

Metabolomics and allergy: Opening Pandora's box



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Understanding disease pathogenic mechanisms is still the best way to improve diagnosis, prognosis, and therapeutic strategies. After decades of biased clinical observations or targeted disease models, where suspected mechanisms were essentially based on the demonstration of a rarely more than 1 ligand to receptor interaction, an unbiased approach based on the analysis of complex databases built on well-defined clinical expressions confronted to systems biology analyses (genomics, transcriptomics, metabolomics, proteomics, etc) has allowed the characterization of essential disease clusters. This has been the hallmark of the development of the field of severe asthma with the definition of essential asthma endotypes,¹ and more recently for atopic dermatitis.² Clusters are linked to potential disease markers, which may eventually become targets for therapeutic intervention. Asthma has been emblematic in this respect, and the current availability of powerful anti-IL-5 or IL-4/IL-13 biologicals is in large part derived from the creative work performed by several severe asthma networks over the recent few years. Therefore, applying this approach—an untargeted approach based on metabolomic analysis of patients' serum—to allergy domains such as food allergy (FA), where progress in terms of disease markers, diagnosis, therapy, and prevention is largely unmet, represents a methodological breakthrough, which may feed novel, unsuspected pathogenic hypothesis and potential therapeutic strategies. This is the difficult path that Talal A. Chatila's group has chosen to follow in a well-documented, substantial, and highly stimulating report in this issue.³ Serum metabolomic profiling by mass spectrometry of children with FA, children with asthma, and healthy controls was performed in an untargeted manner and analyzed by complex but now well-established biostatistical methodologies. The main conclusions indicate that pediatric FA metabolomic signature is associated with a decrease in serum level of sphingolipids and ceramides, lysophospholipids, lysoplasmalogens, diacylglycerol, and fatty acids as major lipid metabolite categories. Quite interestingly, FA appears to shape the metabolic landscape of these children, because the profile of

children with FA and asthma was similar to the profile of children with FA alone, but radically different from the profile of children with asthma alone and of course from the profile of healthy children. Moreover, the metabolomic profile of food-allergic children without or with asthma was closely linked to the severity of allergic reactions to food, and even more so in children with multiple food allergies. In these cases, severe systemic reactions to food were closely associated with changes in tryptophan metabolites, eicosanoids, plasmalogens, and fatty acids. The next point was to question the origin of such altered metabolomic signatures. Several observations pointed out the probable differentially regulated intervention of gut microbiota. Indeed, the specific comparison of children with FA with or without asthma with those with asthma alone showed significant alterations in the metabolism of *secondary* biliary acids as well as aromatic amino acids, products of microbiota-dependent enzymatic conversion in the gut. The natural killer (NK) T/NK_{Ti} (T invariant) cells' role, highlighted in asthma,⁴ was also examined, but NKT cells, at least in terms of frequencies and activation level, were found within the normal range. Taken together, without pinpointing definitive and specific pathogenic mechanisms, this study strongly suggests that the observed modulation in amino acid and lipid metabolites may be strongly dependent on immune cells' interplay with the gut microbiota.

Although this report has still the size of a pilot, cross-sectional study, its remarkable findings open the door to a number of sensitive questions around the pathogenesis of FA *versus* asthma, on the regulation of tolerance, and on the control of allergic reaction severity, and challenges the shaping of the immune programming by immune cell interactions with gut microbiota. Sphingolipids, present in all mammalian cell walls, but also issued from dietary sources, appear as potential key regulators of immune responses leading to specific allergic endotypes, here asthma *versus* FA. The link between sphingolipids, the microbiota, and specific immune-cell subsets such as NKT_i cells is of particular interest. Indeed, *Bacteroides* species in the gut, frequently associated with protective anti-allergic gut microflora, are able to metabolize food-derived sphingolipids, a known ligand of CD1d⁺ NKT_i cells, and to produce them.⁴ Interestingly, children with food sensitization presented with higher NKT_i activation than did children with clinical FA, suggesting a protective role of sphingolipid-activated NKT_i.⁵ Conversely, lower levels of sphingolipids in stools were found in subjects with clinical FA as compared with food-sensitized subjects,⁵ whereas NKT_i cells were found in reduced frequency in children allergic to cow milk.⁶ Alterations in plasma sphingolipids (enhancement of sphingosine-1-phosphate) were associated with severe allergic respiratory reactions.⁷ Nonetheless, the role of NKT_i cells remains undefined in the current report because no differences were observed in terms of frequency at least in children with FA *versus* controls, whereas their frequency was increased as

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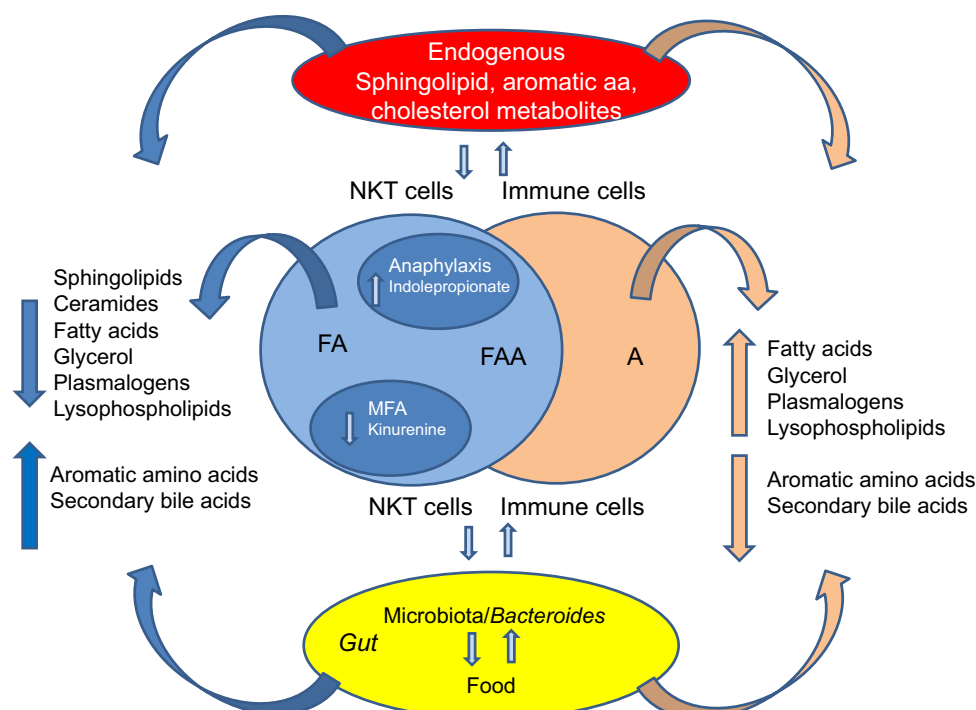


FIG 1. Schematic description of interactions between microbiota and food metabolites leading to differential production of sphingolipids, aromatic amino acids, and biliary acids depending on the phenotypes considered: FA, FA and asthma (FAA), asthma alone (A). Final serum levels of lipids, aromatic amino acids, and biliary acids (indicated on left and right sides of the figure) are the results of both endogenous and microbiota-dependent metabolism. MFA: multiple FA is associated with decreased activity of the kynurenine pathway, and anaphylaxis with enhanced activity of the indolepropionate pathway. aa, Amino acids.

expected from previous studies in those with asthma.⁸ Other findings point out the potential relationship between microbiota differential regulation in FA, such as the association of decreased levels of tryptophan metabolites from the kynurenine and serotonin pathways in children with sensitization to more than 1 food, as well as the association of higher levels of indole tryptophan metabolites with severe food-induced anaphylactic reactions. Both the kynurenine pathway and the indolepropionate pathway are key controllers of mucosal barriers and intestinal homeostasis via microbiota-dependent tryptophan metabolism. Sphingolipid metabolism was also strongly affected in children with FA with severe anaphylaxis. This observation fits well with the fact that sphingomyelins and ceramide metabolites may act as negative regulators of mast cells.⁹ Furthermore, metabolomic differences between children with FA and children with asthma without FA identified significant differences in secondary bile acid ratios, themselves also strongly dependent on gut microbiota composition and function. This suggested, as underlined by Crestani et al, a potential role for intestinal dysbiosis in distinctly contributing to FA or asthma phenotypes, and possibly in generating specific microbiota-induced metabolite signatures, which may serve as disease markers.

These metabolic findings open a quite innovative field of potential nonclassical allergic markers. A better understanding of the interplay between immune system and the microbiota with identification of specific lipid or amino acid metabolite fluctuation may help to better define not only specific pathologic conditions but possibly even to better evaluate in

defined phenotypes their risk of developing allergy. Such information, still largely lacking, will be key to better master the follow-up of our young patients in their march to allergy. There is certainly still a long way to such a goal. At that stage Crestani et al's study is still a relatively small pilot study, but based on high-quality recruitment bases with clinically and biologically well-defined cohorts of patients with FA and asthma. It will need confirmation in longitudinal observations. Long-term, prospective cohorts are needed to firmly establish the metabolomics findings presented here as the expression of basic mechanisms of FA, and not the trivial results of FA itself, in particular, as mentioned by authors, the consequence of restrictive diets. Furthermore, confirmation of these data in a replication cohort should ensure that these novel metabolomics findings can be validated. Anyhow, several exciting observations of this seminal article may shed new light on the field of tolerance regulation, as suggested by the alteration of tryptophan metabolism with possible impact on the breakdown of oral tolerance. The mechanisms leading from sphingolipid metabolism to T_H2 expression will have to be further explored. The observation that the *ORMDL3* gene, strongly associated with allergic asthma, may contribute to the modulation of sphingolipid levels in the lung¹⁰ supports the data presented here, and suggests that similar alterations of sphingolipid metabolism may play a role in the pathogenesis of not only asthma but possibly also FA. From these demonstrations may derive the long-term perspective of therapeutic interventions on sphingolipid metabolites. Dream or not, Crestani et al have here nicely attempted to reconcile

the immune system and the microbiota via crosstalks between immune cells and bacteria-dependent food metabolites, a domain that we will need to become more familiar with (Fig 1). They opened the doors to novel concepts of allergy pathogenesis, and we can bet that further work will lead to other unsuspected boxes and doors.

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