DOI: 10.1111/all.15694

In vivo and ex vivo inflammatory responses of the esophageal mucosa to food challenge in adults with eosinophilic esophagitis

To the Editor,

Elimination diets without the causative foods induce histological and clinical remission in patients with eosinophilic esophagitis (EoE), an allergen-driven type 2 inflammatory disease of the esophagus.[1] However, current tests using skin or serum are poorly predictive of the causative foods,[2] likely because the allergic inflammation may be restricted to the esophagus. We aimed to determine whether in vivo and ex vivo challenge of the esophageal mucosa with whole food extracts could yield clinically and immunologically relevant information about esophageal responses to specific foods.

During endoscopy, the esophageal mucosa of 12 EoE patients was challenged by local injection of 3 common food triggers (cow's milk, wheat, and apple) and 3 foods based on patient's clinical history, and by local flush (i.e., spray) of a mixture of the 6 foods. Acute local responses were monitored for 20 min. Skin prick tests (SPT) and serum IgE measurements were also performed. Esophageal biopsies were exposed to foods in culture to analyze inflammatory mediator production, which was compared with 6 non-EoE controls. Methods are fully described in the Appendix S1. Patient characteristics are provided in Table 1.

All patients were previously diagnosed with EoE and presented with the typical symptoms and endoscopic signs of EoE at time of endoscopy. Of the 11 patients that underwent injections, acute responses characterized by edema, erythema, or smooth muscle contraction (determined by the formation of a muscular ring) at the injection site were observed in 8 patients after injection with apple ($n = 4$), peanut ($n = 4$), wheat ($n = 3$), milk ($n = 2$), tomato ($n = 1$), egg ($n = 1$), and mango ($n = 1$) (Table 1; Figure 1A). In addition, after the end of the endoscopy, 4 patients experienced dysphagia, cramping retrosternal pain or burning sensation that was similar to pain occurring after ingesting those foods. Of the in total 17 foods that induced acute responses following local injections, 9 foods (53%) corresponded with patient's clinical history, 6 (35%) with SPT results and 6 (35%) with serum IgE results. The local flush with a mixture of foods also induced acute responses but, unlike the injections, these responses were barely notable and were observed in only 4 patients (Table S1). Our results confirm the observations of our previous study that esophageal food challenge can trigger local responses in adult EoE patients.[3] However, there was no clear relation between foods that induced a response by mucosal injection, and SPT or serum IgE. The fact that the foods that induce mucosal responses do not necessarily show positive SPT and/or serum IgE results, and the fact that SPT and serum IgE are poorly predictive of the causative foods,[2] indicate that local esophageal challenge may indeed be needed for a better prediction of the causative foods. Nonetheless, given the moderate responsiveness to challenge by flush, the clinical challenges associated with injections, and the invasiveness for patients as endoscopic challenge can induce short lasting but severe symptoms, both challenge tests will not likely become a useful test in clinical practice.

In contrast, a less invasive biopsy-based ex vivo food challenge test may be considered a promising tool for the identification of causative foods in EoE patients. Non-challenged EoE esophageal biopsies maintained in culture for 24 h showed increased production of total IgE (13.7 vs. 0.1 ng/mg, $p = .0002$), IL-5 (12.5 vs. 1.1 pg/mg, $p = .0288$), IL-6 (29.8 vs. 1.5 ng/mg, $p = .0047$), IL-8 (86.6 vs. 23.2 ng/mg, $p = .0069$), IL-13 (28.6 vs. 0.0 pg/mg, $p = .0080$), and MCP-1 (659 vs. 112 pg/mg, $p = .0320$) compared with non-challenged biopsies from controls (Figure 1B). Eotaxin, IL-9, and IFN-γ were below the detection limit. Analysis of protein levels based on peak eosinophil count did not provide additional insights (data not shown). Furthermore, when exposing biopsies to food in culture, an immunological response is triggered that may reflect the inflammatory cascade seen in EoE. Interestingly, IL-5 levels were increased after ex vivo exposure to milk (89.8 vs. 12.5 pg/mg, $p = .0195$), and IL-9 was increased after exposure to apple (132.3 vs. 0.0 pg/mg, $p = .0039$; Figure 1C). To our knowledge, we are the first to report food-specific induction of IL-5, an important factor in eosinophil trafficking,[4] and IL-9, a promotor of mast cell expansion and function,[5] in the inflamed esophagus of EoE patients, highlighting a potential role for both cytokines in the allergen-specific immune response in EoE.

Lastly, we used a machine learning approach[6] to study whether the ex vivo challenge test can better discriminate clinically suspected (as provided in Table 1) from non-suspected foods than the conventional SPT and serum IgE. Indeed, the ex vivo challenge test outperformed SPT/serum IgE with an AUC of 0.64 vs. 0.5 (Figure S1), evidencing sufficient discriminative scores.[7] Performing food re-challenges based on the ex vivo results was beyond the scope of this study.

This study has limitations. Our study was conducted in a small cohort, and the tested foods were not proven by elimination diets.

TABLE 1 EoE patient characteristics and sensitization patterns.

					Patient's history	Selected foods	Skin prick test	Serum sIgE test	Esophageal mucosal injections
Patient ID	Sex	Age (y)	Atopic comorbidity	PEC	Clinically suspected foods	In addition to milk, wheat and apple	Positive responses	Positive responses (kU/L)	Positive responses
1	M	26	Cat[a], dog[a], OAS[a], RhC[a]	45	Wheat, milk, apple, peanut, soy, tomato,	Soy, peanut, tomato	Wheat, soy, peanut, apple	Wheat (0.44), apple (6.54), soy (0.66), peanut (1.16)	Moderate narrowing/ edema **tomato/peanut**
2	F	21	–	24	Milk, apple, hazelnut, cashew, galia melon	Cashew, hazelnut, galia melon	–	–	–
3	M	48	–	5	Wheat, apple, chicken	Soy, peanut, chicken	Wheat	Milk (1.48), wheat (0.49)	Moderate edema/ rings **apple**/peanut, questionable milk
4	M	52	–	100	Wheat, apple, milk, orange, beer	Soy, orange, beer	Milk, beer	Milk (2.93)	Moderate edema **wheat**, questionable **apple**
5	M	44	RhC[a]	50	–	Soy, peanut, tomato[b]	–	–	–
6	M	48	OAS[a], RhC[a]	52	Wheat, apple, beer	Soy, peanut, beer	Peanut, soy, wheat, milk	Peanut (0.72), malt (0.42)	Strong response **peanut/beer,** moderate **wheat**
7	M	37	Hives[a], OAS[a], RhC[a]	14	–	Soy, peanut, egg	Inconclusive due to hives	Wheat (0.55), apple (0.66), peanut (1.11)	–
8	M	26	OAS[a], RhC[a]	30	Wheat, apple, grape, tomato, mango	Mango, grape, tomato	Grape, mango, wheat, apple, tomato	–	Moderate edema **mango**, questionable edema milk
9	F	28	OAS[a], RhC[a]	NA	Wheat, milk, apple, banana	Soy, peanut, banana	Wheat, banana	–	Not performed[c]
10	F	22	RhC[a]	50	Wheat, milk, egg	Soy, peanut, egg	Milk, egg	Milk (2.85), wheat (0.40), egg (0.66)	Moderate edema apple
11	M	41	Asthma, OAS[a], RhC[a]	20	Milk	Soy, peanut, egg	Apple, soy, milk, wheat	Milk (3.08), apple (3.11)	Contractile, muscular ring: apple, wheat and egg
12	M	30	Asthma, OAS[a], RhC[a]	12	Milk, peanut, wheat	Soy, peanut egg	Milk, soy, wheat	Milk (2.38), wheat (1.27), apple (0.59), soy (0.84), peanut (0.85), egg (0.64)	Questionable **peanut**

Abbreviations: –, none; F, female; M, male; NA, not available; OAS, oral allergy syndrome; PEC, peak eosinophil count; RhC, rhinoconjunctivitis; sIgE, allergen-specific IgE.

[a] SPT and/or serum sIgE proven.

[b] Tomato instead of egg was used because egg extract was not available.

[c] Not performed because the patient withdrew consent for the endoscopy. Foods presented in bold correspond with patient's history.

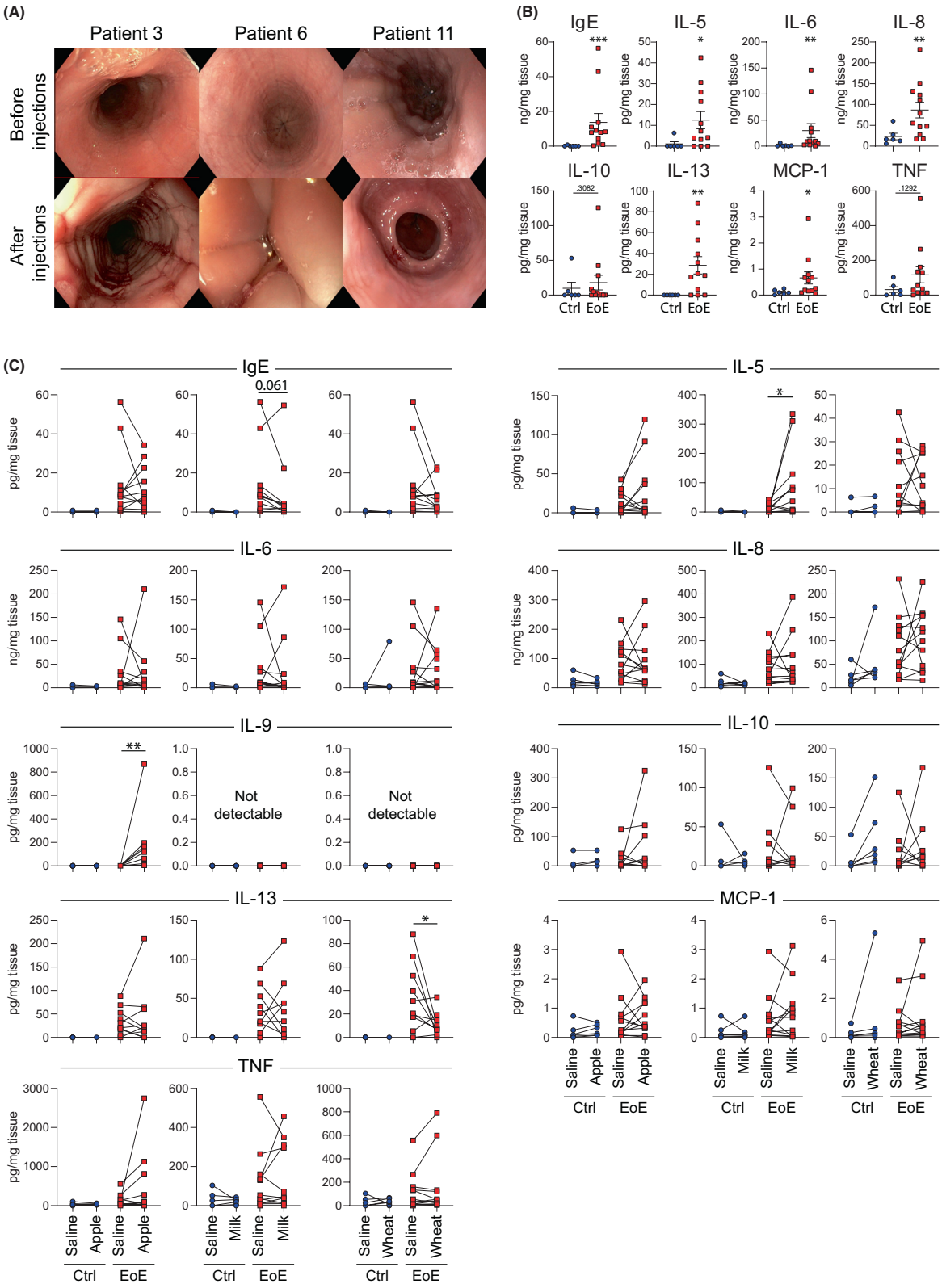

FIGURE 1 In vivo and ex vivo responses to food challenge. (A) Acute responses to mucosal food injections. Patient 3 showed increased edema and more visible rings and furrows after injection of apple and peanut. Patient 6 showed increased edema and erythema after injection of wheat. Patient 11 showed a contractile muscular ring after injection of apple, wheat and egg. (B) Inflammatory protein levels in culture supernatant of non-challenged esophageal biopsies from EoE patients (EoE, $n = 12$) and controls (Ctrl, $n = 6$) cultured for 24 h. Mann–Whitney test: $^{*}p < .05$, $^{**}p < .01$, $^{***}p < .001$. (C) Inflammatory protein levels in culture supernatant of esophageal biopsies from EoE patients (EoE, $n = 12$) and controls (Ctrl, $n = 6$) exposed to saline (negative control) or the common EoE triggers apple, cow's milk or wheat extract for 24 h. Wilcoxon matched-pairs signed rank test: $^{*}p < .05$, $^{**}p < .01$.

Extending the current study in a larger cohort of EoE patients in which causative and safe foods have been identified is needed to shed more light on the usefulness of the ex vivo test to identify causative foods and guide elimination diets. Furthermore, EoE is patchy in biopsies. Normalization of cytokine levels for epithelial/immune cell composition of the biopsies is therefore needed for standardization of the ex vivo test.

In conclusion, we demonstrated that results of food challenge using esophageal tissue provide distinct results from tests using skin and serum and may better reflect clinical response to food exposure. Esophageal biopsy tissue culture is a functional model of EoE and could potentially be used as an ex vivo model for esophageal food challenge to (i) study the food-induced immune response and (ii) identify causative foods to guide elimination diets, and therefore warrants further validation and development.

FUNDING INFORMATION

This research is funded within the Partnership between NWO domain Applied and Engineered Sciences and Danone Nutricia Research and with additional financial support from Topsector Agri and Food, project number 16495 with the acronym LOIRE. AJB is supported by Vidi grant 91718300 from the Netherlands Organization for Scientific Research NWO.

CONFLICT OF INTEREST STATEMENT

AJB received research funding from Norgine, Thelial, SST and received speaker and/or consulting fees from Laborie, Arena, EsoCap, Medtronic, Dr. Falk Pharma, Calypso Biotech, Alimentiv, Sanofi, Reckett, Regeneron and AstraZeneca (all unrelated to this work). RvR received consultancy fees from HAL Allergy, Citeq, Angany, Reacta Healthcare, Mission MightyMe and AB Enzymes, speaker fees from HAL Allergy, ThermoFisher Scientific and ALK, and has stock option of Angany (all unrelated to this work). MTJVA is employed by Danone Nutricia Research. JG and BCAMVE are partly employed by Danone Nutricia Research. All other authors report no conflict of interest.

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