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Increases in plasma IgG4/IgE with trilipid versus paraffin/ petrolatum-based emollients for dry skin/eczema

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To the Editor,

Increasing evidence suggests that allergen sensitization through a dysfunctional skin barrier can result in Type 2 immune activation and subsequent food allergy (FA). It is now hypothesized (dual allergen exposure hypothesis) that early life allergen exposure through the skin induces FA whereas early oral exposure with a diverse diet leads to tolerance. In the

Learning Early About Peanut (LEAP) and Enquiring About Tolerance (EAT) studies, the severity and duration of AD during the first year of life predicted subsequent peanut allergy (PA) and sensitization.^{1, 2} Inflammatory cytokines such as IL-25, IL-33, and TSLP (alarmins) released by epidermal epithelial cells on injury or stress lead to type 2 immune allergic response rather than tolerogenic responses.³ Skin barrier FA prevention studies suggest that regular use of certain prophylactic emollients may aid in primary prevention for AD and subsequent allergic sensitization.⁴⁻⁷ However, recent studies seem to suggest that not all emollients are similar in their ability to correct the skin barrier and to prevent allergic sensitization.⁸ Allergic sensitization is associated with increases in plasma IgE; whereas natural tolerance to protect against allergies is associated with increased plasma IgG4/IgE ratios.⁹ Studies that identify mechanisms by which emollients (trilipid vs. paraffin/petrolatum emollients) normalize the immune system to a protective state of natural tolerance are needed.¹⁰ We hypothesized that infants who received trilipid vs. paraffin/petrolatum -based emollients would show increases in IgG4/IgE ratios and that this would be associated with increases in IL-10 and latency-associated peptide (LAP)+-producing CD4+ T cells and decreases in IL-4-producing CD4+ T cells. Since these infants could be exposed to peanut in house dust or grass in the outdoor environment and thereby sensitized to these allergens (and others not tested) through contact with dry skin/eczema, we further postulated that specific allergen IgG4/IgE ratios could be improved by trilipid vs. paraffin/petrolatum-based emollients.

In this pilot randomized, controlled study at a single site (Stanford) under IRB approval, we compared the effects of 12 weeks of total body application of daily trilipid cream (EpiCeram™, provided by Primus Pharmaceuticals) vs. a paraffin/petrolatum-based cream (Aveeno™), in infants between 4 to 9 months with and without dry skin/AD and measured biomarkers associated with both inflammatory and tolerogenic responses (IgE, IgG4, IL-4 CD4+, IL-10 CD4+ and LAP CD4+ T cells) via published techniques using stored plasma and peripheral blood mononuclear cells from the participants.⁹

We identified 16 infants with and without AD and stratified evenly for AD and FA (defined by food challenge). Infants with any autoimmune diseases, infection, or cancer diagnosis were excluded ([ClinicalTrials.gov NCT03663673](https://clinicaltrials.gov/NCT03663673)). Written consent was obtained from the parents or guardians of the infants. We chose EpiCeram™ because prior studies had indicated that trilipid skin barrier creams mimicking the skin's natural pH and lipid composition (3:1:1 ratio of ceramides, cholesterol and free fatty acids) were superior to conventional moisturizers in improving skin barrier function.⁸ We chose Aveeno because it was the most frequently used emollient in families. All emollients were blinded. The participants/families were instructed not to use any other emollients. They also were asked to minimize bathing to once a day. No other skin treatments were being used at the time of the study. The participants were not on any medications.

All infants were likely exposed to peanut dust as all households listed eating peanut on a regular basis. Peanut allergy was determined by validated food challenge performed in our clinical research unit with trained personnel as per PRACTALL guidelines and they all reacted at 144 mg dose with hives.¹¹ Due to the summer-fall seasons (2019) in which the infants were treated during the 12-week period, they were likely not exposed frequently to

grass pollens. Tables 1 and 2 outline baseline characteristics of infants by treatment group and atopic status, respectively.

Blood was obtained from infants at enrollment and at the end of the study. Total and peanut- and grass-specific IgG4 and IgE were conducted via John Hopkins University laboratories. Methods for characterization of T cell subsets have been detailed previously.¹² The mixed effect linear regression models were fitted to each marker (log-transformed) as a function of intervention group, study time, and their interaction. The significant interaction term represents significant difference between the change of markers over study time across the Aveeno™ and EpiCeram™ groups.

After 12 weeks of treatment, we found that infants had a significant difference on the change of total IgE and total IgG4 across two groups. Those using the trilipid cream had a decreased total IgE, whereas the Aveeno™ group had an increased total IgE ($p=0.0005$). A higher increase in the total IgG4 level was observed in the infants using trilipid cream compared to those using Aveeno ($p=0.020$). No significant differences were observed for the grass-specific IgE or IgG4 or peanut-specific IgE; however, we observed significant changes in peanut-specific IgG4 between groups ($p=0.0004$). Additionally, the total and peanut-specific IgG4/IgE ratios (corrected for by comparing the same unit) also showed the significant difference of change over time across the Aveeno and EpiCeram groups ($p<0.0001$ and $p=0.0004$, respectively) (Figure 1)

We also found differences in immune T cells with treatment. Compared to Aveeno™, EpiCeram™ was associated with significant increases in tolerogenic IL-10+ expressing ($p<0.0001$) and LAP+ expressing CD4+ ($p=0.002$) T cells, and significant decrease in proinflammatory IL-4+ expressing CD4+ T cells ($p=0.004$).

To our knowledge, this is the first study to compare immune markers with use of different emollients. These results build upon previous findings that indicate that trilipid creams are more efficacious than standard paraffin/petrolatum-based ones for the prevention of AD and subsequent FA.⁵ Overall, there was a trend towards decreased IgE and proinflammatory cytokines and increases in tolerogenic T cell subtypes in infants using trilipid creams compared to those using paraffin/petrolatum-based products within a 12 week period. The main limitations of the study are the small sample size and the short period of time of treatment.

These pilot findings improve our understanding of the immune pathogenesis of AD and the role of epithelium and subsequent T cell activation pathways and associated cytokines involved. These are preliminary findings that could be used towards the design of breakthrough translational clinical research and treatment. Our results demonstrate that the hallmarks of allergen tolerance (increases in IgG4/IgE) can be modulated within 12 weeks of emollients meant to improve skin barrier protection. There were differential effects on peanut specific IgG4 (which increased with EpiCeram) versus grass specific IgG4 (no change). The authors speculate that this may be related to different routes of exposure to food (skin) versus inhalant allergens (respiratory tract). We also detected differences in Th2 vs. Th1 shift with decreases in proinflammatory cytokines such as IL-4, and increases in

anti-inflammatory cytokines such as IL-10 and LAP.¹³ It is interesting to note that LAP-expressing T cells have been shown to have immunosuppressive effects mediated via TGF- β ,¹⁴ further studies will focus on the induction of regulatory T cells and other cell subsets during skin barrier emollient usage.

In summary, these results may assist with the design of larger studies for optimization of emollients and identification of biomarkers for monitoring therapy progress. In addition, these data suggest that proper emollient use could potentially prevent atopic diseases and could be a modality added to early introduction of foods in prevention guidelines.

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CONFLICT OF INTEREST STATEMENTS

- Sayantani Sindher: Dr. Sindher reports grants from Aimmune, DBV Technologies, and Regeneron,
- Susan Chan: Dr Chan reports non-financial support from Aimmune and grant support from Novartis.
- Tee Bahnson: Dr. Bahnson reports personal fees from King's College, London, UK, and DBV technologies
- Helen A Brough: Dr Brough reports grants from Food Allergy Research & Education (FARE), being on the UK advisory Board for DBV Technologies and research support from ThermoFisher Scientific.
- Gideon Lack: Dr. Lack holds shares in DBV Technologies, and from Mighty Mission Me, has consulted for Novartis, Sanofi-Genzyme, Regeneron, ALK-Abello, and has received funding from National Institute of Allergy and Infectious Diseases (NIAID, NIH), MRC & Asthma UK Centre, UK Dept of Health through NIHR, National Peanut Board, Action Medical Research, The Davis Foundation, and ICAP Charity.
- Donald Leung: DYML is supported by NIH grants 5AR41256, 1U19AI117673, UL1TR002535 and UM1AI1130780. He is also a recipient of the Edelstein Family Chair of Pediatric Allergy-Immunology at National Jewish Health. DYML has received grant support from Pfizer, Incyte and MedImmune/A-Z; He has also consulted for Sanofi-Genzyme, Regeneron, Aimmune Therapeutics and Janssen Pharmaceuticals.
- Kari C. Nadeau: Dr. Nadeau reports grants from National Institute of Allergy and Infectious Diseases (NIAID), Food Allergy Research & Education (FARE), End Allergies Together (EAT), Allergenix, and Ukko Pharma; Grant awardee at NIAID, National Institute of Environmental Health Sciences (NIEHS), National Heart, Lung, and Blood Institute (NHLBI), and the Environmental Protection Agency (EPA); is involved in Clinical trials with Regeneron, Genentech, Aimmune Therapeutics, DBV Technologies, AnaptysBio, Adare Pharmaceuticals, and Stallergenes-Greer; Research Sponsorship by Novartis, Sanofi, Astellas, Nestle; Data and Safety Monitoring Board member at Novartis and NHLBI; Cofounded Before Brands, Alladapt, ForTra, and Iggenix; Chief Intellectual Office at FARE, Director of the World Allergy Organization (WAO) Center of Excellence at Stanford, Personal fees from Regeneron, Astrazeneca, ImmuneWorks, and Cour Pharmaceuticals; Consultant and Advisory Board Member at Ukko, Before Brands, Alladapt, IgGenix, Probio, Vedanta, Centecor, Seed, Novartis, NHBLI, EPA, National Scientific Committee of ITN and NIH Programs; US patents (patent numbers 62/647,389; 62/119,014; 12/610,940, 12/686,121, 10/064,936, 62/767,444; application numbers S10-392).
- Dr. Shifaa S Alkotob, Dr. Robert Hamilton, Ms Melanie N Shojinaga and Ms. Shu Cao report no COI.

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IMPACT STATEMENT

To our knowledge, this is the first study to compare immune markers with use of different emollients. These results suggest that trilipid creams could be more efficacious than standard paraffin/petrolatum-based emollients for improvement of systemic immune markers in AD and other atopic conditions

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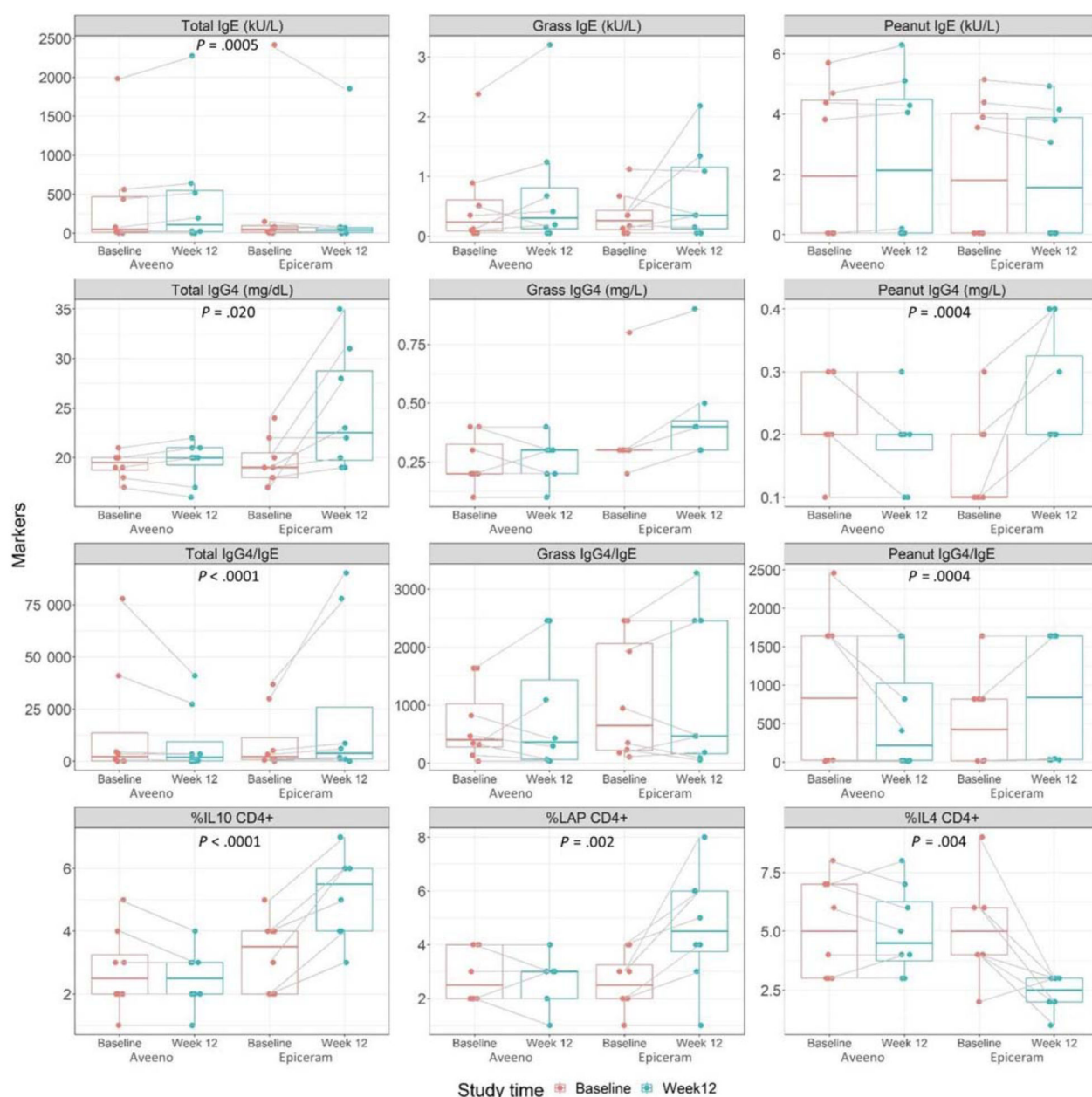


Figure 1.

Boxplot for biomarkers change from baseline to week 12, stratified by intervention group.

Boxplots of biomarkers by intervention group and study time. The mixed effect linear regression models were fitted to each marker (log-transformed) as a function of intervention group, study time, and their interaction. P values represent the significant differences between the change of markers over study time between the Aveeno and EpiCeram groups.

Table 1.

Baseline characteristics

	Total N=16	Randomized Intervention group	
		Aveeno N=8	EpiCeram N=8
Age in months (median [range])	5 [4, 9]	4.5 [4, 8]	5.5 [4, 9]
AD/FA status (%)			
AD-FA+	4 (25%)	2 (25%)	2 (25%)
AD+FA-	4 (25%)	2 (25%)	2 (25%)
AD+FA+	4 (25%)	2 (25%)	2 (25%)
HC	4 (25%)	2 (25%)	2 (25%)
Baseline markers (median [range])			
Total IgE (kU/L)	47.50 [1, 2415]	51.50 [1, 1983]	46 [2, 2415]
Grass IgE (kU/L)	0.26 [0.05, 2.38]	0.23 [0.05, 2.38]	0.26 [0.05, 1.12]
Peanut IgE (kU/L)	1.80 [0.05, 5.71]	1.93 [0.05, 5.71]	1.80 [0.05, 5.15]
Total IgG4 (mg/dL)	19 [17, 24]	19.50 [17, 21]	19 [17, 24]
Grass IgG4 (mg/mL)	0.30 [0.10, 0.80]	0.20 [0.10, 0.40]	0.30 [0.20, 0.80]
Peanut IgG4 (mg/mL)	0.20 [0.10, 0.30]	0.20 [0.10, 0.30]	0.10 [0.10, 0.30]
%IL-10 CD4+	3 [1, 5]	2.50 [1, 5]	3.50 [2, 5]
%LAP CD4+	2.50 [1, 4]	2.50 [2, 4]	2.50 [1, 4]
%IL-4 CD4+	5 [2, 9]	5 [3, 8]	5 [2, 9]

Table 2.

Baseline characteristics by AD/FA group.

	Total N=16	AD/FA Group				P value
		AD-FA+ N=4	AD+FA- N=4	AD+FA+ N=4	HC N=4	
Age in months (median [range])	5 [4, 9]	8.50 [5, 9]	5 [4, 6]	4.50 [4, 7]	4.50 [4, 6]	0.121
Baseline markers (median [range])						
Total IgE (kU/L)	47.50 [1, 2415]	19.50 [15, 24]	355.50 [71, 1983]	259 [79, 2415]	2 [1, 3]	0.005
Grass IgE (kU/L)	0.26 [0.05, 2.38]	0.12 [0.10, 0.35]	0.59 [0.35, 0.89]	0.74 [0.17, 2.38]	0.05 [0.05, 0.05]	0.008
Peanut IgE (kU/L)	1.80 [0.05, 5.71]	4.14 [3.56, 5.71]	0.05 [0.05, 0.05]	4.54 [3.82, 5.15]	0.05 [0.05, 0.05]	0.005
Total IgG4 (mg/dL)	19 [17, 24]	19.50 [17, 21]	18.50 [17, 20]	19.50 [18, 24]	19.50 [18, 22]	0.681
Grass IgG4 (mg/mL)	0.30 [0.10, 0.80]	0.25 [0.10, 0.30]	0.30 [0.20, 0.40]	0.35 [0.20, 0.80]	0.25 [0.20, 0.30]	0.380
Peanut IgG4 (mg/mL)	0.20 [0.10, 0.30]	0.15 [0.10, 0.30]	0.20 [0.10, 0.30]	0.25 [0.10, 0.30]	0.15 [0.10, 0.20]	0.610
%IL-10 CD4+	3 [1, 5]	2.50 [2, 4]	4 [3, 5]	3.50 [2, 5]	2 [1, 2]	0.042
%LAP CD4+	2.50 [1, 4]	3.50 [2, 4]	2.50 [2, 4]	3.50 [2, 4]	2 [1, 2]	0.108
%IL-4 CD4+	5 [2, 9]	4 [3, 4]	6.50 [6, 9]	6.50 [6, 8]	3 [2, 4]	0.007

P value was based on the Kruskal-Wallis test.