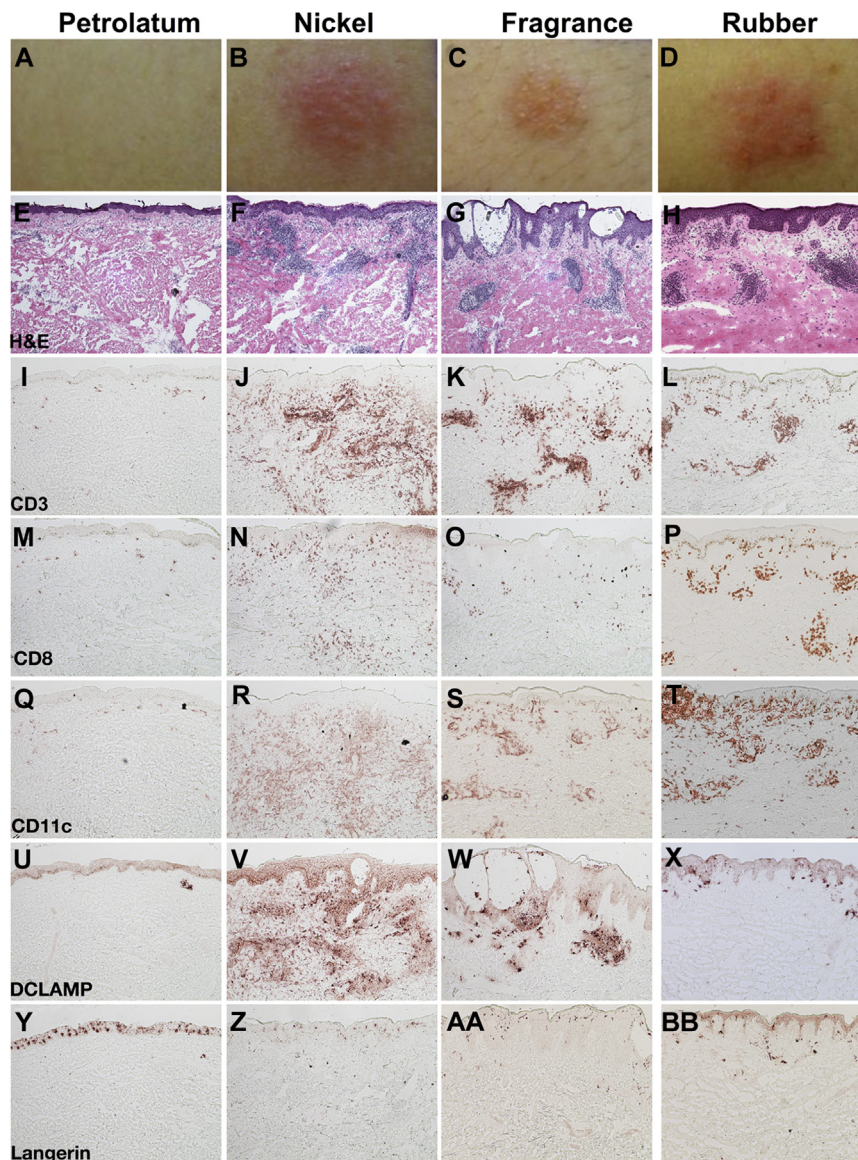
Quality control of microarrays was carried out using standard QC metrics and R package Quality Control. Images were scrutinized for spatial artifacts using Harshlight. Expression values were obtained using the Guanine Cytosine Robust Multi-Array Analysis algorithm. Probe sets with 1 or more sample with expression values of more than 4, and standard deviation (SD) of more than 0.15, were kept for analyses. Expression values were modeled using linear mixed-effect models with allergen group as fixed factor and a random effect for each patient. Fold changes for the comparisons of interest were estimated, and hypothesis testing was conducted using contrasts with the linear models framework in limma package. *P* values from paired *t* tests were adjusted for multiple hypotheses using the false-discovery rate procedure. To clarify whether distinct tissue reactions were not due to clinical scoring differences between allergens, we adjusted for patch score using a linear model in limma package, without significant differences. RT-PCR and IHC data were also analyzed using the linear mixed-effect model. Statistical methodology is detailed in this article's Online Repository at [www.jacionline.org](http://www.jacionline.org).

## RESULTS

### Similar clinical reactions and cellular infiltrates characterize different allergens

Similar clinical reactions with elevated erythematous patches and variable vesicles were observed with all allergens. The intensity of positive patch test results was graded as 1+, 2+, or 3+, as guided by erythema and vesiculation.<sup>34,35</sup> Representative positive reactions to common allergens (nickel, fragrance, and rubber) and a negative reaction to petrolatum (as a control) are depicted in Fig 1, A-D. Small, nonsignificant increases in epidermal thickness were noted on hematoxylin and eosin only with select allergens compared with petrolatum (Fig 1, E-H). IHC revealed significantly larger infiltrates of T cells (CD3<sup>+</sup> and CD8<sup>+</sup>) and DC subsets (myeloid/CD11c<sup>+</sup> and mature/DC-lysosome-associated membrane glycoprotein<sup>+</sup>) across most allergens when compared with petrolatum-occluded skin (*P* < .02 for all except other metals) (Figs 1 and 2; see Table E3 in this article's Online Repository at [www.jacionline.org](http://www.jacionline.org)). Higher infiltrates of resident/CD11c<sup>+</sup> DCs and eosinophils (major basic protein<sup>+</sup>) were also observed in allergen-occluded compared with petrolatum-occluded biopsies (significant only for rubber, *P* < .01) (Fig 2; also see Fig E1 in this article's Online Repository at [www.jacionline.org](http://www.jacionline.org); Table E3).

Despite similar clinical and histologic manifestations among the allergens, some differences were also noted. Overall, nickel demonstrated the greatest magnitude of cellular infiltrates, particularly T cells (CD3<sup>+</sup> and CD8<sup>+</sup>) and CD11c<sup>+</sup> DCs (Fig 2; Table E3). Conversely, other metals (cobalt and potassium dichromate) demonstrated the smallest infiltrates, attaining



**FIG 1.** Representative positive patch reactions to nickel, fragrance, rubber (**B-D**) and a negative reaction to petrolatum (**A**). Hematoxylin and eosin (**E-H**) and staining for CD3 (**I-L**), CD8 (**M-P**), CD11c (**Q-T**), DCLAMP (**U-X**), and Langerin (**Y-BB**), demonstrating increased infiltrates of T cells, DCs, and eosinophils and exfiltration of Langerhans cells in biopsies of allergen- versus petrolatum-occluded skin (histology magnification,  $\times 10$ ).

significance when compared with petrolatum only for mature/DC-LAMP<sup>+</sup> DCs ( $P = .04$ ). Finally, Langerhans cells/Langerin<sup>+</sup> were increased in petrolatum compared with all allergen groups, consistent with their mobilization from the skin on allergen exposure (**Fig 1, Y-BB**).<sup>44-46</sup>

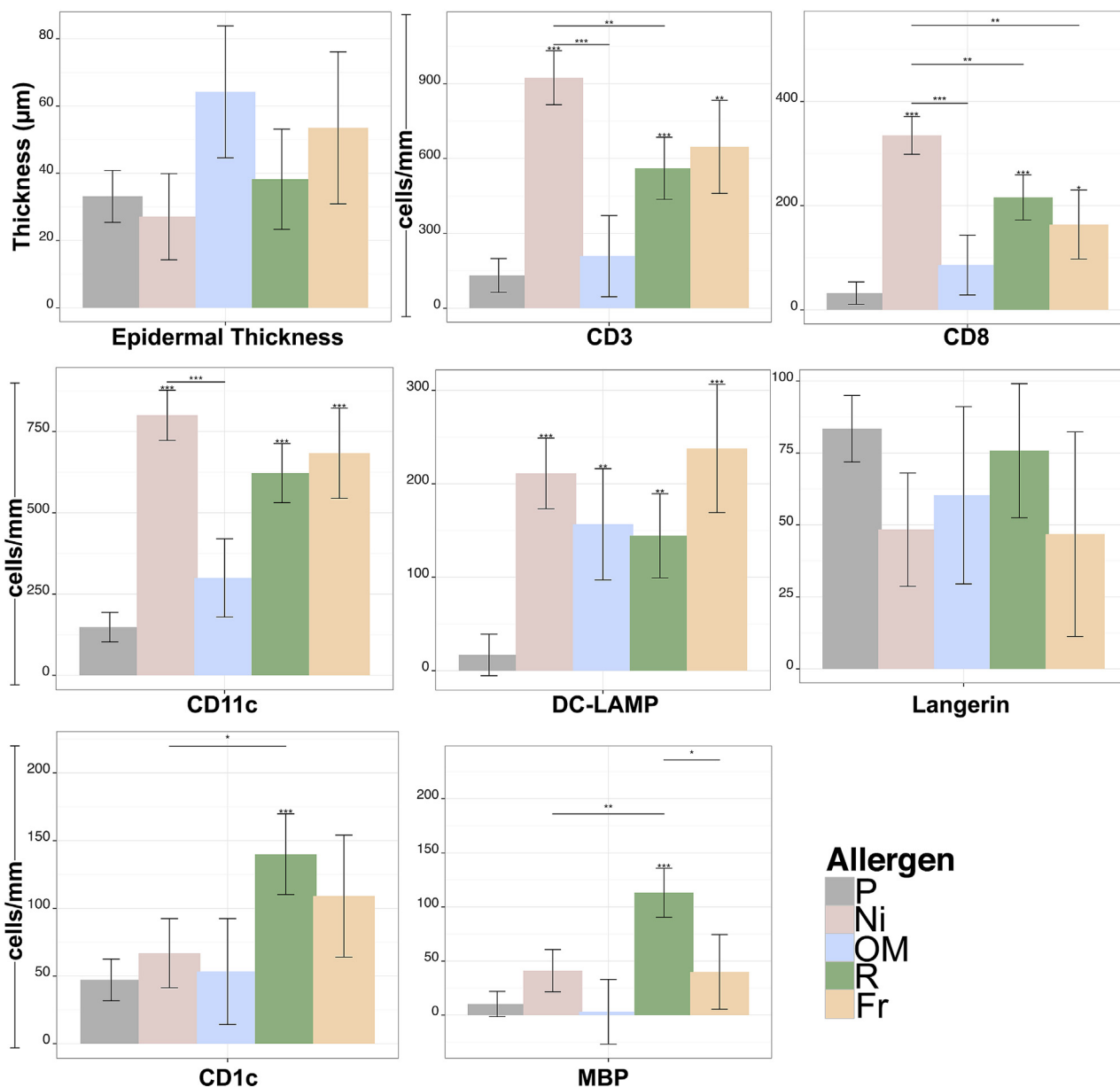
### Clusters of T cells and DCs form close to vesicles in allergen-reactive skin by immunofluorescence

On closer histologic evaluation, we observed cellular clusters in the dermis underneath intraepidermal vesicles (**Fig 1, K; 1 W**). To further explore this observation, immunofluorescence was used to label CD3<sup>+</sup> or CD8<sup>+</sup> T cells (green) and DC-LAMP<sup>+</sup> DCs (red) (see **Fig E2, A-D**, in this article's Online Repository at [www.jacionline.org](http://www.jacionline.org)). Clumps of CD3<sup>+</sup> T cells were intermixed with DC-LAMP<sup>+</sup> DCs (red) just below the vesicles. This colocalization was seen only in allergen-reactive but not in petrolatum-occluded skin (**Fig E2**). A similar clustering pattern,

though less pronounced compared with CD3, was observed with CD8<sup>+</sup> T cells, and some double-positive CD8<sup>+</sup>/DC-LAMP<sup>+</sup> staining was noted, accounting for a select subset of CD8<sup>+</sup> DCs.<sup>47</sup> The visualized T-cell/DC aggregates potentially correspond to skin-associated lymphoid tissues, conceptualized as key players in allergen responses in mice.<sup>48</sup>

### Genomic profiling identifies common and distinct molecular responses to different allergens

Gene arrays were performed to define differentially expressed genes (DEGs) in nickel, rubber, and fragrance compared with petrolatum-occluded skin as represented in the Venn diagram in **Fig 3, A** (with criteria of fold change  $>2$  and false-discovery rate  $< .05$ ). Because other (non-nickel) metals induced only limited genomic changes, these were excluded from this analysis (see **Methods**). We identified a core set of 149 (145 upregulated and 4 downregulated) DEGs in all allergen groups versus

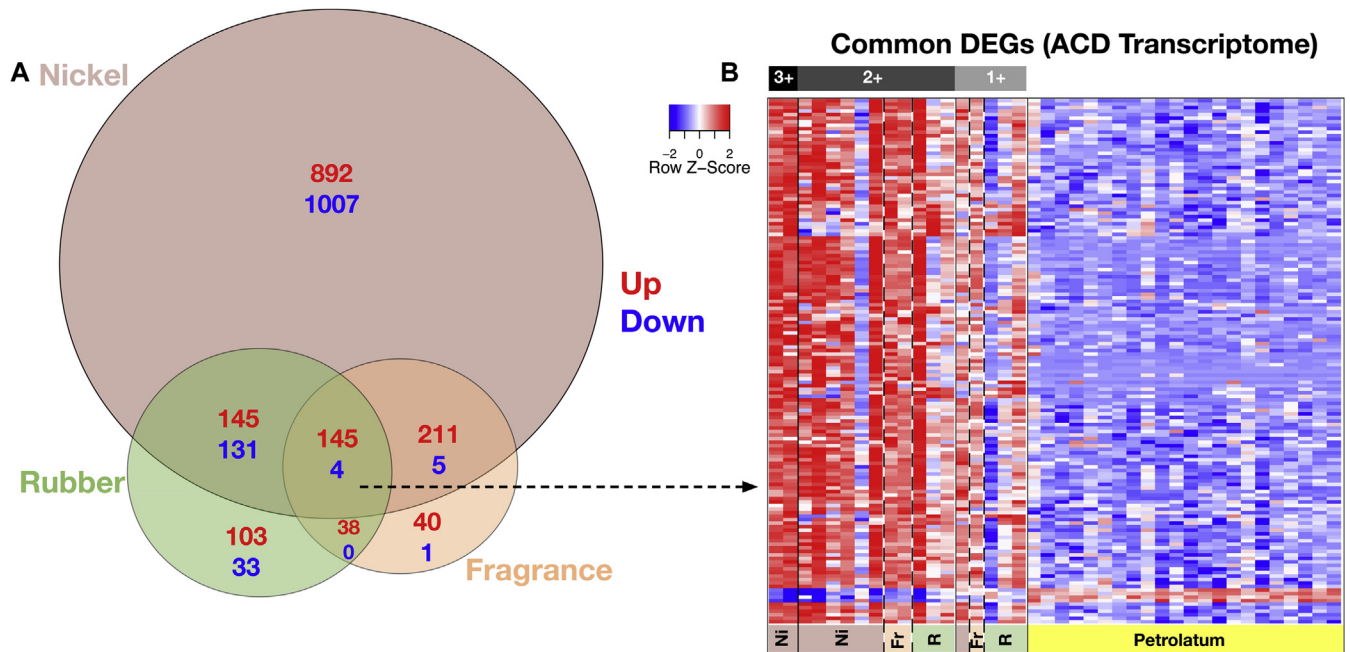


**FIG 2.** Epidermal thickness and cell counts for CD3, CD8, CD11c, DC-LAMP, Langerin, CD1c, and major basic protein in each allergen group were compared with petrolatum and versus nickel. Epidermal thickness was not significantly changed among allergens versus petrolatum. In general, nickel demonstrated the largest infiltrates whereas samples in contact with other metals exhibited much smaller cellular infiltrates. Fr, Fragrance; MBP, major basic protein; Ni, nickel; OM, other metals; P, petrolatum; R, rubber. Mean  $\pm$  SEM. \* $P < .05$ , \*\* $P < .01$ , and \*\*\* $P < .001$ .

petrolatum. This common gene set defines the ACD phenotype or transcriptome (Fig 3, A and B; see Table E4 in this article's Online Repository at [www.jacionline.org](http://www.jacionline.org)). The ACD transcriptome is presented as a heatmap in Fig 3, B, grouped by allergen type and stratified by clinical scoring (red: upregulated genes, blue: downregulated genes; Table E4). The ACD transcriptome consists of immune genes, including markers of T-cell-related genes (granzyme B, inducible T-cell costimulator, CD2, ITK, IL2RA, CCR7, CD80, and CD3D), immune regulators (cytotoxic T-lymphocyte antigen 4 [CTLA4], BATF3, and programmed cell death 1 ligand 1/CD274), DC antigens (CD11c/integrin alpha X/CD11c and CD1b), general inflammatory markers (matrix

metalloproteinase 12), and several chemokines (chemokine, CXC motif, ligand 1 [CXCL1], chemokine, CC motif, ligand 17 [CCL17], CCL19, and chemokine, CC motif, ligand 26 [CCL26]) (Fig 3, A and B; Table E4). For these common genes, nickel generally exhibited the greatest differences from petrolatum followed closely by fragrance, while the latter demonstrated greater differential expression of major T<sub>H</sub>2-related chemokines (CCL17 and CCL26) (see Table E4). We also noted that the intensity gradient of the genomic signal loosely paralleled the clinical grading (Fig 3, B).

Beyond the 149 common genes, the genomic changes induced by individual allergen groups were notably different. These



**FIG 3.** Common and unique DEGs for nickel, rubber, and fragrance compared with petrolatum with direction of dysregulation (*red*/upregulated; *blue*/downregulated; **A-B**). A core set of 149 DEGs in all allergen groups versus petrolatum (intersection of 3 circles, **A**) was used to define the allergic contact dermatitis transcriptome (depicted as a heatmap in **B**; *red*/upregulated; *blue*/downregulated; samples arranged by clinical scoring and allergen group). Allergen-dependent genomic changes were more impressive, particularly in nickel (**A**). *Fr*, Fragrance; *Ni*, nickel; *P*, petrolatum; *R*, rubber.

allergen-specific changes were most impressive with nickel, which generated 1899 distinct DEGs (2951 probes), followed by rubber (136 genes, 174 probes) and fragrance (41 genes, 47 probes) (Fig 3, A; see Tables E5-E7 in this article's Online Repository at [www.jacionline.org](http://www.jacionline.org)). Some genes were commonly activated by nickel and rubber (276 genes) but not with fragrance, while conversely 216 genes were activated in nickel and fragrance but not in rubber (Fig 3, A). To identify unique molecular changes to individual allergen groups, we analyzed DEGs in each group versus petrolatum, showing a similar pattern of correlation between molecular and clinical intensity (see Fig E3 and Table E8 in this article's Online Repository at [www.jacionline.org](http://www.jacionline.org)). We noted that innate/IFN/ $T_H1$ -related (chemokine, CXC motif, ligand 9 [CXCL9], chemokine, CXC motif, ligand 10 [CXCL10], chemokine, CXC motif, ligand 11 [CXCL11], 2'-5' oligoadenylate synthetase like, IL-6, IL-1B, IL12RB2, myxovirus resistance 1, and Toll-like receptor 7) and  $T_H17$ -related genes (chemokine, CXC motif, ligand 2 [CXCL2], IL23A, and peptidase inhibitor 3 [PI3]) were commonly induced by nickel, whereas  $T_H2$ -related genes (tumor necrosis factor ligand superfamily, member 4 [OX40L]) were particularly induced by fragrance. Rubber notably induced proliferation-promoting genes (cyclin B1-2/CCNB1-2 and cell division cycle associated 2) and IL-22, the  $T_H22$  cytokine (Fig 3; Tables E5-E8).

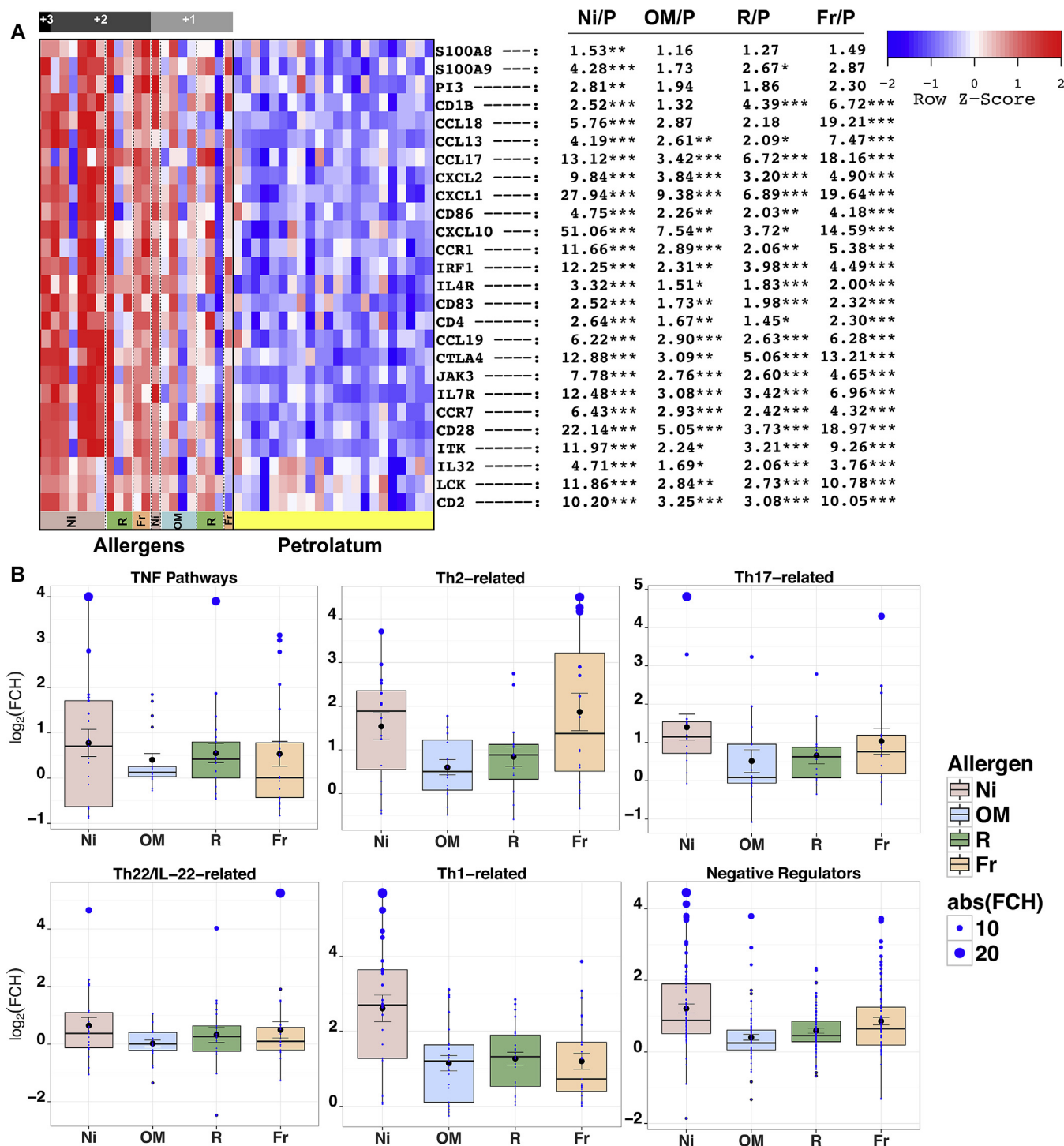
### Individual allergen groups show a trend toward differential immune skewing on gene arrays

To further evaluate for differential immune polarization between various allergen groups, we analyzed an inflammatory gene subset within the DEGs in individual allergen groups versus petrolatum. Major T-cell-, DC-, and cytokine and

chemokine-related genes were significantly upregulated in allergen-reactive samples, and gene induction seemed to parallel clinical scoring (Fig 4, A; Table E8). Nickel, representing the majority of 2+/3+ reactions, generally exhibited the highest induction of inflammatory markers than did other metals, fragrance, or rubber (Fig 4, A; Table E8). Particularly impressive increases were observed in nickel for T-cell markers (granzyme B, CD28, CD2, LCK, CCR7, and JAK3),  $T_H1$ /IFN-related genes (CXCL10), and  $T_H17$ -related genes (PI3, CXCL1, and CXCL2) ( $P < .01$ ; Fig 4, A; Table E8).

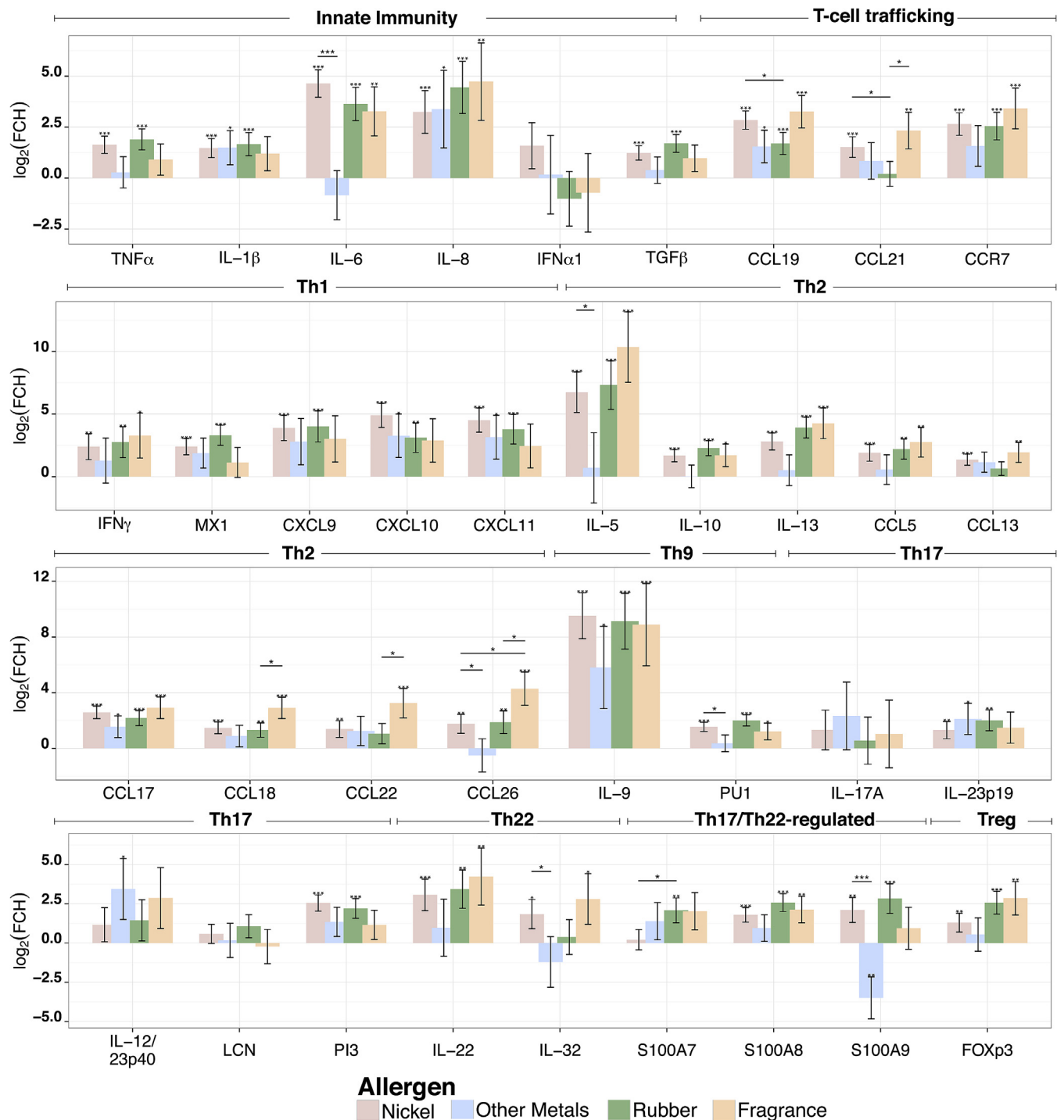
Despite the overall stronger genomic signal with nickel, select immune pathways were more highly induced with other allergens (ie,  $T_H2$ -inducing chemokines—chemokine, CC motif, ligand 13 [CCL13], CCL17, and chemokine, CC motif, ligand 18 [CCL18]—were highest in fragrance). We next assessed the extent to which individual allergens activated major inflammatory and regulatory pathways represented in the gene arrays using previously defined gene lists for these axes (Table E8).<sup>36,38,41,43</sup>

The box-plot graphs in Fig 4, B, demonstrate that different allergens appear to selectively upregulate certain immune pathways to a greater degree than do others. For example, nickel strongly induced TNF- (ie, IL-1B),  $T_H1$ /IFN- (ie, CXCL9, CXCL10, CXCL11, and 2'-5' oligoadenylate synthetase like),  $T_H17$ - (ie, CXCL1, CXCL2, IL-23A, and PI3/elafin), and  $T_H22$ -related genes (ie, S100-calcium binding protein A9 [S100A9] and IL-32) as well as negative immune regulatory genes (ie, LILRB2, CTLA4, CD274/programmed cell death 1 ligand 1, and PTPRC/CD45) (Fig 4, B). Fragrance potently induced  $T_H2$  chemokines (ie, CCL13, CCL17, CCL18, chemokine, CC motif, ligand 22, and CCL26) and negative regulators (CTLA4), and rubber generally resembled fragrance, albeit with smaller inductions. Finally, non-nickel metals generally showed



weaker immune activation. These findings imply that individual allergens can induce polar immune responses, suggesting that ACD should not be considered a single immunologic phenomenon (Fig 4, B; mean/median, and major representative pathways by allergen type). Because metals such as nickel

are recognized by T-cell receptors in conjunction with MHC-related proteins, we investigated whether there is an association between HLA haplotypes and the allergen groups by assessing all HLA markers present in our gene-array results. Consistent with recent publications, nickel and other

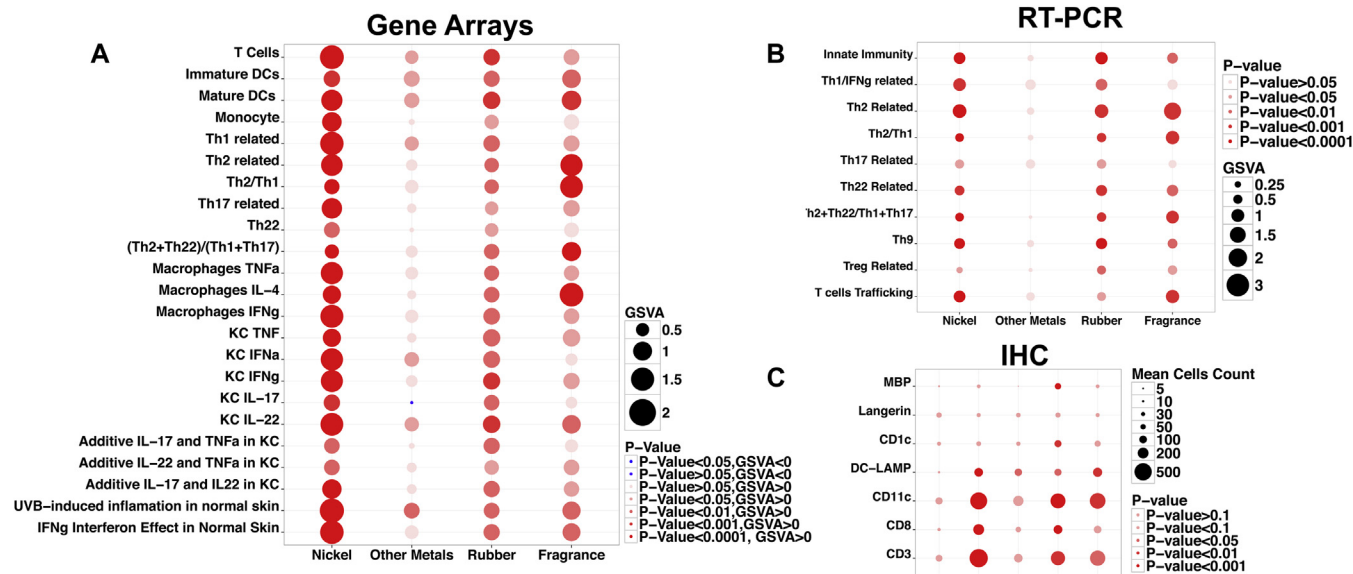


**FIG 5.** Real-time PCR was used to evaluate the expression of major cytokines and inflammatory markers; data were grouped according to allergen type and by the major inflammatory and regulatory pathways represented. Fold changes (*FCH*) and *P* value of allergen versus petrolatum (directly above the SEM bars) and nickel versus the other allergens are indicated if significant. \**P* < .05, \*\**P* < .01, and \*\*\**P* < .001.

metals demonstrated increased expression of HLA-DPA1, HLA-DPB1LA-D<sup>49-51</sup> as well as HLA-DOB, HLA-DMB, and HLA-DMA. Interestingly, we also found significantly increased expression of HLA-DOB and HLA-DPA1 in rubber and HLA-DQB2 in fragrance (see Fig E4 in this article's Online Repository at [www.jacionline.org](http://www.jacionline.org)).

### Genomic profiling by RT-PCR confirms allergen-specific immune polarization

To confirm differential immune activation in an allergen-dependent manner and to extend the analysis to low-abundance cytokines that are poorly detected by gene arrays, mRNA expression of representative inflammatory, innate, and various



**FIG 6.** Bubble plots comparing different measures of the immune response across different allergens. Bubble diameter proportional to the gene-set variation analysis (GSVA) score for gene arrays (A) and RT-PCR (B) or mean cell counts for IHC (C). Larger bubbles reflect higher magnitudes of expression/cellular infiltrates, and the color of each bubble indicates the significance (*P* value). *Fr*, Fragrance; *Ni*, nickel; *OM*, other metals; *P*, petrolatum; *R*, rubber.

$T_H$ -induced genes was measured (Fig 5; see Table E9 in this article's Online Repository at [www.jacionline.org](http://www.jacionline.org)). Overall, very significant increases in many inflammatory products were observed in all allergen groups (although less evident for other metals) compared with petrolatum, with nickel and fragrance showing the greatest increases. Markers of innate immunity (IL-6 and IL-8) were significantly upregulated for most allergens. T-cell trafficking genes (CCL19 and CCR7) were upregulated in all allergens, with the greatest inductions in fragrance.

Mirroring the gene arrays, higher mRNA expression of some innate (IFN- $\alpha$ 1 and IL-6) and  $T_H$ 1-related (ie, IFN- $\alpha$ 1, CXCL10, and CXCL11) genes was observed in nickel than in other allergen groups. Conversely, fragrance exhibited greater expression of most  $T_H$ 2-related cytokines (IL-5 and IL-13) and chemokines (CCL5, CCL13, CCL17, CCL18, chemokine, CC motif, ligand 22, and CCL26),  $T_H$ 22-related genes (IL-22 and IL-32), and regulatory T cells (FOXP3).  $T_H$ 9-related genes (IL-9 and PU-1) were similarly increased in nickel, rubber, and fragrance. In line with gene-array data, metals other than nickel generated the weakest mRNA changes than did other allergens, with the exception of  $T_H$ 17-regulated cytokines (IL-17A, IL-23p19, and IL-12/23p40), which were most elevated in other metals. Although rubber usually showed smaller mRNA inductions than did the more potent inducers, it exhibited similar or greater expression of select  $T_H$ 17- (IL-23p19, lipocalin2/LCN, and PI3/elafin),  $T_H$ 22 (IL-22), and  $T_H$ 17/ $T_H$ 22-regulated genes (S100A7/A8/A9). As illustrated for IL-5 and IL-9 in Fig E5 (in this article's Online Repository at [www.jacionline.org](http://www.jacionline.org)), activated T cells are the likely source of these polar cytokine responses.

### Pathway analysis of genomic data suggests that different allergens induce distinct immune reactions

Fig 6 offers bubble-plot overviews of gene-array, RT-PCR, and IHC data, classified by allergen type. Bubble diameters are

proportional to the gene-set variation analysis score (for gene arrays and RT-PCR) or mean cell counts (for IHC). Larger bubbles reflect higher magnitudes of immune responses/cellular infiltrates; significance is indicated by color intensity (false-discovery rate/*P* value).

To dissect the effects of each allergen group on major cells or pathways, we evaluated their relative genomic scores for previously published lists of major T-cell, DC, monocyte, keratinocyte, T-cell axes, and immune gene sets (Fig 6, A).<sup>36,38,41,43</sup> Overall, nickel consistently demonstrates the highest immune activation among all allergens, confirming it as a potent immune activator. As visualized by bubbles representing innate immunity (ie, TNF or IFN- $\alpha$ -treated keratinocytes or macrophages),  $T_H$ 1,  $T_H$ 17, and  $T_H$ 2/ $T_H$ 1 and  $T_H$ 2+ $T_H$ 22/ $T_H$ 1+ $T_H$ 17 ratios, nickel exhibits potent induction of innate immunity and  $T_H$ 1/ $T_H$ 17 polarization. In contrast, fragrance shows strong  $T_H$ 2 skewing, a much smaller  $T_H$ 1/ $T_H$ 17 polarization, and a  $T_H$ 22 polarization component. These findings are also supported by the RT-PCR bubble plot (Fig 6, B), where fragrance exhibits greater  $T_H$ 2/ $T_H$ 1 and  $T_H$ 2+ $T_H$ 22/ $T_H$ 1+ $T_H$ 17 ratios than does nickel. Similar to fragrance, but with a lesser magnitude, rubber shows  $T_H$ 2+ $T_H$ 22 immune deviation (Fig 6, A and B). Nickel's higher immune activation is also reflected by its IHC counts (Fig 6, C) with the greatest T-cell (CD3<sup>+</sup> and CD8<sup>+</sup>) and DC (CD11c<sup>+</sup> and DC-LAMP<sup>+</sup>) infiltrates; moderate infiltrates characterized fragrance and rubber, while other metals had only modest infiltrates (Fig 6, C).

To evaluate consistency between the clinician-dependent scoring and molecular markers of inflammation, we performed a Spearman rank correlation analysis between clinical scoring and cellular infiltrates and the immune gene sets listed in Fig 6, A-C.<sup>36,38,41,43</sup> The most impressive correlations with clinical scoring were demonstrated by IHC with cellular infiltrates, particularly T cells ( $r = 0.75/CD3^+$ ,  $r = 0.68/CD8^+$ ), CD11c<sup>+</sup> DCs ( $r = 0.64$ ), and eosinophils/major basic protein<sup>+</sup> ( $r = 0.56$ )



( $P < .01$ ; see Table E10 in this article's Online Repository at [www.jacionline.org](http://www.jacionline.org)). The strongest correlations with clinical scoring on gene arrays were found with T-cell-related ( $r = 0.71$ ), and T-cell pathways genes, including  $T_H17$ -related ( $r = 0.49$ ),  $T_H1$ -related ( $r = 0.47$ ), and  $T_H22$ -related ( $r = 0.43$ ). High correlations were also detected with IL-22 and IFN- $\gamma$ -induced genes in keratinocytes ( $r = 0.62$  and  $0.53$ , respectively;  $P < .05$ ; see Table E11 in this article's Online Repository at [www.jacionline.org](http://www.jacionline.org)). For RT-PCR, correlations with scoring were strongest for  $T_H2$ - and  $T_H22$ -related products ( $r = 0.46$  and  $0.45$ , respectively;  $P < .04$ ).

## DISCUSSION

Distinct hapten-specific immune polarizations to potent sensitizers have been demonstrated in murine CHS models, including a  $T_H2$  bias for fluorescein isothiocyanate<sup>52</sup> and  $T_H1$ /IFN- $\gamma$  polarity for dinitrofluorobenzene and TNCB.<sup>2,10,53-55</sup> Nevertheless, because mice do not mount inflammatory responses to clinically relevant allergens,<sup>2,9,12,13</sup> and because the relevance of murine models to human ACD is unclear,<sup>8,9,12</sup> we used patch testing to common allergens to evaluate for polar T-cell polarization in humans.

Past human ACD studies largely involved blood analyses of hapten-specific effector T cells. These studies predominantly focused on nickel and suggested polar responses on hapten re-exposure in PBMCs. Nickel-specific responses were shown to be predominantly  $T_H1$ -polarized,<sup>22-24,27,56-59</sup> with additional  $T_H2$  and  $T_H17$  components compared with controls.<sup>17,23,30,60</sup> Characterization of specific effector responses to other allergens is limited.<sup>61</sup> One study noted a  $T_H2$ -biased response to paraphenylenediamine, while another described mixed polarity by chromium.<sup>61-63</sup> Attempts at using peripheral blood as an *in vitro* diagnostic tool for different allergens have also been mixed, suggesting inherent limitations to using circulating cells to assess cutaneous reactions to contact sensitizers.<sup>63-66</sup> The pertinence of peripheral data is particularly uncertain considering that relevant DC populations are absent in blood.<sup>9,29</sup>

Few human ACD studies include skin analyses. However, these evaluated a limited set of markers, grouped immune responses to different allergens as a single phenomenon, or focused on a single allergen (particularly nickel), discounting the potential for allergen-specific differences.<sup>8,9,14-17,59,67</sup> Elevated IL-1 and IL-36 family cytokines and associated molecules were noted in biopsies of positive patch test results to multiple allergens.<sup>68</sup> Another study, characterizing tissue responses to 9 different allergens, detected high levels of IL-17, IL-23, IFN- $\gamma$ , and IL-4,<sup>15</sup> while CCL17 and CXCL10 were significantly elevated in collective samples of nickel- and fragrance-allergic skin.<sup>67</sup> A nickel-specific skin study identified selective increases in inflammatory markers by gene arrays and RT-PCR (ie, granzyme B, CCR7, S100A7, and CCL19), but did not address T-cell polarization, cytokine production, and clinical correlations.<sup>69</sup> Another skin-based nickel-specific study focused on CCL17 and CCL27, noting significantly increased CCL17.<sup>70</sup>

Our study expands the understanding of T-cell activation and associated cytokines in allergen-reactive skin. It is the first human study to define shared and unique ACD transcriptomes for clinically relevant allergens, determine allergen-specific polar immune responses, and associate clinical intensity with immune activation. Although the common ACD transcriptome consists of

only 149 genes (~30% immune-related), a much larger set was uniquely altered by different allergens, most notably nickel. Although marked immune responses were noted with all allergen groups, specific allergens more potently induced certain pathways. Nickel significantly increased  $T_H1$ /IFN and innate immune responses, consistent with its selective binding to Toll-like receptor 4, which preferentially induces  $T_H1$  activation by DCs.<sup>71-73</sup> It also induced significant  $T_H17$  skewing, consistent with PBMCs data,<sup>17</sup> and  $T_H22$ . Fragrance, and to a lesser extent rubber, demonstrated a strong  $T_H2$  bias and some  $T_H22$  polarization, with much smaller  $T_H1$ / $T_H17$  contributions. This differs from a report that noted significant  $T_H1$  and minimal  $T_H2$  effector responses in peripheral blood to fragrance,<sup>61</sup> thus emphasizing the importance of studying effector responses to topical sensitizers directly in skin. Interestingly, induction of negative immune regulators paralleled the inflammatory responses, being highest with nickel, though regulatory T cell-related FOXP3 was highest in fragrance. In addition, although associations between several HLAs with nickel and other metals were previously reported,<sup>49-51</sup> our data associate the expression of other HLA genes with rubber and fragrance.

The polar immune responses seen with various allergens might serve as a model for atopic dermatitis (AD), another common inflammatory skin disease. These eczematous processes are closely related; patients with AD exhibit increased ACD reactions, possibly due to impaired barrier function, with allergens potentially triggering AD flares.<sup>74-76</sup> Recent work demonstrated that patients with intrinsic AD were frequently sensitized to metals (nickel and cobalt),<sup>76,77</sup> while our recently published study identified significantly higher  $T_H1$ / $T_H17$ / $T_H22$  polarization in intrinsic AD than in extrinsic AD.<sup>38</sup> In this report, we identify high expression of  $T_H1$ / $T_H17$ / $T_H22$  genes in nickel-inflamed skin and  $T_H17$  markers in other metals, suggesting the possibility that these allergens may be potential triggers for intrinsic AD and may thus serve to simulate this subtype.

In contrast to metals, patch tests to dust mite and other environmental allergens induce  $T_H2$ -polarized responses, simulating acute AD.<sup>78-80</sup> Similarly, the  $T_H2$ / $T_H22$  polarization we noted for fragrance might be of particular relevance to AD because fragrances are common sensitizers in atopics.<sup>76,81</sup> In addition, because acute AD is a  $T_H2$ / $T_H22$ -polarized process,<sup>42</sup> fragrance might provide an experimental tool for simulating AD,<sup>82</sup> potentially expanding our understanding of AD and its triggers.

Finally, although much of our study focused on effector T-cell functions in the elicitation phase, it also raises the concept of inflammatory skin-associated lymphoid tissue, which has recently reemerged following mice data.<sup>48</sup> This model showed that dermal DCs rather than Langerhans cells were central to CHS responses.<sup>2,83</sup> Our histologic data, which revealed close aggregates of dermal DCs and T cells in reactive human skin, introduces the possibility for inflammatory skin-associated lymphoid tissue in which DCs might be able to stimulate T cells locally rather than in nearby lymph nodes, producing nearby vesicles as seen in our samples.

Our study offers new insights into the pathogenesis of human ACD. It defines the common transcriptome of clinically relevant sensitizers in human skin and identifies unique immune pathways preferentially activated by different allergens, suggesting that ACD cannot be considered a single entity. At the mechanistic

level, it suggests that only some sensitizers (fragrance) show classic “allergic”  $T_H2$  polarization as might be implied by the term “allergic contact dermatitis,” while others (nickel) generate a  $T_H1/T_H17$  polarity. Collectively, our findings provide a novel framework for understanding effector responses to clinically relevant sensitizers, and may help guide future human studies of ACD as well as intrinsic and extrinsic AD.

**Clinical implications: The polar responses seen with nickel ( $T_H1/T_H17$ ) and fragrance ( $T_H2/T_H22$ ) could provide a model system to study immune polarizations in acute intrinsic and extrinsic atopic dermatitis.**

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