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Patch testing of food allergens promotes Th17 and Th2 responses with increased IL-33: A pilot study

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Background

Food allergy/FA affects ~3.5% of adults and 8% of children(s1). It involves both immediate, IgE-mediated(1)(s2), and T-cell-mediated, delayed-type hypersensitivity/DTH reactions(2)(s3,s4). While the gold standard for diagnosis is oral challenge(s5), prick tests/SPT and specific serum-IgE/sIgE levels are often used to detect IgE sensitization(2)(s6). Food patch tests/FPT, in which epicutaneous application of food allergens/FALs elicits T-cell-mediated responses, may be useful for diagnosing late-phase FA reactions/DTH(1,3,4)(s2,s7), but their underlying immune mechanisms in skin are poorly characterized(5,6)(s8-10).

Therapeutic strategies for FA are lacking. While oral immunotherapy has shown promise(s11,s12), safety and efficacy concerns were raised(s13-15). A promising alternative is epicutaneous immunotherapy/EPIT, which provides allergen exposure through skin-associated lymphoid tissue/SALT(s16), but molecular changes it induces in skin remain uncharacterized. Understanding FPT's skin molecular profile may provide pathogenic information and accelerate therapeutic development.

Questions Addressed

We aimed to characterize immune responses of FPT in comparison with those of common contact allergens/CCAs(7).

Experimental Design

Twenty-five patients with self-reported FA and/or positive SPT and/or sIgEs were patched-tested with 17 common FALs and 15 CCAs. Patch tests were applied for 48hrs and clinically graded at 72hrs. For each positive FPT, 6mm biopsies from the reaction site and unexposed healthy skin were taken under IRB-approved protocols. As negative controls,

three healthy volunteers without FA were patch-tested with peanut and beef (representative FALs). Immunohistochemistry, immunofluorescence, and RT-PCR were performed as described(7)(s17). For additional details, see Supplementary Methods.

Results

Twelve patients (6 female, 6 male; ages 28-63yrs) had positive FPTs (TableS1), including beef (n=1), codfish (n=1), barley (n=2), celery (n=1), lentil (n=5), and peanut (n=2; TableS1). Overall, clinical reactions produced by FALs had lower intensity than those induced by CCA, with most FPT reactions graded 1+, compared to 2+/3+ reactions common to CCAs (e.g. nickel; representative clinical and immunohistochemistry images in Fig.S1A-X). Similar to other CCAs(7), FPT skin displayed increased infiltrates, including significantly increased CD11c⁺, DC-LAMP⁺, and CD1c⁺ dendritic cells/DCs; and major basic protein/MBP⁺/eosinophils (p<0.05; Fig.1A), with some T-cell increases (Fig.1A). Beef showed the highest increases in most cellular infiltrates (Fig.S1Y). Most FPTs showed focal decreases in epidermal CD1a⁺/Langerhans cells/LCs, with the presence of dermal CD1a⁺/LCs, suggesting epidermal to dermal migration as seen with CCA (Fig.S2A-B)(s18).

Using microarrays to compare gene expression induction by FPT vs. CCA, we found weaker inductions in FPT (Fig.1B, Fig.S3A-B). Fold change/FCH>2.0 in FPT vs. control and false discovery rate/FDR<0.05 defined differentially expressed genes/DEGs. In FPT, we identified 157 DEGs (150 up-, 7 down-regulated) vs. 1524 CCA DEGs (844 up-, 680 down-regulated). Only 5 DEGs were unique to FPT (TableS2), with the majority of FPT DEGs being a subset of CCA DEGs (Fig.S3A). Among top FPT DEGs were T-cell (CD28, LTF), DC (CD1b), and IL-17-regulated (DEFB4, S100A9, CXCL1, PI3/Elafin) genes (TableS3).

We also investigated whether FALs have different genomic profiles, as shown for CCAs(7)(s19). Because only a few samples were available for some foods, we grouped FPTs with similar profiles by hierarchical clustering (Fig.S4) by RT-PCR (including 52 markers representing multiple pathways), producing four groups: 1. Beef/codfish (n=2), 2.

Barley/celery (n=3), 3. Peanut (n=2), 4. Lentil (n=5). A heatmap of FPTs vs. control skin is presented (Fig.1C; individual barplots in Fig.S5). Beef/codfish had the highest Th17 and Th17/Th22 up-regulation (lipocalin2/LCN2, PI3, S100A7/8/9; $p<0.05$, Fig.1C), with a small Th2 component (CCL13, CCCL18, CCL22; $p<0.05$). High Th17 increases (IL-17A, IL-17F) were seen in beef/codfish and peanuts, and significant Th17 pathway enrichment was also seen in a Gene Set Variation Analysis/GSVA in both groups (Fig.S6). Overall, barley/celery had the largest Th2 component (e.g., IL-13, CCL22, IL-5, CCL26; $p<0.05$). Large IL-5 induction in this group was consistent with increased MBP⁺/eosinophils in barley (Fig.S1). Lentil produced the weakest reactions. IL-9 showed large increases in most foods (Fig.1C).

We confirmed that patients with positive FPT reactions did not merely show an irritant reaction to applied food allergen, using RT-PCR for representative innate and T-helper markers. Significant increases in cytokines were found in positive FPTs vs. non-allergic subjects patch-tested with beef and peanut. The strongest differences were elevated IL-9, Th17, and Th2 genes (Fig.S7, TableS4).

Although FPTs were weaker than CCA reactions, IL-33 showed higher inductions with FPT than with two potent CCAs (nickel, fragrance; Fig.2A)(7)(s19). Among foods, IL-33 expression was greatest in peanut (Fig.S8A). Genes related to IL-33 signaling, including innate (IFN α 1, IL-1 β , IL-6, IL-8)(43,44) and Th2-related (IL-4, IL-15, IL-13)(8)(s20), showed parallel increases in peanut (Fig.S8A). Indeed, IL-33 has been recently shown in cultures and animal models to participate in peanut sensitization(5,9). A GSVA of IL-33 pathway genes showed the highest enrichment in peanut ($p<0.01$), with no significant enrichment in any other food group (Fig.S8C). To determine which cells express IL-33 in positive peanut FPT, we performed IL-33 immunostaining, showing diffuse epidermal staining, with greater staining in peanut-tested vs. control skin (Fig.2B-C), and no staining with isotype control (Fig.2D). We also performed double immunofluorescence of IL-33 with CD3⁺/T-cells, CD11c⁺/DCs, and CD1a⁺/LCs, confirming strong keratinocyte staining and lack of co-localization of IL-33 with T-cells, dermal DCs, and LCs (Fig.2E-G).

Conclusions

FPT elicits inflammatory responses characterized by cellular infiltration, with inductions of Th17, Th2, and Th9-related genes. Genomic profiling shows that FPT-induced genes are a subset of those activated by CCAs (i.e., nickel) (Fig.S3A). Non-allergic controls failed to show activation of inflammatory mediators with beef or peanut FPTs.

Much effort has been devoted to developing allergen-specific immunotherapies/SIT, which involve repeated administrations of sensitizing allergens(s12,s13,s21-23). Many routes for SIT have been pursued, including recently, EPIT(s12,s21-23). In principle, the skin route is advantageous, because it is non-invasive, with rare systemic responses(s22), and has high abundance of antigen presenting cells with tolerogenic functions(s24,s25). If EPIT produces similar responses to FPTs, tolerance induction can be explained by potentially titrating FAL-induced immune responses. Our characterization of FPT responses provides a starting point for investigating immune modulation by EPIT.

The differential polarization with individual foods might provide further avenues for targeted therapeutics for FA, with different food subsets showing elevated Th17, Th2, IL-9, and IL-33. IL-33 and Th9/IL-9 have been reported to play a role in peanut-induced allergy(5,6,9). The reason for increased IL-33 in peanut vs. other foods is not yet apparent.

This preliminary study has several limitations: a small sample size of positive FPTs requiring grouping different foods with similar genomic profiles for statistical power; despite affecting children more frequently, this study included only adult patients; we did not have concurrent SPTs for this cohort. Overall, our study has shown that FPT skin reactions show a cell-mediated allergic response characterized by eosinophil infiltration, and production of “allergic” cytokines such as IL-5, IL-9 and IL-33.

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Figure Legends

Fig.1. A. Cell counts of CD11c, DC-LAMP, CD1c, CD3, CD8, MBP, and CD1a in food, nickel, and fragrance groups with control skin. * $p<0.01$; ** $p<0.05$; *** $p<0.01$. **B.** Heatmap of microarray expressions of food DEGs in food allergens, nickel, and other common contact allergens compared to control (heatmap with 29 control patients available in Fig.S3B), with diminished red in food implying less immune activation. Ba=barley, Bf=beef, Cl=celery,

Cd=codfish, Pea=peanut, Fr=fragrance, Co=cobalt, P=PPD, TM=thiuram mix. **C.** Heatmap of FCH vs. control of RT-PCR expressions. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Fig.2. A. IL-33 FCH of expression vs. control measured by RT-PCR for food, nickel, and fragrance groups. Representative stains of IL-33 in control (**B**) and peanut patch-tested (**C**) skin, and IgG1 isotype control in peanut patch-tested skin (**D**), showing greater diffuse basal and suprabasal epidermal staining in peanut patch-tested skin. Representative double immunofluorescent staining of IL-33 (red) with CD11c (green; **E**), CD3 (green; **F**), and CD1a (green; **G**).

Figure 1

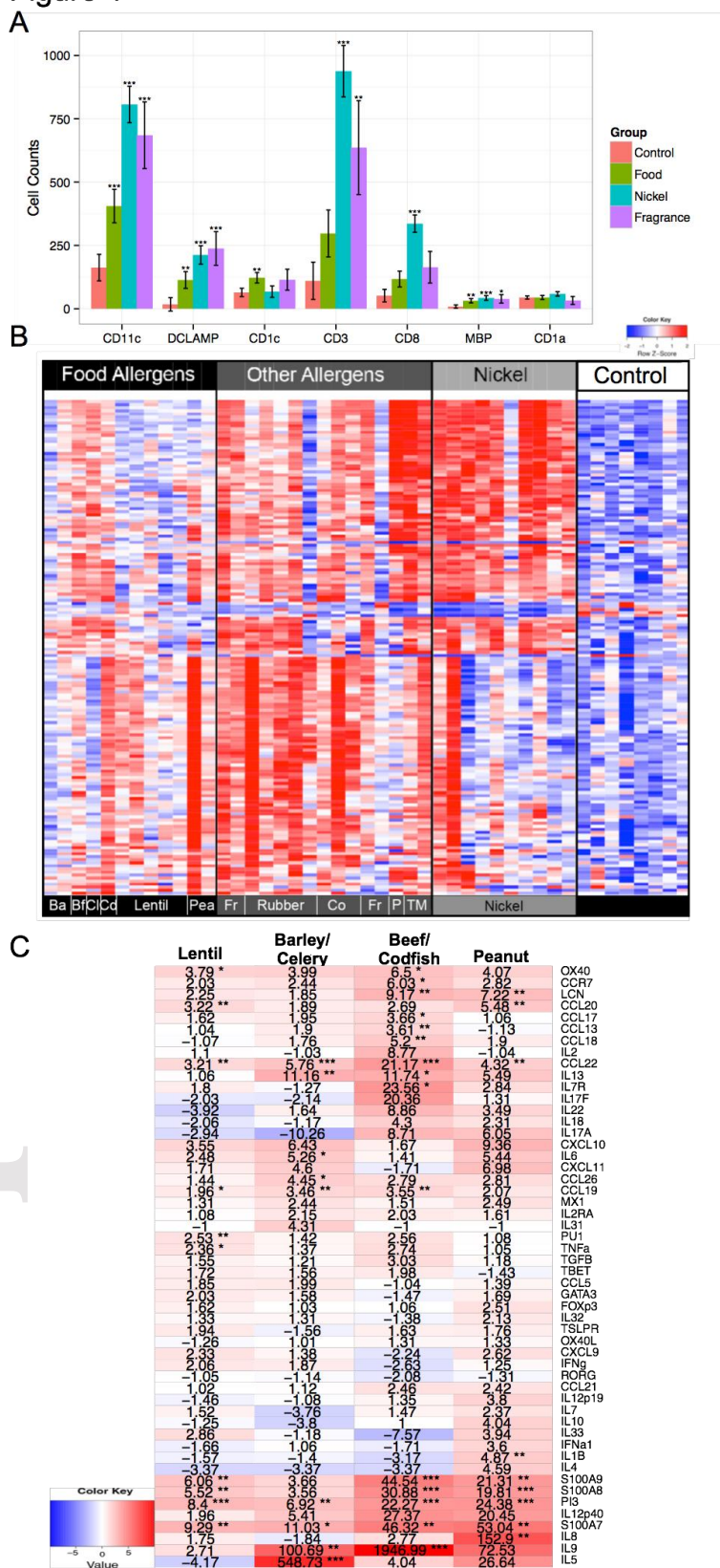


Figure 2

