# 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, $T_H1/T_H17$ and a $T_H22$ component. Fragrance, and to a lesser extent rubber, demonstrated a strong $T_H2$ bias, some $T_H22$ polarization, and smaller $T_H1/T_H17$ 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%.[1-3] 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.[1-6] 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 rechallenge, producing visible dermatitis with peak inflammatory reactions at 72 and 24 hours in human and mice, respectively.[7-10] Patch testing is the criterion standard procedure to diagnose ACD.[11] 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.[2,12,13] 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.[8,9,14-17] In murine CHS

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J.G.K., M.S.-F., J.C.d.R., M.R., T.C., and N.D. were supported by the National Center for Research Resources (NCRR) (grant no. 5UL1RR024143-02), 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.
Disclosure of potential conflict of interest: Y. D. Estrada has received research support from the National Institutes of Health. J. G. Krueger has received consultancy fees from Centocor, Lilly, and Pfizer and has received research support from Amgen, Centocor, Lilly, Merck, and Pfizer. E. Guttman-Yassky has received research support from Novartis, Janssen, Leo-Pharma, Bristol Meyers Squib, Dermira, Celgene, and Regeneron and has received personal fees from Leo-Pharma, Genentech, Dermira, Celgene, Stiefel/GSK, Pfizer, Medimmune, Anaptysbio, and Regeneron. The rest of the authors declare that they have no relevant conflicts of interest.
Received for publication December 20, 2013; revised March 14, 2014; accepted for publication March 18, 2014.
Available online April 25, 2014.
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0091-6749/$36.00
© 2014 American Academy of Allergy, Asthma & Immunology
http://dx.doi.org/10.1016/j.jaci.2014.03.009

*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,[9] implying the possibility that effector immune activation pathways could also be allergen specific in human ACD.

Both $CD4^+$ and $CD8^+$ T cells can act as effectors in type IV delayed-type hypersensitivity and CHS reactions (although $CD4^+$ T cells appear to dominate human effector responses).[14,18] 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 $T_H1$-dominated or a mixed $T_H1/T_H2$ response, ACD is increasingly recognized as involving production of $T_H17$ and $T_H22$ cytokines.[14-21]

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

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[33] 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+.[34,35] 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).

### 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). 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.[36-43] Expression values were normalized to hARP. Affymetrix U133Plus 2.0 arrays (Affymetrix, Santa Clara, Calif) were used for gene arrays, as previously described.[36-43]

### 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.

## 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.[34,35] 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 ($CD3^+$ and $CD8^+$) and DC subsets (myeloid/$CD11c^+$ and mature/DC-lyosome-associated membrane glycoprotein$^+$) 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). Higher infiltrates of resident/$CD1c^+$ DCs and eosinophils (major basic protein$^+$) 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; 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 ($CD3^+$ and $CD8^+$) and $CD11c^+$ DCs (Fig 2; Table E3). Conversely, other metals (cobalt and potassium dichromate) demonstrated the smallest infiltrates, attaining

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