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Slow alignment of GMO allergenicity regulations with science on protein digestibility

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ABSTRACT

The current science on food allergy supports the dual allergen exposure hypothesis where sensitization to allergenic proteins is favored by dermal and inhalation exposure, and tolerization against allergy is favored by exposure in the gut. This hypothesis is bolstered by the epidemiological evidence showing that regions where children are exposed early in life to allergenic foods have lower rates of allergy. This led medical experts to replace the previous recommendation to exclude commonly allergenic foods from the diets of young children with the current recommendation that such foods be introduced to children early in life. Past beliefs that lowering gut exposure would reduce the likelihood that a protein would be allergenic led regulators and risk assessors to consider digestively stable proteins to be of greater allergenic risk. This resulted in international guidance and government regulations for newly expressed proteins in genetically engineered crops that aligned with this belief. Despite empirical results showing that allergens are no more digestively stable than non-allergens, and that gut exposure favors tolerization over sensitization, regulations have not come into alignment with the current science prompting developers to continue to engineer proteins for increased digestibility. In some rare cases, this could potentially increase sensitization risk.

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Pediatric Recommendations on Exposure to Foods

The frequency of food allergy has increased in recent years.[1] Children are especially vulnerable to food allergy, some of which is transitory and some of which may persist lifelong.[2,3] Medical experts previously recommended exclusion of commonly allergenic foods from young children to prevent sensitization to the allergenic proteins that they contain. Recently, it was found that in regions where commonly allergenic foods were introduced to children early in life, fewer individuals developed allergies to these same foods. Apparently, early introduction of commonly allergenic foods favors tolerization against allergy rather than sensitization. This led to the modern recommendation by medical experts to expose children to common allergenic foods early in life.[4] It is now thought that sensitization to food allergens may actually occur primarily due to dermal and/or inhalation exposure to food dust, whereas exposure to these same allergens in the gut favors tolerization against allergy (dual allergen exposure hypothesis).[5,6]

Current Regulatory Allergenicity Guidance for Genetically Engineered Crops

In a related area of science, it was previously thought that allergenic proteins were more resistant to gastrointestinal digestive enzymes compared with non-allergenic proteins due to predicted increased exposure in the gut.[7,8] It followed that international regulatory guidance and regional regulations for genetically engineered crops treated digestion-resistant newly expressed proteins as an allergenic risk.[9–13] As an example, the digestively stable insecticidal Cry9c protein variant (modified to be stable)[14] expressed in genetically engineered StarLink™ maize was not approved for food use by the US Environmental Protection agency (EPA) due to a perceived allergenic risk even though it

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was sourced from *Bacillus thuringiensis* (Bt), a bacterium widely distributed in the environment and widely used as a microbial insecticide without reports of allergy, and that the Cry9c protein shows low amino acid homology to known allergens.[15] Such regulations and examples prompted developers of genetically engineered crops to select proteins that were rapidly digested under simulated gastrointestinal conditions for expression by transgenes and/or to engineer newly expressed proteins to be more digestible.[16]

However, it is now apparent that no correlation exists between the digestive stability of proteins and their allergic status.[17–19] In fact, one study using a mouse model found that engineering a known digestively stable allergen to be more digestible prevented tolerization against that protein resulting in sensitization.[20] Similar to the aforementioned situation for allergenic food exposure in children, digestive stability likely increases gut exposure favoring tolerization against allergy. This realization has caused several research groups to suggest that digestive stability should no longer be considered a risk factor when conducting an allergenicity assessment for newly expressed proteins in genetically engineered crops.[21–23]

However, unlike the updated guidance by medical experts to expose young children to allergenic foods early in life, regulatory guidance for the safety assessment of newly expressed proteins in genetically engineered crops has not been updated to align with the current understanding that digestively stable proteins are not at greater risk for becoming allergens. Currently, digestively stable newly expressed proteins in genetically engineered crops are widely perceived and regulated as an allergenic risk. For example, the European Food Safety Authority (EFSA) recently held a workshop, in part to reconsider the value of digestion in the weight-of-evidence assessment of the allergenicity of novel food proteins.[24] After the workshop, EFSA acknowledged that the evidence for digestive stability as a direct predictor of allergenic risk for food proteins is weak and that it is critical to consider the feasibility and practicality of inclusion of this parameter in the weight-of-evidence assessment; nevertheless, EFSA inexplicably continued to endorse the validity of including digestibility in the weight-of-evidence assessment of allergenicity.[25] Consequently, developers continue to be motivated to engineer digestively unstable proteins for expression in genetically engineered crops, potentially increasing the risk of sensitization.

Elicitation Risk

Some accurately point out that reducing exposure in the gut via digestion should reduce the elicitation risk in individuals already sensitized to a food allergen (or cross-reactive proteins).[19] However, bioinformatic screening of all candidate newly expressed protein amino-acid sequences for similarity to known allergens is a very reliable indicator of the cross-reactive risk within those already sensitized to known allergens because very few truly novel allergens are discovered each year (high sequence homology within groups of cross-reactive allergens).[25–28] Additionally, the few new unique sequences are likely of minor clinical importance (low frequency of occurrence and not resulting in severe reactions) since they are primarily added to the database because they react with IgE antibodies, which is an often required attribute, but not sufficient in itself to indicate clinical relevance.[29] When a sequence is found to exceed the highly conservative amino acid thresholds for similarity to a known allergen, targeted IgE serum screening is typically conducted.[30] The use of powerful bioinformatic tools, a comprehensive allergen database (https://comparedatabase.org/), and a knowledge of the allergenic status of the organism from which the protein was sourced, in combination with targeted IgE serum screening, has resulted in no documented cases of allergenicity to any newly expressed protein in any commercialized genetically engineered crop.[31] Independent of the digestive stability of the candidate protein, these screening results are used to assess the potential elicitation risk for the protein. Thus, digestive stability is not used in any practical scenario as a differentiator for the acceptability of the elicitation risk for a newly expressed protein in a genetically engineered crop.[32,33] Notwithstanding, digestion information for known allergens can help researchers understand the steps potentially involved in clinical allergy, but this does not translate to understanding the

allergenic potential of proteins with unknown allergenicity such as those newly expressed in genetically engineered crops.[34,35]

Slow Regulatory Alignment with Current Science

Government regulations are often slow to align with scientific advances due to the bureaucratic processes typically involved in updating official regulations and guidance. This is exacerbated when official international guidance is the basis for regional regulation. However, precedent for deviating from international guidance has occurred. For example, EFSA ceased requiring that contiguous short-amino-acid exact matches with known allergens for newly expressed proteins in genetically engineered crops be used as a bioinformatic threshold to indicate cross-reactive allergenic risk when the science indicated that such matches were not useful in this endeavor.[10,36–38] Furthermore, resistance to change can sometimes create additional barriers to alignment of regulation with the most current science, especially when some perceive removing a regulatory hurdle, even when not protective for risk, as a weakening of regulation. This slow response is also a partial consequence of prescriptive regulations that are ill suited to adaptation or interpretation by regulators when the science advances, thus requiring an official regulation update to obtain scientific alignment for the risk assessment. Finally, some argue that a digestion assay may eventually be developed that is more physiologically relevant, and that results from such a future assay may better correlate with the allergenic potential of different proteins.[11] However, predicating the inclusion of digestibility results generated using current assays in regulations, based on the premise that an assay developed in the future may be predictive of allergenic risk, seems both unwarranted and inconsistent with the dual allergen exposure hypothesis where gut exposure favors tolerization against allergy. Together, these factors have thus far prevented our knowledge that digestibility does not correlate with the allergenic status of proteins and does not predict the allergenic risk for newly expressed proteins in genetically engineered crops from enabling regulatory alignment with the current science.

Recommendations

Under current prescriptive regulations for genetically engineered crops, it is important for regulatory bodies to prioritize the updating of regulations to align with the current science. In this case, digestive stability should be removed as a risk factor within the weight-of evidence allergenicity assessment of newly expressed proteins in genetically engineered crops since it carries no weight scientifically (and could, in rare cases, result in increased sensitization risk). It is also important for authors of regulation to anticipate scientific progress such that regulations are not so prescriptive as to impede safety assessors from aligning with the current scientific understanding before regulations can be formally updated. The principle of science-based risk assessment should continue to be the foundation of regulation.

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