

(DGLA) and arachidonic acid (AA), promote the T_H1 phenotype and thereby protect infants against atopic respiratory disease. We agree with Dr Das. Indeed, it is now well established that both the amount and the type of dietary fatty acids influence the fatty acid composition of cell membranes.³ The cell membrane fatty acid content of immune system cells, in turn, influences the profile of lipid mediators produced by those cells, which then directs the production of specific cytokines. The lipid mediator and cytokine microenvironment where antigen presentation takes place is critical for determining whether a naive T cell becomes T_H1 or T_H2 .⁴ However, the effects of AA metabolites on T-cell subtype and the immune response are complex. AA can give rise to various eicosanoids, some of which are thought to be mediators of atopic disease and promote the T_H2 phenotype. For example, prostaglandin E_2 , the major prostaglandin produced from AA in the lungs, can inhibit production of T_H1 cytokines (IL-2 and IFN- γ) in murine and human $CD4^+$ T cells and thus favor a T_H2 cytokine response.⁵ In contrast, prostaglandin E_2 can also inhibit T_H2 cytokine and IgE production by B cells.⁶ These data highlight the need for further study into the relation between LCPUFAs, eicosanoids, and T_H1 and T_H2 cytokines in general and in breast milk versus formula-fed infants in particular.

In a recent review of fatty acids and T_H1/T_H2 balance, Harbige³ suggests that LCPUFAs in the diet might promote development of the thymus and thus protect infants against immune disease. The thymus is the main source of T cells and the site where they diversify and are programmed into an effective primary T-cell repertoire.³ During in utero and early postnatal development, there is a striking accumulation of AA in the mouse thymus. This has been shown to be vital for thymocyte growth, differentiation, and apoptosis. Harbige³ postulates that accumulation of AA is linked to early thymic programming in relation to autoreactive T cells and tolerogenic mechanisms. Breast milk, which is rich in linoleic acid (the precursor of AA), is an important source of AA during early postnatal development. Deficiency of AA at this critical time could hinder the development of the thymus and induce long-lasting or permanent effects and thus increase an infant's susceptibility to immune disease.

Previously, we observed that breast-feeding was especially protective against ETS-related asthma and recurrent wheeze in children up to 6 years of age. It is possible that these protective effects occur through modulation of the immune system by LCPUFAs in breast milk. However, smoking itself (either active or passive) is a strong modulator of the immune system. Many different types of studies (in vitro and in vivo) have shown that cytokines are skewed toward the T_H2 phenotype when cells or animals are exposed to tobacco products, extracts, or simulated smoke. In one ex vivo human study, IL-2-secreting and IFN- γ -secreting cells were almost nondetectable in the bronchoalveolar lavage fluid of smokers compared with nonsmokers.⁷ In another study, IL-4 levels were increased in a dose-dependent manner in the peripheral blood monocytes from light and heavy smokers compared to

nonsmokers.⁸ Few such studies concerning the toxic effects of ETS on the immune system have been conducted in infants or children. This is unfortunate because the effects of ETS are far more severe at these early ages. The immune systems in infants and children are immature, and their capacity to produce cytokines is markedly diminished. In addition, all infants are slightly skewed toward a weak T_H2 phenotype at birth, regardless of whether they have atopy later in life or not. By skewing infants toward a T_H1 phenotype and/or by promoting normal thymic development, LCPUFAs in breast milk could counteract the immunotoxic effects of ETS.

This discourse focuses attention on the importance of LCPUFAs contained in breast milk in modulating the immune system during early development. Infant formulas are continuously being modified as important components of breast milk are identified. Formulas supplemented with LCPUFAs, in amounts similar to those in human milk, are now available, and their use has been shown to improve visual acuity in infants compared with the use of nonsupplemented formulas. It will be interesting in the future to compare the status of atopic conditions in infants fed LCPUFA-supplemented formulas with those fed breast milk or the nonsupplemented formulas.

Patricia C. Chulada, PhD, MHS^a

Michelle A. Carey, PhD^b

Darryl C. Zeldin, MD^{a,b}

^aOffice of Clinical Research

^bLaboratory of Pulmonary Pathobiology

National Institute of Environmental Health Sciences

National Institutes of Health

Research Triangle Park, NC

REFERENCES

1. Chulada PC, Arbes SJ Jr, Zeldin DC. Breast-feeding, passive smoking, and asthma and wheeze in children. *J Allergy Clin Immunol* 2003;112:808-9.
2. Das UN. Breast-feeding, atopy, and asthma. *J Allergy Clin Immunol* 2004;113:1002.
3. Harbige LS. Fatty acids, the immune response, and autoimmunity: a question of n-6 essentiality and the balance between n-6 and n-3. *Lipids* 2003;38:323-41.
4. Vieira PL, de Jong EC, Wierenga EA, Kapsenberg ML, Kalinski P. Development of Th1-inducing capacity in myeloid dendritic cells requires environmental instruction. *J Immunol* 2000;164:4507-12.
5. Betz M, Fox BS. Prostaglandin E_2 inhibits production of Th1 lymphokines but not of Th2 lymphokines. *J Immunol* 1991;146:108-13.
6. Pene J, Rousset F, Briere F, Chretien I, Bonnefoy JY, Spits H, et al. IgE production by normal human lymphocytes is induced by interleukin 4 and suppressed by interferons gamma and alpha and prostaglandin E_2 . *Proc Natl Acad Sci U S A* 1988;85:6880-4.
7. Hagiwara E, Takahashi K-I, Okubo T, Ohno S, Ueda A, Aoki A, et al. Cigarette smoking depletes cells spontaneously secreting Th1 cytokines in the human airway. *Cytokine* 2001;14:121-6.
8. Byron KA, Varigos GA, Wootton AM. IL-4 production is increased in cigarette smokers. *Clin Exp Immunol* 1994;95:333-6.
doi:10.1016/j.jaci.2003.12.014

Starlink genetically modified corn and allergenicity in an individual

To the Editor:

Sutton et al¹ have proposed that the double-blind, placebo-controlled food challenge (DBPCFC) approach is

the ultimate proof of the true allergenicity of Starlink or other transgenic proteins. However, the conclusions based on this approach to the genetically modified corn Starlink containing the Cry9C gene, as arrived at by Sutton et al,¹ need critical evaluation.

The patient investigated by Sutton et al¹ had complained of 3 allergic reactions after consumption of processed foods containing the transgenic corn at different time periods. However, when the DBPCFC test was conducted with the raw Starlink corn sample that was powdered and placed in a gelatin capsule, the patient did not have any symptoms. Similar observations were also seen in the skin prick test performed on the patient with the same corn specimen extract.

A factor that needs to be considered here is the influence of the food matrix. It is relevant to point out that the patient in question had originally consumed processed corn products while he experienced the reactions. The effect of other ingredients or food additives present in the processed corn products on the properties of the Cry9C protein needs to be examined because Martin-Orue et al² had earlier raised the possible influence of food matrices, which are the components of the human diet, on the extent of transgenic DNA degradation and also the ability to form complexes with proteins, including digestive enzymes.

The inconsistencies in the results of the Cry9C analysis also need to be considered. When the patient had submitted the corn chip samples for analysis to the US Food and Drug Administration (FDA), they were found to be positive for the Cry9C DNA but not the Cry9C protein. However, analysis of the corn specimen by the patient showed positive results for the Cry9C protein. It is not clear from the observations of Sutton et al¹ whether the corn specimen used for the DBPCFC study was positive for the Cry9C DNA, the Cry9C protein, or both.

According to the ELISA test developed by the Centers for Disease Control and Prevention and the FDA, the blood sample of the patient did not have IgE antibodies directed against the Cry9C protein. While developing the method, the FDA used the recombinant Cry9C protein expressed in *Escherichia coli* and not the plant-derived protein as the antigen. According to Kuiper et al³ the epitopes present on Cry9C in maize might not be present in the nonglycosylated *E coli*-derived protein. They also recognized that a specific goat antiserum against Cry9C was included in the ELISA because of the nonavailability of human serum containing the IgE antibody to Cry9C, which could have resulted in a lack of specificity shown by the ELISA test for human anti-Cry9C IgE. Perhaps a comparative study of the IgE binding could have been carried out with ELISA by using both the microbially derived and the plant-derived Cry9C proteins.

Thus the scientific community has to rigorously investigate many of these concerns before concluding unequivocally that the transgenic proteins in Starlink corn are not allergenic, and such unequivocal evidence could be used as a model for proving the safety of genetically modified foods.

Vasanthi Siruguri, MSc, PhD
Sesikeran B, MD
Ramesh V. Bhat, MSc, PhD
National Institute of Nutrition
Indian Council of Medical Research
Hyderabad-500007, India

REFERENCES

1. Sutton SA, Assaad AH, Steinmetz C, Rothenberg ME. A negative double-blind, placebo-controlled challenge to genetically modified corn. *J Allergy Clin Immunol* 2003;112:1011-2.
2. Martin-Orue SM, O'Donnell AG, Arino J, Netherwood T, Gilbert HJ, Mathers JC. Degradation of transgenic DNA from genetically modified soya and maize in human intestinal simulations. *Br J Nutr* 2002;87:533-42.
3. Kuiper HA, Kleter GA, Noteborn HPJM, Kok EJ. Assessment of the food safety issues related to genetically modified foods. *Plant J* 2001;27:503-28.

doi:10.1016/j.jaci.2004.01.571

Reply

To the Editor:

We would like to thank Siruguri et al¹ for emphasizing several important points related to transgenic foods and their potential for allergenicity.

The authors suggest that processing of the chips could have uncovered an antigenic epitope that might not have been present in the raw corn. This is based on evidence that cooking, heating, or baking can change protein conformation. This is true, yet the effects of processing are variable. Some antigens, such as Ara h 1 and Ara h 2, become more allergenic,² whereas others, such as BSA, become less allergenic.³ Accordingly, it is difficult to predict the effect that making a tortilla chip has on the allergenicity of the Cry9c protein. However, a study in 1997 showed that corn-derived *Bacillus thuringiensis* (Bt) protein was resistant to alteration by both heat and simulated mammalian gastric fluid.⁴ This would suggest that the protein remains intact on processing and consumption.

In addition, on dry milling Starlink corn, 40% of the Cry9c protein content is lost, and additional processing, such as masa milling in corn chips, can reduce the protein content to undetectable levels.⁵ This is, in our opinion, the most likely reason that the US Food and Drug Administration (FDA) did not find the Cry9c protein in the sample provided by the subject. It was also a reason not to use the corn chips for our food challenge. The corn chips to which the patient reacted were made available by the consumer's group Friends of the Earth. Unfortunately, they had been in storage for many months and were unsuitable for oral consumption. We chose to prepare our own Starlink corn sample by using material that was certified by an academic laboratory at Iowa State University. This sample was only Starlink corn and was not intermixed with wild-type corn. The amount of Cry9c protein and DNA was therefore much larger in our sample than in the corn chips contaminated with Starlink. We also pulverized the Starlink corn only to get it into the capsules; there was no heating or processing of the samples.

the ultimate proof of the true allergenicity of Starlink or other transgenic proteins. However, the conclusions based on this approach to the genetically modified corn Starlink containing the Cry9C gene, as arrived at by Sutton et al,¹ need critical evaluation.

The patient investigated by Sutton et al¹ had complained of 3 allergic reactions after consumption of processed foods containing the transgenic corn at different time periods. However, when the DBPCFC test was conducted with the raw Starlink corn sample that was powdered and placed in a gelatin capsule, the patient did not have any symptoms. Similar observations were also seen in the skin prick test performed on the patient with the same corn specimen extract.

A factor that needs to be considered here is the influence of the food matrix. It is relevant to point out that the patient in question had originally consumed processed corn products while he experienced the reactions. The effect of other ingredients or food additives present in the processed corn products on the properties of the Cry9C protein needs to be examined because Martin-Orue et al² had earlier raised the possible influence of food matrices, which are the components of the human diet, on the extent of transgenic DNA degradation and also the ability to form complexes with proteins, including digestive enzymes.

The inconsistencies in the results of the Cry9C analysis also need to be considered. When the patient had submitted the corn chip samples for analysis to the US Food and Drug Administration (FDA), they were found to be positive for the Cry9C DNA but not the Cry9C protein. However, analysis of the corn specimen by the patient showed positive results for the Cry9C protein. It is not clear from the observations of Sutton et al¹ whether the corn specimen used for the DBPCFC study was positive for the Cry9C DNA, the Cry9C protein, or both.

According to the ELISA test developed by the Centers for Disease Control and Prevention and the FDA, the blood sample of the patient did not have IgE antibodies directed against the Cry9C protein. While developing the method, the FDA used the recombinant Cry9C protein expressed in *Escherichia coli* and not the plant-derived protein as the antigen. According to Kuiper et al³ the epitopes present on Cry9C in maize might not be present in the nonglycosylated *E coli*-derived protein. They also recognized that a specific goat antiserum against Cry9C was included in the ELISA because of the nonavailability of human serum containing the IgE antibody to Cry9C, which could have resulted in a lack of specificity shown by the ELISA test for human anti-Cry9C IgE. Perhaps a comparative study of the IgE binding could have been carried out with ELISA by using both the microbially derived and the plant-derived Cry9C proteins.

Thus the scientific community has to rigorously investigate many of these concerns before concluding unequivocally that the transgenic proteins in Starlink corn are not allergenic, and such unequivocal evidence could be used as a model for proving the safety of genetically modified foods.

Vasanthi Siruguri, MSc, PhD
Sesikeran B, MD
Ramesh V. Bhat, MSc, PhD
National Institute of Nutrition
Indian Council of Medical Research
Hyderabad-500007, India

REFERENCES

1. Sutton SA, Assaad AH, Steinmetz C, Rothenberg ME. A negative double-blind, placebo-controlled challenge to genetically modified corn. *J Allergy Clin Immunol* 2003;112:1011-2.
2. Martin-Orue SM, O'Donnell AG, Arino J, Netherwood T, Gilbert HJ, Mathers JC. Degradation of transgenic DNA from genetically modified soya and maize in human intestinal simulations. *Br J Nutr* 2002;87:533-42.
3. Kuiper HA, Kleter GA, Noteborn HPJM, Kok EJ. Assessment of the food safety issues related to genetically modified foods. *Plant J* 2001;27:503-28.

doi:10.1016/j.jaci.2004.01.571

Reply

To the Editor:

We would like to thank Siruguri et al¹ for emphasizing several important points related to transgenic foods and their potential for allergenicity.

The authors suggest that processing of the chips could have uncovered an antigenic epitope that might not have been present in the raw corn. This is based on evidence that cooking, heating, or baking can change protein conformation. This is true, yet the effects of processing are variable. Some antigens, such as Ara h 1 and Ara h 2, become more allergenic,² whereas others, such as BSA, become less allergenic.³ Accordingly, it is difficult to predict the effect that making a tortilla chip has on the allergenicity of the Cry9c protein. However, a study in 1997 showed that corn-derived *Bacillus thuringiensis* (Bt) protein was resistant to alteration by both heat and simulated mammalian gastric fluid.⁴ This would suggest that the protein remains intact on processing and consumption.

In addition, on dry milling Starlink corn, 40% of the Cry9c protein content is lost, and additional processing, such as masa milling in corn chips, can reduce the protein content to undetectable levels.⁵ This is, in our opinion, the most likely reason that the US Food and Drug Administration (FDA) did not find the Cry9c protein in the sample provided by the subject. It was also a reason not to use the corn chips for our food challenge. The corn chips to which the patient reacted were made available by the consumer's group Friends of the Earth. Unfortunately, they had been in storage for many months and were unsuitable for oral consumption. We chose to prepare our own Starlink corn sample by using material that was certified by an academic laboratory at Iowa State University. This sample was only Starlink corn and was not intermixed with wild-type corn. The amount of Cry9c protein and DNA was therefore much larger in our sample than in the corn chips contaminated with Starlink. We also pulverized the Starlink corn only to get it into the capsules; there was no heating or processing of the samples.

The fact remains that Cry9c DNA, and not protein, was detected in the sample of corn chips that the study subject provided to the FDA. The historical piece of information that the subject detected protein in a sample of chips to which he reacted is intriguing but is less important than the FDA's results. Siruguri et al¹ cite Martin-Orue et al⁶ as evidence that the subject's reported allergic reaction might be due to dietary factors interacting with Cry9c rather than the Cry9c protein's innate allergenic potential. However, the model in the study only evaluated nucleic acids and not proteins, which are the most common allergens causing IgE-mediated reactions. Furthermore, the cited study was designed to evaluate the plausibility that transgenic DNA might persist long enough to become incorporated into gut flora and did not evaluate transgenic protein degradation or allergenicity. Although it is plausible that the food matrix affects protein degradation and allergenicity, the biologic role of this process has not been clearly established. For example, individuals with peanut allergy tend to react to Ara h 1 and Ara h 2 whether it is in peanut butter, baked goods, or raw peanuts; individuals also react whether they ingest the antigen or it is touched to their mucosa.

Finally, a distinction needs to be made between a protein's allergenic potential and the occurrence of allergy in a single patient. Scientists are currently unable to reliably predict whether a protein will be an allergen. Many allergenic proteins have similar properties; however, there is not one single property or combination of properties that globally predicts allergenicity. Until reliable methods of allergen predictability are established, we will continue to make educated guesses concerning when and how a protein might be an allergen. We therefore

advocate the double-blind, placebo-controlled food challenge as the method of choice to prove allergenicity in an individual.

Steven A. Sutton, MD
Amal H. Assa'ad, MD
Christine Steinmetz, RN
Marc E. Rothenberg, MD, PhD
Division of Allergy/Immunology
Cincinnati Children's Hospital Medical Center
3333 Burnet Ave
Cincinnati, OH 45229

REFERENCES

1. Siruguri V, Sesikeran B, Bhat RV. Starlink genetically modified corn and allergenicity in an individual. *J Allergy Clin Immunology* 2004;113:1003-4.
2. Maleki SJ, Chung SY, Champagne ET, Raufman JP. The effects of roasting on the allergenic properties of peanut proteins. *J Allergy Clin Immunology* 2000;106:763-8.
3. Fiocchi A, Restani P, Riva E, Mirri GP, Santini I, Bernardo L, et al. Heat treatment modifies the allergenicity of beef and bovine serum albumin. *Allergy* 1998;53:798-802.
4. Peferoen M. In Vitro digestibility and heat stability of the endotoxin Cry9C of *Bacillus thuringiensis*. In Vitro Digestibility MRID No. 44258108. Available at: <http://www.epa.gov/pesticides/biopesticides/pips/old/cry9c/der-44258108a.htm>. Accessed December 31, 2003.
5. SAP 2000b. Assessment of scientific information concerning StarLink corn. FIFRA Scientific Advisory Panel, SAP Report No. 2000-06, December 1, 2000. Available at: <http://www.epa.gov/oscpmont/sap/2000/november/one.pdf>. Accessed December 31, 2003.
6. Martin-Orue SM, O'Donnell AG, Arino J, Netherwood T, Gilbert HJ, Mathers JC. Degradation of transgenic DNA from genetically modified soya and maize in human intestinal simulations. *Br J Nutr* 2002;87:533-42.

doi:10.1016/j.jaci.2004.01.572