Distinct transcriptome profiles differentiate nonsteroidal anti-inflammatory drug–dependent from nonsteroidal anti-inflammatory drug–independent food-induced anaphylaxis

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Background: Lipid transfer protein (LTP), an abundant protein in fruits, vegetables, and nuts, is a common food allergen in Mediterranean areas causing diverse allergic reactions. Approximately 40% of food-related anaphylaxis induced by LTPs requires nonsteroidal anti-inflammatory drugs (NSAIDs) as a triggering cofactor.

Objective: We sought to better understand the determinants of NSAID-dependent and NSAID-independent LTP-induced anaphylaxis (LTP-A).

Methods: Selection of patients was based on a proved clinical history of NSAID-dependent or NSAID-independent anaphylaxis to LTPs, positive skin prick test response to LTPs, and serum LTP IgE. Whole-transcriptome (RNA sequencing) analysis of blood cells from 14 patients with NSAID-related LTP-A (NSAID-LTP-A), 7 patients with LTP-A, and 13 healthy control subjects was performed to identify distinct gene expression signatures.

Results: Expression of genes regulating gastrointestinal epithelial renewal was altered in both patient sets, particularly in those with LTP-A, who also presented with gene expression profiles characteristic of an inflammatory syndrome. These included altered B-cell pathways, increased neutrophil activation markers, and increased reactive oxygen species levels. Increased expression of the IgG receptor (CD64) in patients with LTP-A was mirrored by the presence of LTP-specific IgG_1 and IgG_3. Conversely, patients with NSAID-LTP-A were characterized by reduced expression of IFN-γ–regulated genes and IFN-γ levels, as well as upregulated expression of adenosine receptor 3 *(ADORA3)* and genes related to adenosine metabolism.

Conclusions: Gene ontology analysis suggests disturbances in gut epithelial homeostasis in both groups with LTP-A, with potential integrity breaches in patients with LTP-A that might explain their distinct inflammatory signatures. Differential regulation in patients with LTP-A and those with NSAID-LTP-A of the IFN-γ pathway, IgG receptors, and *ADORA3* might provide the pathogenic basis of their distinct responses. (J Allergy Clin Immunol 2015;■■■:■■■-■■■.)

Key words: *Anaphylaxis, food allergy, lipid transfer protein syndrome, nonsteroidal anti-inflammatory drugs, transcriptome analysis*

Anaphylaxis is a systemic allergic reaction that is rapid in onset and can cause death.[1] Food allergens are the major triggers for anaphylaxis, accounting for 33% to 56% of all cases and up to 81% of cases in children.[2-4] Lipid transfer protein (LTP) is a relevant plant panallergen described as the most frequent cause of food-induced anaphylaxis in the Mediterranean basin.[5,6] Patients with LTP allergy are most frequently characterized by the presence of specific IgE to peach LTP (Pru p 3) but commonly show sensitization and reactions to multiple plant foods containing LTPs.[5,7-9] Often, an anaphylactic episode to an LTP occurs under the influence of a cofactor, such as physical exercise, alcohol, and/or a medication, especially a nonsteroidal anti-inflammatory drug (NSAID).[6,9-11] In an Italian cohort 78% of patients with food-dependent exercise-induced anaphylaxis were sensitized to LTPs,[10] and in a Spanish cohort with NSAID- and exercise-related food allergy, LTP sensitization was demonstrated in 92% of patients.[11] Furthermore, in another study almost half of the cases of LTP-induced anaphylaxis (LTP-A) were related to cofactors, with NSAIDs involved in 36% of reactions.[9] The enhancing effect of NSAIDs in food-induced anaphylaxis is well documented,[12,13] and it extends beyond LTPs to other common allergens, such as shellfish,[14] sunflower seeds,[15] gliadin,[16,17] and peanut.[18]

Some reports have associated anaphylaxis with expression of genes that regulate the innate and adaptive immune system.[2,19] However, the transcriptional profiles associated with either cofactor-dependent or independent anaphylaxis to the same food allergen have not been examined. Here we used next-generation sequencing to characterize the gene expression landscape under unchallenged conditions of patients with LTP-related anaphylaxis

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Supported by the Division of Intramural Research Programs within the National Institute of Allergy and Infectious Diseases and the National Institute of Arthritis and Musculoskeletal and Skin Diseased, National Institutes of Health and by Ministerio de Economia y Competitividad–Instituto de Salud Carlos III (FIS-PI11/01326), Spain. R.M.-C. was the recipient of the "Rio Hortega" Fellowship, Ministerio de Economia y Competitividad, Instituto de Salud Carlos III, Spain.

Disclosure of potential conflict of interest: The authors declare that they have no relevant conflicts of interest.

Received for publication March 16, 2015; revised May 6, 2015; accepted for publication May 29, 2015.

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0091-6749

http://dx.doi.org/10.1016/j.jaci.2015.05.042

Abbreviations used

ADORA3:	Adenosine receptor 3
α-gal:	Galactose α-1,3-galactose
HV:	Healthy volunteer
IPA:	Ingenuity Pathway Analysis
LTP:	Lipid transfer protein
LTP-A:	LTP-induced anaphylaxis (NSAID independent)
NSAID:	Nonsteroidal anti-inflammatory drug
NSAID-LTP-A:	NSAID-related LTP-induced anaphylaxis
Pru p 3:	Peach lipid transfer protein
RNA-Seq:	RNA sequencing
ROS:	Reactive oxygen species
RPKM:	Reads per kilobase of exon per million mapped reads
SPT:	Skin prick test

who differ in their dependency on NSAIDs for the manifestation of anaphylaxis to gain insights into the mechanisms influencing their distinct responses. The analysis reveals important differences in the type of immunity controlling the *in vivo* environment in these patient subsets and suggests that FcγRI receptors and LTP-specific IgG might play a contributory role in anaphylaxis to LTPs, whereas adenosine receptor 3 *(ADORA3)* might relate to reactions involving NSAIDs.

METHODS

Further details on the methods used in this study can be found in the Methods section in this article's Online Repository at www.jacionline.org.

Study population

Patients were recruited based on a clinical history of anaphylaxis (defined as in Sampson et al[1]) elicited by LTP-containing plant foods, positive skin prick test (SPT) responses to LTPs, and/or the presence of LTP (Pru p 3)–specific serum IgE (Table I). LTP-sensitized patients were divided into 2 groups: (1) patients with a clinical history of anaphylaxis caused by a food source containing LTP (LTP-A) and (2) patients with a clinical history of anaphylaxis to LTPs that is only observed when the culprit food was consumed in proximity to the ingestion of an NSAID (up to 2 hours before the reaction onset; NSAID-related LTP-A [NSAID-LTP-A]). Patients with NSAID-LTP-A had no anaphylaxis when separately ingesting a food source containing LTPs or an NSAID. Six (43%) of these patients also reported anaphylaxis to LTPs after exercise (see Table E1 in this article's Online Repository at www.jacionline.org). Patients with a clinical history of NSAID-induced asthma or urticaria were excluded from the study. Oral or nasal challenge with aspirin or NSAIDs[20] to confirm tolerance to NSAIDs was performed in some patients with a nonconclusive clinical history. None of these patients had LTP-related reactions or consumed NSAIDs at the time samples were collected.

Healthy volunteers (HVs) had no clinical history of food allergy or drug reactions and had negative SPT responses to LTP-containing plant foods and other common food allergens. Informed consent was obtained from all participating subjects. The study was approved by the local ethics committee of the hospital clinic (Barcelona, Spain).

Transcriptome sequencing and analysis

Whole-blood samples were used as a noninvasive source for RNA because it has been demonstrated that whole blood cell RNA is an adequate genomic tool to investigate organ biomarkers[21] and disease pathogenesis.[22] Blood was collected in PAXgene Blood RNA or DNA tubes and processed as described in the Methods section in this article's Online Repository. Poly-A RNA was reverse transcribed into cDNA, and approximately 200-bp fragments were ligated to sequencing adapters by using the TruSeq RNA (Illumina, San Diego, Calif) system, followed by 15 cycles of PCR amplification per the manufacturer's specifications. The cDNA libraries were sequenced with the Genome Analyzer HiSeq 2000 (Illumina) by using a 50-bp read length. The RNA sequencing (RNA-Seq) data were aligned to the hg18 reference genome with TopHat software by using options (-no-coverage-search and –G) to specify the RefSeq gene model. FastQC was used to evaluate sequence quality. Transcript expression levels were estimated by using Partek GS to determine the reads per kilobase of exon per million mapped reads (RPKM). A gene was considered expressed if the RPKM value was 0.1 or greater. Ingenuity Pathway Analysis (IPA; Ingenuity, Mountain View, Calif) was used for pathway analysis. This study used the high-performance computational capabilities of the Biowulf Linux cluster at the National Institutes of Health (Bethesda, Md; http://biowulf.nih.gov).

Statistical analysis

Sequencing results were analyzed with Partek GS 6.6 software and expressed in RPKM values. RPKM values were $\log_2$ transformed with an offset by 0.001, and then ANOVA was performed to determine differential gene expression comparing patient groups versus HVs (Partek GS 6.6). Statistical analyses for all other measurements were performed with GraphPad Prism software (GraphPad Software, La Jolla, Calif). All measurements other than RNA-Seq were done in duplicates or triplicates. Comparison between groups was performed by using a nonparametric test (Mann-Whitney test), unless otherwise indicated. P values of less than .05 were considered statistically significant.

RESULTS

Study population

A total of 21 patients and 13 healthy subjects with ages ranging from 28 to 45 years were recruited (Table I). All patients had positive SPT responses to peach LTP (Pru p 3) and presented LTP (Pru p 3)–specific IgE in serum, as measured by using ImmunoCAP (Table I and see Table E1).

Whole-transcriptome analysis

Alignment of sample reads to the human genome database using TopHat showed that 75% to 92% of reads from each sample were properly mapped to the human genome. Sequencing resulted in an average of 40,821,991 reads mapped per subject, which corresponded to a total of 20,650 expressed genes. Transcriptome comparison between the different groups indicated broader expression changes in patients with LTP-A than in those with NSAID-LTP-A. Further details on these comparisons can be found in the Methods section in this article's Online Repository. Of note, transcript abundance in blood might reflect differences in transcriptional regulation or in blood cell composition, both of which are valuable predictors of underlying pathology.[22,23] The numbers of blood cell subtypes in both patient cohorts were in the normal range, and no differences were found between patients with LTP-A and patients with NSAID-LTP-A (see Table E2 in this article's Online Repository at www.jacionline.org).

Differentially expressed genes in both patient subsets are associated with gastrointestinal diseases and epithelial alterations

Cancer and gastrointestinal diseases were identified as the main diseases/disorders associated with differentially expressed genes in both NSAID-LTP-A–specific (Table II) and LTP-A–specific

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