



Confirmation of a predicted lack of IgE binding to Cry3Bb1 from genetically modified (GM) crops

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ABSTRACT

Some GM crops including MON863 corn and stack varieties contain Cry3Bb1 protein. Cry3Bb1 is very important from the standpoint of assessing the safety of GM crops. In this study Cry3Bb1 was assessed from the standpoint of possible binding to IgE from allergy patients. First, an ELISA that was improved in our laboratory was used to test serum samples from 13 corn allergy patients in the United States with recombinant Cry3Bb1 expressed in *Escherichia coli*, and serum samples from 55 patients in Japan with various food allergies were also assayed. Two samples from the Japanese allergy patients were suspected of being positive, but Western blotting analysis with purified Cry3Bb1 indicated that the binding between IgE and Cry3Bb1 was nonspecific. Ultimately, no specific binding between IgE and recombinant Cry3Bb1 was detected. Next, all proteins extracted from MON863 corn and non-GM corn were probed with IgE antibodies in serum samples from the corn allergy patients by Western blotting, but the staining patterns of MON863 and non-GM corn were similar, meaning that unintended allergic reactions to MON863 are unlikely to occur. Our study provides additional information that confirms the predicted lack of IgE binding to Cry3Bb1 in people with existing food allergies.

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1. Introduction

Cry3Bb1 is an insecticidal protein produced by *Bacillus thuringiensis* that kills corn rootworm larvae. The Cry3Bb1 coding sequence was introduced into the corn genome by Monsanto to develop MON863 corn, a grain that is protected against feeding damage by the corn rootworm. Several stack varieties containing the Cry3Bb1 gene were also developed to simultaneously protect corn from different species of insects.

There has been controversy about the safety of GM crops. International regulatory authorities consider it necessary to demonstrate that new crops developed by applying biotechnology methods are as safe as conventional crops, and they have published Codex guidelines (2003) for pre-market research on GM crops. The Codex guidelines have adopted a weight-of-evidence approach, and there are five key elements in the assessment. The first is whether the source of the gene is an organism that is a frequent cause of allergic reactions. The second element is a bioinformatic element, which consists of sequence searches for matches with >35% identity over 80 amino acids (or of >50% overall identity for more realistic risks) between the protein of interest and all known allergens. The third is IgE testing to determine whether IgE antibodies bind the introduced protein. The fourth element is stability

testing to determine whether the protein expressed is highly resistant to digestion by pepsin, and the fifth is the abundance and stability of the protein in the food.

There is no international agreement in regard to post-marketing studies of GM crops. The authors think that surveillance studies of the allergenicity of GM crops are needed in order to achieve better acceptance of GM crops. Some people think that the pre-market study of Cry3Bb1 was sufficient and that our study was unnecessary. However, we think it is meaningful to provide as many data concerning the safety of GM crops as possible. This study is one of a series of studies we have been conducting to confirm the predicted lack of IgE binding to novel proteins in genetically modified crops. We performed an IgE binding test of recombinant Cry3Bb1 and an extract of MON863 as surveillance research. However, *B. thuringiensis*, which biosynthesizes Cry3Bb1, does not usually induce allergic reactions, and the Cry3Bb1 sequence does not match that of any known allergens. Moreover, Cry3Bb1 is rapidly and extensively degraded by pepsin. That means that IgE binding tests are not obligatory according to the Codex guidelines. Based on the international criteria, our study was optional. However, it adds information regarding the safety assessment of GM corn containing Cry3Bb1.

In this study, we assessed the binding characteristics to recombinant Cry3Bb1 of IgE antibodies in serum samples of allergy patients. An ELISA that was improved in our laboratory was used to conduct the tests, and the results showed that the IgE antibodies

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in the serum samples of corn allergy patients in the United States and patients with various food allergies in Japan did not specifically bind to recombinant Cry3Bb1.

IgE antibodies from corn allergy patients were also tested for their binding profiles to all proteins extracted from MON863 and a non-GM corn counterpart, but the staining patterns of the two corn extracts were similar.

In addition to the existing body of evidence indicating that MON863 is as safe as non-GM corn, the results of this study confirmed the predicted lack of binding to Cry3Bb1 by IgE from persons with corn and other food allergies.

2. Methods

2.1. Cloning and expression of Cry3Bb1

Genomic DNA was extracted from MON863 GM corn with a DNeasy Plant Mini Kit (Qiagen, Tokyo, Japan), and the Cry3Bb1 coding sequence introduced into MON863 was amplified by the polymerase chain reaction (PCR). The amplicon was then subcloned into plasmid vector pT7-Blue-T (Merck, Darmstadt, Germany), and the nucleotide sequence was confirmed. An NdeI site and XhoI site were introduced at the 5' end and at the 3' end, respectively, of the Cry3Bb1 coding sequence by PCR. The Cry3Bb1 gene fragment was then subcloned into pCold1 (Takara, Ohtsu, Japan) and transformed into pG-Tf2/BL21 (Takara). Expression of the recombinant protein was induced with 0.1 mM isopropyl-1-thio- β -D-galactopyranoside (IPTG). A fusion peptide consisting of 16 amino acid residues including a His tag was added to the N-terminus of the recombinant Cry3Bb1.

2.2. Purification of recombinant Cry3Bb1

After induction of expression of the recombinant protein, 250 ml of the *Escherichia coli* culture was subjected to centrifugation. The *E. coli* pellet was washed in 25% sucrose, 0.1 M Tris-HCl (pH 8.0), and 1 mM EDTA, and it was then resuspended in 3 ml of 10 mM Tris-HCl (pH 8.5) (buffer A) supplemented with protease inhibitors and disrupted by sonication. The suspension was centrifuged, and the supernatant was recovered and applied to a 5-ml HiTrapQ HP column (GE Healthcare, Buckinghamshire, England). The proteins that were adsorbed were eluted in a stepwise manner with 25 ml of buffer A, followed by successive 5 ml volumes of buffer A plus 0.1 M, 0.2 M, 0.3 M, 0.4 M, and 0.5 M NaCl. The fractions eluted with 0.2 M NaCl and 0.3 M NaCl were combined, mixed with imidazole solution to a final imidazole concentration of 20 mM, and then loaded onto a 5-ml HisTrap HP column (GE Healthcare). The proteins adsorbed were eluted in a stepwise manner with a 25 ml volume of 10 mM sodium phosphate (pH 7.4), 0.25 M NaCl (buffer B) plus 20 mM imidazole, and then successive 5 ml volumes of buffer B plus 40 mM, 100 mM, 200 mM, 300 mM, and 500 mM imidazole. The fractions eluted with 200 mM, 300 mM and 500 mM imidazole were combined, and then concentrated with Centrprep YM-50 (Millipore, Bedford, MA, USA). Imidazole was removed with a 5-ml HiTrap Desalting column (GE Healthcare) and buffer containing 0.1 M Tris-HCl (pH 7.0) and 0.1 M NaCl. During purification each fraction was analyzed by SDS-PAGE and Coomassie brilliant blue (CBB) staining. Polyclonal rabbit antibody Bt Cry3B AP was purchased from Fitzgerald Industries International, Inc. (Concord, MA, USA) and used for the immunoblot analysis.

2.3. Serum samples

Commercially available serum samples from a total of 13 corn allergy patients in the US (PlasmaLab International, Everett, WA,

USA) were purchased in 2005 and 2006. Information on the serum samples is shown in Table 1.

In 2003 and later serum samples were obtained from 55 Japanese patients with food allergies who exhibited signs of allergy clinically and were positive for allergen-specific IgE when tested by the Immuno-CAP method (Pharmacia Diagnostic, Uppsala, Sweden). Information on the serum samples is shown in Table 2.

It could not be confirmed if the corn allergy was diagnosed clinically by double-blind, placebo-controlled food challenge (DBPCFC).

Sera from three healthy donors (specific-IgE-negative) in Japan were used as controls. Informed consent had been obtained from all of the food allergy patients and healthy donors. This study was approved by the Institutional Review Board of the National Institute of Health Sciences.

2.4. ELISA

Assay plates (96 wells) were coated with purified Cry3Bb1 (0.2 μ g/ μ L 50 mM sodium carbonate buffer [pH 9.6]/well) at 4 °C overnight. After washing with PBS containing 0.05% Tween 20 (PBS-T), the plates were blocked for 2 h at room temperature (RT) with PBS containing 0.1% casein, and then washed with PBS-T and incubated overnight at 4 °C with serum samples diluted 20-fold in 0.1% casein-PBS. Next, the wells were washed with PBS-T containing 1 M NaCl as described previously (Takagi et al., 2006). The wells were then exposed for 1 h at RT to peroxidase-conjugated goat anti-human IgE antibodies (Nordic Immunology, 1:1000) in 0.1% casein-PBS and washed. Substrate solution (TMB reagent; BD Biosciences, San Diego, CA, USA) was added, and color development was allowed to continue for 5 min. Colorimetric intensity (OD₄₅₀–OD₅₇₀) was measured according to the manufacturer's protocol, and the control was measured after adding buffer alone. The control value has already been subtracted from the values shown in Figs. 2 and 3. The experiment was carried out twice, and serum samples were measured in duplicate on each plate. A serum sample was judged to be ELISA-positive, when the absorbance of the sample was greater than the value of the mean plus 5 SD of the control serum values (Yagami et al., 1998).

Coating of the plates with recombinant Cry3Bb1 was confirmed by binding with rabbit antibody Bt Cry3B AP (Fitzgerald Industries International, Inc.) and by failure to bind with normal rabbit serum.

The inter variability and intra-assay variability of the ELISA data were 10.3% and 7.6%, respectively.

Table 1

Serum characteristics. All serum samples were from American patients with corn allergy and were purchased from PlasmaLab International. They were all reactive to multiple food allergens. The anti-Cry3Bb1 IgE ELISA values are after subtraction of the mean buffer control value. The anti-Cry3Bb1 IgE ELISA values of the healthy donors were 0.0173, 0.0231, and 0.0385, respectively.

Serum No.	Anti-Cry3Bb1 IgE ELISA (OD ₄₅₀₋₅₇₀)	Immuno-CAP for corn (IU/ml)	Allergens other than corn which bind to IgE
1	0.0295	18.5	Potato, chicken serum proteins
2	0.0350	5.1	
3	0.0260	5.4	
4	0.0225	19.3	
5	0.0560	13.8	
6	0.0270	4.1	
7	0.0135	13.9	
8	0.0200	4.3	
9	0.0270	10.2	
10	0.0090	31.5	Chestnut, walnut
11	0.0090	28.0	
12	0.0175	15.3	
13	0.0135	4.9	

Table 2

Serum characteristics. All serum samples were from Japanese patients with food allergy symptoms. FA, food allergy; AD, atopic dermatitis; ND, not determined. The anti-Cry3Bb1 IgE ELISA values shown are after subtracting the mean buffer control values. The anti-Cry3Bb1 IgE ELISA values of the healthy donors were 0.0227, 0.0409, and 0.0394, respectively.

Serum No.	Total IgE	Anti-Cry3Bb1 IgE ELISA (OD _{450–570})	Main diagnosis
1	75	0.0325	FA (egg, milk), AD
2	23.9	0.0135	FA (egg), AD, asthma
3	253	0.0380	FA (egg), AD, asthma
4	130	0.0365	FA (egg), AD
5	241	0.0935	FA (egg), AD
6	335	0.0215	FA (egg), asthma
7	130	0.0280	FA (egg), AD, asthma
8	185	0.0675	FA (egg), AD, allergy to cedar pollen
9	809	0.0275	FA (egg), AD
10	20,500	0.0260	FA (egg), AD, anaphylaxis to milk, allergy to cedar pollen
11	52	0.0200	FA (wheat), AD
12	30	0.0105	FA (egg), AD
13	64	0.0290	FA, allergy to cedar pollen
14	234	0.0225	FA (egg), asthma
15	221	0.0160	FA (egg)
16	23	0.0145	FA (egg, wheat, milk)
17	122	0.0320	FA (egg)
18	1060	0.0175	FA (egg), AD
19	7560	0.0490	FA (egg), AD
20	177	0.0265	FA (egg, milk), AD
21	1900	0.0180	FA (egg), AD
22	77	0.0260	FA (egg), AD, asthma
23	1050	0.0415	FA (egg, wheat), AD
24	215	0.0365	Allergy to cedar pollen, oral allergy syndrome
25	129	0.0685	Allergy to house dust, AD
26	2340	0.0830	FA (egg), asthma, AD
27	4040	0.0595	FA (egg), asthma, AD
28	72	0.0455	FA (egg), AD
29	324	0.0860	FA (egg), AD
30	697	0.0490	FA (egg), asthma
31	4460	0.0495	FA (egg), AD
32	80	0.0590	FA (egg)
33	231	0.0485	FA (shrimp), asthma
34	1366	0.0650	FA (egg), AD
35	338	0.0515	FA (egg, cheese), AD
36	459	0.0325	FA (egg), AD
37	12,700	0.0370	FA (egg), AD
38	154	0.0730	FA (egg, milk)
39	9050	0.0575	FA (egg white), AD, photosensitivity reaction
40	3740	0.0455	FA (egg, milk), asthma, AD
41	596	0.0495	FA (egg, shrimp, mayonnaise, tuna), AD
42	271	0.0605	FA (egg, milk), asthma, AD
43	978	0.0325	FA (egg), AD
44	271	0.0840	FA (egg), AD, asthma
45	288.4	0.0300	FA (egg), AD
46	9726	0.0490	FA (egg), AD
47	4000	0.1075	FA (egg), AD
48	949.4	0.0330	FA (egg), AD, asthma
49	ND	0.0395	FA (egg), AD
50	512	0.0665	FA (egg), AD
51	ND	0.0550	FA (egg, wheat), AD
52	5363	0.0870	FA (egg)
53	ND	0.0615	FA (egg)
54	ND	0.0215	FA (egg)
55	2703	0.0180	FA (egg)

2.5. Serum IgE Western blot analysis of GM and non-GM corn extracts

GM corn (MON863) and closely genetically related non-GM corn were purchased at the same time. The GM corn and non-GM corn were ground into powder and stirred in PBS at 4 °C overnight. The suspension was then centrifuged at 100,000g for 1 h at 4 °C, and the supernatant was collected and used as an extract.

The protein concentration of the extract was measured with a BCA protein assay kit (Pierce, Rockford, IL, USA).

The proteins in each corn extract were separated by SDS-PAGE on 2D-gel (5–20% acrylamide, XV PANTERA Gel; DRC, Tama, Tokyo, Japan). 2D-gel was used because there were no empty spaces between lanes and the nitrocellulose membrane could be easily cut into narrow strips. The samples were run at 100 mA in Tris–glycine buffer supplied by DRC with an electrophoresis apparatus, ELICA (DRC). The proteins were transferred overnight onto a nitrocellulose membrane in 25 mM Tris, 192 mM glycine, 10% methanol at 32 mA with a transfer apparatus, MINI2-D (Bio-Rad, Hercules, CA, USA). The filter was blocked in 0.5% casein–PBS at RT for 1 h. After cutting the membrane vertically into strips 0.3 cm wide, each strip containing 40 µg sample of protein was incubated for 1 h at RT with 4- or 40-fold diluted serum, and then overnight at 4 °C. After washing with PBS-T, the membrane strips were incubated with 500-fold diluted peroxidase-conjugated donkey anti-human IgE for 1.5 h at RT, and after washing again they were stained with substrate solution (Immunostain HRP-100; Konica, Tokyo, Japan).

3. Results

3.1. Expression and purification of Cry3Bb1

The 2.0-kb Cry3Bb1 coding sequence was cloned from MON863 GM corn. Recombinant Cry3Bb1 had an apparent molecular mass of 75 kDa when analyzed by Western blotting, and that value was consistent with its calculated molecular mass.

Cry3Bb1 needed to be prepared in a soluble form for use as an ELISA coating antigen. Several combinations of vectors and host *E. coli* strains were compared, and a sufficient amount of soluble recombinant protein was found to be expressed when pCold1 and pG-Tf2/BL21 were used as the vector and the host, respectively.

Even when protease inhibitors were used at the highest concentrations according to the manufacturers' instructions, recombinant Cry3Bb1 was gradually cleaved during purification. Since trace amounts of proteases derived from the *E. coli* host probably digested the recombinant Cry3Bb1, a rapid purification procedure had to be used with small columns attached to syringes. Recombinant Cry3Bb1 was purified in two steps, an anion exchange chromatography step followed by a chelate chromatography step, and 630 µg of recombinant Cry3Bb1 was obtained from 250 ml of *E. coli* culture. As shown in Fig. 1 (lanes 13–15), the resulting fractions were homogeneous after SDS-PAGE and visualization by Coomassie brilliant blue (CBB) staining. The identity of the purified protein was confirmed to be Cry3Bb1 by Western blotting with polyclonal antibody to Cry3Bb.

3.2. ELISA

ELISA was performed with recombinant Cry3Bb1 as the coating antigen to test for the presence of Cry3Bb1-specific IgE antibodies. Serum samples from 13 corn allergy patients and from 55 patients with various food allergies were tested. The distribution of the ELISA values is shown in Figs. 2 and 3. The absorbance of the serum samples from two of the food allergy patients in Japan exceeded the mean value plus 5 SD of the control serum values, and IgE Western blotting was performed on both samples. The IgE antibodies in neither sample bound to recombinant Cry3Bb1 (data not shown), indicating that the binding demonstrated by ELISA was nonspecific. No IgE antibodies that were specific for recombinant Cry3Bb1 were found in any of the serum of the patients in either group.

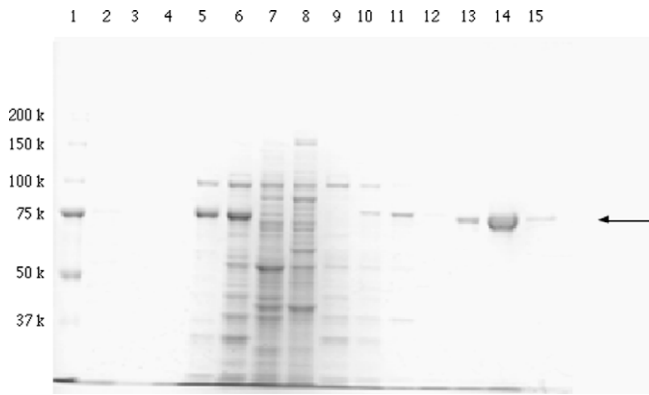


Fig. 1. Purification of Cry3Bb1 expressed in *E. coli*. Lane 1, molecular weight marker. Lanes 2–8, fractions from the HiTrapQ HP column: lanes 2 and 3, non-binding fractions; lanes 4–8, fractions eluted with 0.1 M, 0.2 M, 0.3 M, 0.4 M, and 0.5 M NaCl, respectively. Lanes 9–15, fractions from the HiTrap HP column: lanes 9 and 10, non-binding fractions; lanes 11–15, fractions eluted with 40 mM, 100 mM, 200 mM, 300 mM, and 500 mM imidazole, respectively. The SDS-PAGE gel was stained with CBB. Recombinant Cry3Bb1 is indicated by an arrow.

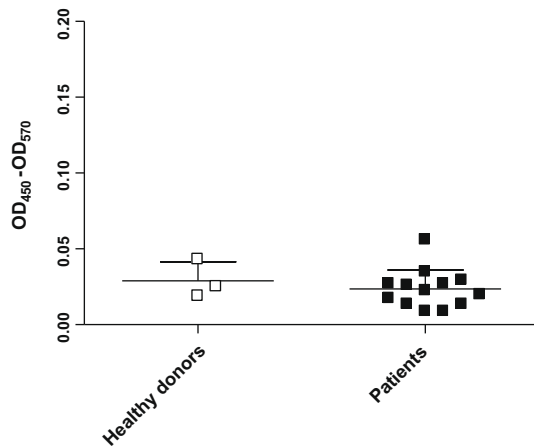


Fig. 2. Data obtained by ELISA of 16 serum samples. Serum samples from 13 corn allergy patients in the US and three healthy donors were diluted 20-fold and assayed for anti-Cry3Bb1 IgE. The mean value (long horizontal line) and standard deviation (short line above) are indicated for each group.

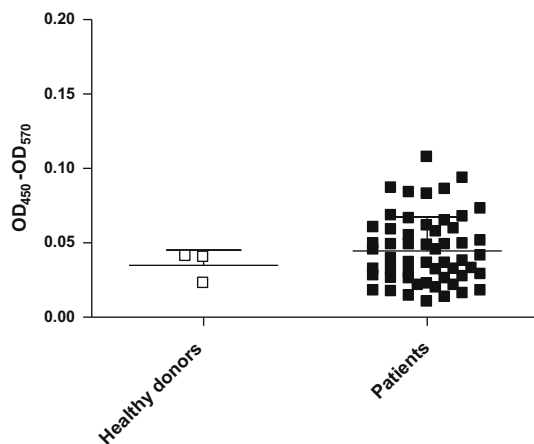


Fig. 3. Data obtained by ELISA of 58 serum samples. Serum samples from 55 patients in Japan with various food allergies and from three healthy donors were diluted 20-fold and assayed for anti-Cry3Bb1 IgE. The mean value (long horizontal line) and standard deviation (short line above) are indicated for each group.

3.3. Western blot analysis of GM and non-GM corn extracts with IgE from corn allergy patients

Serum P1 and serum P2, which were positive for corn-specific IgE (13.8 and 13.9 IU/ml, respectively), were used for immunoblotting. Both sera were from American patients. The staining patterns of the extracts of MON863 corn and of non-GM corn are shown in Fig. 4, and they were similar. Comparison with the results of the analysis of the non-GM corn extract showed that no new proteins were detected by the analysis of the MON863 extract. No protein in the corn extracts was detected near 75 kDa, which is the molecular weight of Cry3Bb1 and indicated by an arrow in Fig. 4. No proteins were recognized when serum from the healthy control was tested.

When serum samples P1 and P2 were diluted 40-fold, the IgE did not react with any protein in the corn extracts. When serum sample P1 was diluted 4-fold, the IgE bound to a 17-kDa protein, as shown in Fig. 4, lanes 5 and 12. When serum sample P2 was diluted 4-fold, the IgE reacted with an 11-kDa protein and a 17-kDa protein, as shown in Fig. 4, lanes 7 and 14. These proteins were tentatively assigned to known allergens according to their apparent molecular weight by searching a database. The 17-kDa protein was consistent with profilin (14 kDa, Gibbon et al., 1998) or thioredoxin h1 protein (14 kDa, Weichel et al., 2005), and the 11-kDa protein corresponded to a nonspecific lipid transfer precursor (12 kDa, Tchang et al., 1988). The total protein profiles of the corn extracts were revealed by staining blot strips with CBB and are shown in Fig. 4, lanes 1 and 8. A strong band was detected near 17 kDa and was thought to be recognized by serum P1 and serum P2 in lanes 5, 7, 12, and 14. Many proteins in the 10–15 kDa range were detected in lanes 1 and 8, and it was impossible to identify the 11-kDa protein recognized by serum sample P2 as the protein detected by CBB staining.

4. Discussion

As of February 2008, a total of 88 varieties of seven different plants have been authorized as GM crops for food use in Japan, and 36 varieties of GM corn had been authorized for food use in Japan (<http://www.mhlw.go.jp/english/topics/foodsafety/dna/dl/02-02-02.pdf>). CP4-EPSPS (5-enolpyruvyl-shikimate-3-phosphate synthase from *Agrobacterium* sp. Strain CP4), PAT (phosphinothricin acetyltransferase), or *B. thuringiensis* insecticidal toxins have been introduced into most of these GM crops in addition to antibiotic-resistance genes as selection markers.

Assessing the safety of Cry3Bb1 is very important, because it is contained in MON863 and three stack varieties of GM crops authorized for food use in Japan. The safety of Cry3Bb1 has been investigated in the form of a pre-market study.

Hammond et al. studied the serum chemistry, body weight, and other features of rats fed MON863, and concluded that MON863 is as safe and nutritious as conventional varieties of corn (Hammond et al., 2006). George et al. analyzed the chemical composition of MON863 and conventional corn and reported that it was the same (George et al., 2004).

Food allergy is one of the major reasons for assessing the safety of GM crops. However, even though food allergy has been studied intensively, since Cry3Bb1 has never been in our food supply, it is very hard to predict whether it will induce an allergic response. Many scientists are interested in the potential allergen-like properties of Cry3Bb1, and several studies have been already performed. Leach et al. and Hileman et al. found no homology between Cry3Bb1 and known protein allergens, and Betz et al., Leach et al., and Hileman et al. showed that Cry3Bb1 is readily digested by simulated gastric fluid (Betz et al., 2000; Hileman et al., 2001; Leach et al., 2001).

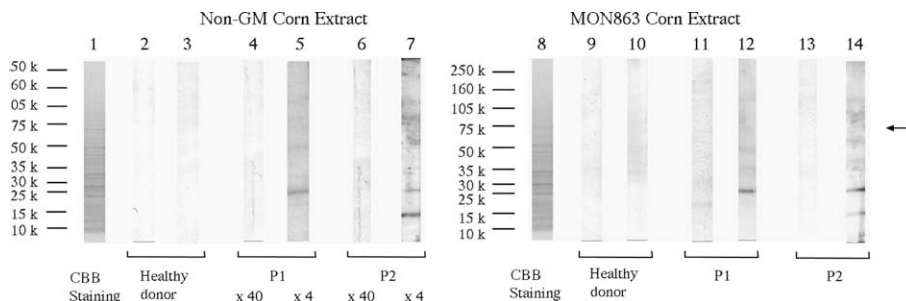


Fig. 4. IgE-Western blotting of sera with GM corn and non-GM corn extracts. Whole extracts from a GM corn strain, MON863 (Cry3Bb1-introduced), and from a non-GM corn strain were separated by SDS-PAGE and blotted onto a nitrocellulose membrane. The membrane strips were allowed to react with 4- and 40-fold diluted serum samples from two patients (P1 and P2) who were allergic to corn and from a healthy donor. The total protein profiles for the corn extracts was revealed by staining blot strips with CBB and are shown in lane 1 (non-GM corn extract) and lane 8 (MON863 extract). The expected location of Cry3Bb1 is indicated by an arrow.

No international agreements have yet been reached regarding post-marketing studies of GM crops. The authors think that GM crops should be assessed carefully in order to facilitate acceptance of GM crops. In the present study we performed a surveillance study of Cry3Bb1 and MON863 from a new standpoint, an IgE binding test. We used ELISA and Western blotting because these two methods have been used widely for allergenicity tests in the past.

First, we studied the ability of IgE antibodies from food allergy patients to bind recombinant Cry3Bb1. IgE antibody binding to an allergen is the first and important step in an allergic response.

The serum samples from the 13 corn allergy patients that were used in this study were purchased from Plasmalab International after MON863 maize began to be cultivated in the US. Corn allergy is very rare in Japan, and only one serum sample from a corn allergy patient has ever been collected in Japan. As an alternative, serum samples from 55 patients in Japan with various food allergies were also tested, because the IgE levels of such patients tend to be higher, and they are at higher risk for allergy to Cry3Bb1.

In order to monitor specific IgE antibodies in human sera we developed an ELISA-based method in which nonspecific binding of IgE antibodies to antigen is reduced by an additional wash with 1 M NaCl. We previously reported determining the CP4-EPSPS, Cry9C, PAT, and Cry1Ab are not allergenic based on the results of using this method (Takagi et al., 2006; Nakajima et al., 2007), and we used the same improved ELISA method again to test the allergenicity of recombinant Cry3Bb1 in the present study. This study is one of a series of studies we have been conducting to confirm the predicted lack of IgE binding to novel proteins in genetically modified crops.

We use a two-step procedure to perform the IgE binding test in our laboratory, and the first step is the ELISA. Since our ELISA procedure is simple and requires less serum, it is suitable as a preliminary screening method for a large numbers of serum samples. When a serum sample is suspected of being positive, it is tested by Western blotting or ELISA inhibition, which is more reliable, but more labor intensive. Those methods are suitable for small numbers of serum samples.

Two serum samples from Japanese patients were suspected of being positive by the improved ELISA and were analyzed by Western blotting, but the results indicated that the binding was non-specific. No specific binding of recombinant Cry3Bb1 by IgE antibodies was detected in this study.

Even if specific serum IgE to a protein were detected, binding to the protein by IgE would not necessarily induce IgE receptor cross-linking on mast cells or basophils or degranulation of the cells. If IgE antibodies specific to Cry3Bb1 were detected, further study would be necessary, such as a basophil degranulation test, skin test, or oral challenge. The serum IgE test is the first step in allergenicity evaluation.

There are two main considerations for the safety of GM crops and food allergy. First, the new protein might elicit allergic reactions in people with existing allergy. Second, the new protein might be a new allergen. This study addresses the first issue, and no IgE binding to Cry3Bb1 was found in patients with existing food allergies including corn allergy. Addressing the second consideration would require analysis of antibody responses in sufficient numbers of atopic patients with confirmed, long-term, repeated exposure to Cry3Bb1.

If insertion of the transgene containing the Cry3Bb1 coding sequence into the corn genome changes the status of chromatin near a gene encoding an allergen, it may unintentionally upregulate the allergen. This risk needed to be assessed, and our next study is designed to do so. The binding profiles of IgE antibodies from corn allergy patients were tested in regard to all proteins extracted from a GM corn strain, MON863, and a non-GM corn strain. Different sera yielded different staining patterns, but a similar staining pattern was obtained when an extract of MON863 and an extract of non-GM corn were compared. The results of this experiment showed that allergic reactions to other allergens besides Cry3Bb1 are unlikely to occur.

In conclusion, IgE antibodies from corn allergy patients and patients with various food allergies did not bind specifically to recombinant Cry3Bb1. The IgE antibodies from the corn allergy patients yielded the same binding profiles in relation to extracts of MON863 and of non-GM corn. MON863 is as safe as non-GM corn from the standpoint of no specific binding between IgE antibodies and recombinant Cry3Bb1.

We could not demonstrate exposure to Cry3Bb1 by the patients who provided the serum samples, meaning that this study cannot be strictly described as a post-marketing study. The results of this study support the findings of the existing safety assessment and provide additional information regarding the safety of GM corn containing Cry3Bb1.

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