

A negative, double-blind, placebo-controlled challenge to genetically modified corn

To the Editor:

Starlink corn is a genetically engineered corn developed by Aventis CropScience (Research Triangle Park, NC) to express an insecticidal protein Cry9c (also known as Bt protein). This protein is derived from the bacterium *Bacillus thuringiensis* and is directed at lepidopteran and coleopteran insects. Starlink corn also contains genes from *Streptomyces hygroscopicus*, which make the corn tolerant to glufosinate herbicides. In 1998, the US Environmental Protection Agency (EPA) approved registration of Starlink for restricted use as animal feed. In September 2000, the consumers' group Friends of the Earth announced that traces of Starlink corn DNA were found in taco shells made by Kraft Foods (Phillip Morris, Northfield, Ill), signifying an inadvertent introduction of this transgenic food into the human food supply.¹ This initiated the eventual recall of several hundred corn products by the US Food and Drug Administration (FDA), as well as larger concerns about how this transgenic food had entered the human food supply.

On June 11, 2001, the Centers for Disease Control and Prevention (CDC) published an investigation into human health effects associated with the transgenic corn. The investigation found that 28 subjects had experienced apparent allergic reactions after ingestion of the transgenic corn. An ELISA to detect IgE antibodies directed against Cry9c revealed that none of the 17 subjects who submitted serum samples had detectable IgE antibodies to this protein.² In July 2001, the US EPA Scientific Advisory Panel (SAP) reviewed data associated with the FDA's assay method of the submitted specimens. The panel was concerned that the assay lacked sufficient sensitivity to conclude that the sera tested contained no Cry9c-specific IgE antibodies.³ The US EPA SAP had previously formulated criteria to assess the

potential allergenicity of proteins. Cry9c was evaluated by these criteria, and it was determined that Cry9c had a medium likelihood of being an allergen and that these properties were more concerning than other transgenic Cry proteins already in the human food supply—eg, Cry1Ac.³ The allergenicity was based on Cry9c's resistance to acid treatment and to protease digestion, molecular weight (68.7 kd), glycosylation, and the immunologic response detected in the blood of the Brown Norway Rat.⁴

We report the results of a double-blind, placebo-controlled food challenge (DBPCFC) in a patient who reported allergic reactions to Starlink corn products and who had been enrolled in the CDC/FDA evaluation. The patient is a 58-year-old male who complained of at least 3 allergic reactions after consumption of products thought to contain the transgenic corn. In September 2000, the patient consumed a meal with tortillas, beans, rice, beef, and plantains with cheese. Fifteen minutes after finishing the meal, he experienced diarrhea and later pruritis. He self-administered diphenhydramine and intramuscular epinephrine (0.3 mg at a concentration of 1:1000) that was prescribed over the phone by a physician. He had eventual resolution of his symptoms. In April 2001, he consumed corn chips and 3 hours later, developed pruritis, hives, and numbness of his lips. He later developed perioral and periocular swelling. He again self-administered an intramuscular injection of epinephrine, which cleared the symptoms with no further evolution. He sent a sample of these corn chips to the US FDA. In June 2001, the Department of Health and Human Services contacted the patient to let him know that a sample of corn chips that he allegedly reacted to tested positive for Cry9c DNA but negative for Cry9c protein. A separate letter from the Department of Health and Human Services stated that his blood sample submitted to the CDC and FDA for analysis under the previously mentioned CDC investigation did not have IgE antibodies directed against the Cry9c protein.

The patient subsequently challenged himself to corn chips that he reported being Cry9c protein-positive by a QuickStix Strip assay. The QuickStix Strip assay was designed by EnviroLogix (Portland, Me) before Starlink corn's discovery in the human food supply. It is for use by workers in the grain industry to test large shipments of corn for the presence of Cry9c protein. The QuickStix Strip assay is designed to extract and detect the presence of >0.125% of the Cry9c protein in ground corn samples.⁵ He again had itching after consumption of the corn chips. Forty minutes later, he developed a hive that was documented by an emergency room physician. In August 2001, an independent allergist performed intradermal skin testing on the subject to an extract of Starlink corn, and he had a positive reaction at a 1:10 dilution, which consisted of a 5-mm wheal with local pruritis. The patient never eliminated corn products from his diet.

Because of the confusion surrounding this case and the potential allergenicity of Cry9c in general, we admitted the patient to our facility for a DBPCFC to Starlink corn. Informed consent was obtained from the patient.

The patient was admitted to the hospital for 3 days. Skin-prick tests were done with a commercially available corn extract (Hollister-Stier, Spokane Wash), an extract of wild-type corn, and an extract of Starlink corn. The extracts were prepared by adding 1 g of the corn flour to 10 mL albumin saline. The extract was agitated overnight at 37°C. The material was subsequently centrifuged, and the supernatant was used for skin testing. Wild-type and Starlink corn, kindly provided by Dr Charles R. Hurburgh, Jr, of Iowa State University Extension Seed Testing Laboratory, were obtained. They were certified free and positive, respectively, for the presence of Starlink protein. These corn specimens and lactose placebo were ground to powder and placed in gelatin capsules. Neither the patient nor the medical staff involved had any knowledge of which product he was being administered. He fasted before all challenges except for his antihypertensive candesartan cilexetil (Atacand, AstraZeneca LP) 16 mg PO QD. Each day, he received a total of 21 g of the randomized test material at gradually increasing doses over a 210-minute period. The dose of 21 g was chosen because it approximates the amount of corn flour in a tortilla. The patient was continually evaluated for signs of allergic reaction.

Skin tests were placed by prick with a positive histamine control and a saline control. The histamine was positive, with a 6-mm wheal and a 20-mm flare. The saline control was negative. All of the corn skin tests were uniformly negative. Throughout the entire 3-day period of the DBPCFC, the patient never developed any hives, respiratory symptoms, hypotension, vomiting, or diarrhea. At the end, it was revealed that he had received the Starlink corn on day 2 of the DBPCFC.

Genetic modification is meant to improve the quality and quantity of agricultural products. Whether intentionally or unintentionally, these genetically modified foods have been and will be entering the human food supply. Transgenic foods have created many fears and concerns with the public, most notably the possibility of causing allergic reac-

tions. It is incumbent on the scientific community to rigorously investigate these concerns and submit to consumers evidence-based decisions on the risks and benefits.

It is well established that the DBPCFC is the gold standard for evaluating a causative relation between a food and reported reactions. Because the criteria used by the US EPA SAP to suggest the potential allergenicity of a protein are not well standardized and validated, we thought that a DBPCFC was warranted for this patient. We propose that a DBPCFC is likely to remain the ultimate proof of the true allergenicity of this and other transgenic proteins in the future.

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