Lack of cross-reactivity between the *Bacillus thuringiensis* derived protein Cry1F in maize grain and dust mite Der p7 protein with human sera positive for Der p7-IgE

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**Abstract**

Cry1F protein, derived from *Bacillus thuringiensis*, is effective at controlling lepidopteran pests and a synthetic Cry1F transgene was transferred into maize. For the safety assessment of genetically modified food crops, the allergenic potential of the introduced novel trait(s) is evaluated. Because no single parameter is currently predictive of allergic potential, a ‘weight of evidence’ approach has been proposed. As part of this assessment, the amino acid (aa) sequence of the Cry1F protein was compared to a database of known allergens using recommended criteria. The Cry1F protein did not show significant similarity or a match of eight contiguous identical aa with any allergen. However, a single six contiguous aa match was identified between Cry1F and the Der p7 protein of the dust mite, *Dermatophagoides pteronyssinus*. To investigate whether Cry1F was cross-reactive with Der p7, sera from 10 dust mite allergic patients containing Der p7-specific IgE antibody were used to compare IgE-specific binding. No evidence of cross-reactivity was observed between Cry1F and Der p7. This study provides in vitro IgE sera screening data, that when considered in the context of other bioinformatic data [Hileman R.E., Silvanovich, A., Goodman R.E., Rice E.A., Holleschak G., Astwood J.D., Hefle S.L., 2002. Bioinformatic methods for allergenicity assessment using a comprehensive allergen database. Int. Arch. Allergy Immunol. 128, 280–291; Stadler, M.B., Stadler, B.M., 2003. Allergenicity prediction by protein sequence. FASEB J. 17, 1141–1143.], adds further evidence arguing against the use of a six contiguous identical amino acid search to identify potential cross-reactive allergens. Cry1F is heat labile, rapidly hydrolyzed in an in vitro pepsin resistance assay, not glycosylated and not from an allergenic source. Taken together, these data indicate a lack of allergenic concern for Cry1F.

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**Keywords:** Bioinformatics; *Bacillus thuringiensis*; Transgenic; Maize; IgE sera screening; Dust mite; Safety assessment

1. Introduction

Currently, the most widely grown GM crops are soybeans, maize, canola, and cotton containing one or more targeted genes that bestow insect resistance or herbicide tolerance or a combination of these traits (Konig et al., 2004). Insect resistant plants are desirable because of enhanced pest control, reduced need for chemical insecticides, and increased yields. Some insect resistance crops have been produced by genetic modification with genes from the gram-positive bacterium *Bacillus thuringiensis* commonly found in soil. A number of different strains of *B. thuringiensis* have been found to produce crystal proteins or inclusion bodies that are very effective in controlling certain species of insect pests (Aronson and Shai, 2001). In over 40 years of commercial use as biopesticides, *B. thuringiensis* insecticidal proteins have been used safely with no adverse reports of human health or environmental effects (McClintock et al., 1995; Siegel, 2001).

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The insecticidal crystal Cry1F protein was originally derived from \textit{B. thuringiensis} var. \textit{aizawai} strain PS811 and is very effective at controlling lepidopteran pests. A synthetic truncated Cry1F transgene optimized for plant expression was transferred into maize. The amino acid sequence derived from the synthetic gene is identical to the native Cry1F protein. The expressed Cry1F protein has 605 amino acids with a molecular weight of \( \approx 68 \) kDa. Insect-resistant transgenic maize product that contains the Cry1F gene that encodes the Cry1F protein was grown commercially in the US for the first time in 2003.

In contrast to traditional foods a rigorous safety assessment process exists for GM crops \cite{Cockburn2002}. The safety assessment process of GM food focuses on an evaluation of the allergenic and toxic potential of the introduced novel trait(s), as well as the wholesomeness of the GM crop. The approach by which allergy assessment has been conducted involves the use of a step-wise, decision tree \cite{FAOWHO2001, Metcalfe1996}. However, because no single parameter has been shown to be predictive of allergic potential, a ‘weight of evidence’ approach has been proposed to assess protein allergenicity \cite{Codex2003}. The evaluation of protein allergenicity is currently based upon that which has been associated with allergens such as amino acid sequence similarity, the resistance of the protein to pepsin in vitro \cite{Astwood1996, Thomas2004}, the stability of the protein to heating and processing conditions, the source of the gene encoding the transgenic protein, and with a basis for cross-reactivity, in vitro IgE sera screening studies \cite{Codex2003, FAOWHO2001}. Importantly, in vitro digestibility methods do not reproduce in vivo gastric conditions \cite{Mendelsohn2003}. However, resistance to pepsin in vitro has been correlated with protein allergenicity \cite{Astwood1996}, although the relationship is not absolute \cite{Fu2002}.

The Cry1F protein was found to hydrolyze within one minute in an in vitro pepsin resistance assay \cite{Evans1998}. The Cry1F protein also loses immunoreactivity after heat processing and degrades quickly under pressures and heat commonly used in commercial processing of maize \cite{Mayes1999}. In addition, the Cry1F protein is not glycosylated and \textit{B. thuringiensis} \cite{Evans1998}. The lack of data concerning the allergenic potential of \textit{B. thuringiensis} was recently reconfirmed in an April 2005 EPA Scientific Advisory Panel \cite{SAP2005} in which the panel states it “….was not aware of any report of \textit{B. thuringiensis} being an allergen.” Bernstein et al. \cite{Bernstein1999} examined the use of \textit{B. thuringiensis} and work-related allergic reactions. In regard to this study, the EPA SAP Panel concluded that the authors “….reported positive skin tests in some individuals exposed to an intact source organism (BT), not a crystal protein per se…..” Taken together, these data indicate a lack of allergenic concern for the Cry1F protein.

As part of the ‘weight-of-evidence’ assessment, the amino acid sequence of the Cry1F protein was also compared to a database of known allergens using criteria established by the ILSI-IFBC \cite{Metcalfe1996} and FAO/WHO Expert Consultation on Allergenicity of Foods Derived from Biotechnology \cite{FAOWHO2001}. FAO/WHO has recommended that IgE cross-reactivity between a novel protein and a known allergen be considered a possibility when there is more than 35% identity over a segment of 80 amino acids. However, others have reported that for cross-reactivity to occur, a high degree of homology is needed, likely in excess of 50–70%, over significant spans of the target protein and allergen \cite{Aalberse2000}. To further exclude the possibility of cross-reactivity with a known allergen, a stepwise contiguous identical amino acid segment search is recommended with known allergens to identify amino acid sequences that may represent linear IgE-binding epitopes \cite{Metcalfe1996}. Eight contiguous amino acid matches between a novel protein and a known allergen(s) have been recommended to identify sequences that may represent linear epitopes \cite{Metcalfe1996}, while FAO/WHO \cite{FAOWHO2001} has suggested the use of a six contiguous amino acid match.

When comparing the amino acid sequence of Cry1F to that of known allergens, no significant similarity (i.e., >35% identity over an 80 amino acid window) or potential cross-reactive epitopes using an eight contiguous identical amino acid search were identified. However, a single six contiguous identical amino acid match was observed between the Cry1F protein and the Der p7 protein of the dust-mite, \textit{Dermatophagoides pteronyssinus}. \textit{D. pteronyssinus} belongs to the Pyroglyphidae family of mites, which are considered, from an allergy standpoint, to be one of the more important mite species in domestic dwellings \cite{Stewart1995}. Dust mite allergy in general has been demonstrated to be an independent risk factor for the development of asthma \cite{Peat1996, Wahn1997}. The prevalence of dust mite allergy in the general population has been estimated to be in the range of 9–16% \cite{Sidenuis2001}. Der p7 is a 198 amino acid protein with a molecular weight of approximately 22 kDa. The function of Der p7 in the dust mite is unknown. However, most other dust mite allergens are hydrolases that correspond to those commonly associated with digestion in vertebrates and invertebrates \cite{Chua1988, Heyman1989, Lake1991}. Der p7 has been reported to be a major allergen (i.e., one to which more than 50% of individuals sensitive to that substance react in IgE-specific immunoassays) \cite{Shen1995}. To date, the IgE-binding epitopes on the Der p7 protein have not been characterized.

While it seemed unlikely that a single six contiguous amino acid match represented a biologically meaningful observation since these occur commonly amongst unrelated proteins \cite{Hileman2002, Stadler2003}, the ability of serum from dust mite allergic subjects to recognize the Cry1F protein in maize grain was further investigated in an IgE sera-screening study.
2. Materials and methods

2.1. Comparison of the amino acid sequence of the Cry1F protein to known protein allergens

The amino acid sequence of the Cry1F protein (expressed by maize line 1507) was compared to a database of known allergens using criteria established by the ILSI-IFBC (Metcalf et al., 1996) and FAO/WHO Expert Consultation on Allergenicity of Foods Derived from Biotechnology (FAO/WHO, 2001). The database contained 2033 entries and was constructed at Pioneer Hi-Bred International (Pioneer) by compiling protein allergen sequences identified by using keyword searches [i.e., allergen(s) and isoallergen(s)] from published, curated protein databases of known or putative allergen sequences that included (Gendel, 1998; King et al., 1994; Metcalf et al., 1996) as well as the Swiss-Prot/TrEMBL, PIR, and GenPept, nr datasets. Current matching criteria include eight or more contiguous amino acids that are identical to any segment of any known allergen (food, inhalant, or contact allergen) or greater than 35% identity over an 80 amino acid segment of a protein.

The sequence alignment programs used in this study were part of Version 10.2 of the GCG (Accelrys, San Diego, CA) Wisconsin package run on a SGI Origin 3000 computer under the Irix Release 6.5 operating system. The SSearch program in the GCG software suite utilizing a Smith Waterman algorithm (Smith and Waterman, 1981), in conjunction with a modified identity matrix (allorone14.cmp) that eliminates positive scoring contributions from non-identical but conserved residues, was employed to search for multiple sequences that shared similarities above 50% identity with the Cry1F protein over an 80 °C.

To evaluate whether there was an identity of greater than 35% over any 80 amino acid residues between the Cry1F protein and the Pioneer Allergen Database, input sequences were first normalized using the MID function of Microsoft Excel to extract all possible overlapping 80 residue peptides from the query peptide sequence. The resulting 526 peptides of 80 residues each were placed into FASTA format and subjected to FASTA (Pearson and Lipman, 1988) analysis against the Pioneer allergen dataset. Because of the inability of the GCG FASTA implementation to accept multiple sequences, a Perl script was used to submit the sequences one at a time to the program and concatenate the resulting output. The default word size (2), gap creation and extension penalties (12 and 2, respectively), the default identity matrix (bloom 50), and a cut-off E value of 1.0 were used in the searches.

2.2. Preparation of maize extracts

Maize flours [one sample each of Cry1F containing maize and a nontransgenic maize line (33P6 #2 yellow maize)] were provided by Pioneer (Des Moines, Iowa) and extracted separately by continuous agitation with PBS and a protease-inhibitor cocktail without EDTA (Roche, Indianapo-lis, IN) overnight at 4 °C. After centrifugation at 2500 rcf for 15 min at 4 °C, the supernatant was collected, and then centrifuged at 12,000 rcf for 3 min. The Coomassie Plus Protein Assay (Pierce, Rockford, IL) was used to determine the protein concentrations. All extracts were stored at −80 °C.

2.3. Serum samples from dust mite allergic patients

A total of 20 sera from 12 Taiwanese and 8 US patients with allergy to dust mite based on clinical histories and skin prick testing were screened by immunoblot for the presence of IgE antibodies to the Der p7 protein. The Taiwanese serum samples were kindly provided by Dr. Tsai of Kaohsiung Hospital, Taipei, Taiwan and the US samples by Dr. Hugh A. Sampson. The immunoblots were generated using a highly purified (i.e., 99%) recombinant Der p7 protein. Immunoblotting was done with a Der p7-specific monoclonal antibody (WH8), kindly provided by Dr. Shen of the

| Amino acid | A | B | C | D | E | F | G | H | I | K | L | M | N | P | Q | R | S | T | V | W | X | Y | Z |

* indicates a background matrix score.
Department of Medical Research, Veterans General Hospital, Taichung, Taiwan, and with the 20 dust mite allergic patient sera. While all patients had elevated serum dust mite-specific IgE antibodies, as measured by the Pharmacia UniCAP System FEIA (Pharmacia and Upjohn Diagnostics), only 10 patients [50%] demonstrated Der p7-specific antibodies via immunoblot. All serum samples were stored at −80 °C until used in the immunoblots of Cry1F containing maize and non-transgenic maize. Normal (i.e., not atopic) human sera (Pharmacia, Peapack, NJ) were used as negative controls.

2.4. SDS–PAGE analysis

For SDS-analyses, proteins were separated by SDS–PAGE (gradient 4–12% gels; Nu-Page Bis–Tris System, Invitrogen, Carlsbad, CA) following the protocol from the manufacturer. Ten micrograms of each maize protein extract and 50 ng of purified Der p7 were loaded per well. The resolved proteins were transferred to Immobilon-P membranes (Millipore, Bedford, MA) and then stained with 2.2% Coomassie blue for total protein analyses. For molecular weight determination MultiMark Multi-Color standard (Invitrogen, Carlsbad, CA) were utilized. An immunoblot was conducted for its sensitivity. In comparative trials, we have been able to measure food-specific antibody in the low picogram range by immunoblot in contrast to the low nanogram range by ELISA.

2.5. Probing immunoblots with Cry1F monoclonal antibodies

For detection of the Cry1F protein band in separated maize proteins, immunolabeling was performed using a murine monoclonal IgG antibody-specific to the Cry1F protein (Pioneer, Des Moines, Iowa). The Immobilon-P membranes were blocked in 5% non-fat dry milk for 15 min at room temperature. Monoclonal antibodies were diluted 1:1000 in 1% bovine serum albumin, 10% normal goat serum in PBS–TWEEN and incubated for one hour at room temperature with gentle agitation. After 1 h immunoblots were washed with PBS and then labeled with anti-mouse IgG (H + L) HRP conjugate (Promega, Madison, WI). After gentle agitation for 30 min at room temperature and washing with PBS, immunoblots were incubated with ECL Western blotting detection reagents (Amersham Biosciences, UK) for 1 min and exposed to Kodak Biomax Imaging Film for 30 s.

2.6. Probing immunoblots with dust mite-allergic patient sera

For detection of IgE binding to the separated maize proteins, immunolabeling was performed with patient sera from 10 dust mite/Der p7 IgE positive-allergic patients. Normal human serum (Pharmacia, Peapack, NJ) was used as a negative control. Similar to the Raybourne et al. (2003) ELISA study in which a known human positive control serum containing IgE specific to Cry9c antigen was not available, a human serum positive control containing IgE specific to Cry1F was also not available. However, recombinant Der p7 protein was included in the study to evaluate the IgE detection reagents. Patient and control sera were diluted 1:5 in PBS–TWEEN plus 1% bovine serum albumin (BSA) and 10% normal goat serum, and were incubated with immunoblots with gentle agitation at room temperature. After 2 h immunoblots were briefly rinsed with PBS and then labeled with [125I]-goat anti-human IgE (DiaMed, Windham, ME) diluted 1:5 in PBS–TWEEN plus 1% BSA with 0.16 mCi/ml of total activity of [125I]-as per the manufacturer. [125I]-conjugated antibodies were used because we have found this to be the most sensitive method for detecting antigen-specific IgE [unpublished observation]. Imaging film can be developed for up to 3 days without background interference. After gentle agitation for 1 h at room temperature and washing with PBS, immunoblots were mounted on filter paper and exposed to Kodak BioMax Imaging Film for 1–3 days. Protein images were scanned with CanoScan D125OU2F and composed using Adobe Photoshop and IN Design software.

3. Results

3.1. Amino acid sequence comparisons

No significant similarity as defined by FAO/WHO (2001) was demonstrated with any of the allergens contained in the Pioneer Allergen Database. In addition, no contiguous identical 8 amino acid matches were observed between the Cry1F protein and known allergens. However, a single 6 amino acid match was identified between the Cry1F protein and the Der p7 protein of the dust mite, D. pteronyssinus (Walters and Cressman, 1998) (Fig. 1).

The six-residue peptide (i.e., TLTSFE) identified between the Cry1F and Der p7 proteins was subsequently compared against all of the protein sequences in the Genpept dataset and PIR-NREF datasets. The output from the Genpept search identified 24 proteins that contained the target 6-residue sequence, while the PIR search identified 22 proteins, which were all contained in the Genpept set. The identified proteins, albeit some hypothetical, included those derived from humans, bacteria, viruses, mice, cows, and reptiles. Some examples include the following: red clover necrotic mosaic virus, bovine gastrin-binding protein-like precursor fragment, Bacillus cereus acetyltransferase, and full-length cDNA clone of placenta of Homo sapiens. These data indicate that the sequence of 6 amino acids in question occurs in a number of other proteins.

3.2. IgE-binding study with Cry1F containing maize grain and sera from dust mite allergic patients containing IgE to the Der p7 protein

Utilizing a mouse monoclonal antibody-specific for Cry1F, Fig. 2 demonstrates that the Cry1F maize tested contained the expressed Cry1F protein, whereas the non-transgenic maize did not. As depicted in Fig. 3, the sera from 10 dust mite allergic subjects had IgE antibodies that bound to Der p7, a 26 kDa protein band in lane 3. However, none of these subjects had IgE antibodies recognizing Cry1F, a 61 kDa protein expressed in maize. Thus, there was sufficient Cry1F protein in the maize extract to be read-
illy identified by Cry1F-specific antibody, and sufficient Der p7-specific IgE antibody in the human sera to cause an identifiable reaction with the Der p7 protein. Based on these data, there was no evidence that dust mite allergic individuals possess cross-reactive IgE antibodies to corn transfected with the Cry1F protein.

4. Discussion

The comparison of homology of amino acid sequence and structure to known allergenic proteins is an important endpoint in the evaluation of allergenicity of genetically modified foods (Codex, 2003; FAO/WHO (2001)). Walters and Cressman (1998) compared the sequence similarity of the Cry1F protein to that of known allergens and gliadins. Based on criteria recommended by the ILSI-IFBC (Metcalfe et al., 1996) and FAO/WHO (2001), no significant similarity (i.e., >35% identity over an 80 amino acid window) or eight contiguous amino acid matches were demonstrated between the Cry1F protein and allergens contained in the Pioneer Allergen Database.

However, a six contiguous amino acid screen was performed and a single match was identified between the Cry1F protein and the Der p7 protein from dust mite. To further investigate whether the Cry1F protein was cross-reactive with the Der p7 protein, an IgE-binding assay was conducted with sera from dust mite allergic patients that contained IgE antibody-specific to the Der p7 protein. From a risk perspective, the plant expressed Cry1F protein was the most appropriate choice as the test substance for our sera screening study, because it is identical to the protein that will be present in the food. The use of the plant expressed Cry1F protein in this study: (1) focused the safety assessment on the food item (maize); (2) evaluated Cry1F protein in the natural context of other maize proteins (i.e., the food matrix); and (3) evaluated the Cry1F protein in amounts relative to consumer exposure.

Our data indicate that there was sufficient Cry1F protein in the maize extract to be readily identified by Cry1F-specific antibody, and sufficient Der p7-specific IgE antibody in the human sera to cause an identifiable reaction with the Der p7 protein. Based on the results of this study, there was no evidence of allergic cross-reactivity between the Cry1F protein and Der p7.

As reported by Codex (2003) and FAO/WHO (2001), the source of the gene as well as sequence homology data need to be considered before proceeding with serum screening with the novel protein. If the protein was derived from
an allergenic source or was ‘homologous’ to a known allergen, it is highly recommended that the IgE-binding properties of the protein be further investigated using sera from individuals that have IgE-specific to the source of the gene or to the sequence matching allergen(s) ( Codex, 2003; FAO/WHO, 2001; Metcalfe et al., 1996). An example of the utility of IgE sera screening for identifying potential allergens involves the methionine-rich 2S albumin from the Brazil nut that had been introduced into transgenic soybeans to improve their amino acid profile for animal feed (Nordlee et al., 1996). Since the Brazil nut is a recognized allergenic food, the 2S albumin protein was evaluated to determine if it was allergenic by conducting IgE-binding studies with sera from individuals allergic to Brazil nuts. The 2S albumin protein bound IgE from the Brazil nut allergic individuals. Skin-prick testing with Brazil nut and transgenic soybean extracts confirmed the protein to be a major allergen. Based on this study, the 2S albumin transgenic soy product was never commercialized (Nordlee et al., 1996).

Conducting ‘scientifically meaningful’ IgE serum screens are problematic due in part to the lack of standardized methods and the availability of well-characterized human serum samples from a sufficient number of patients since the incidence of individuals with specific allergies is quite low (i.e., 1–2% of adults and 4–6% of children) (Goodman and Leach, 2004). In the case of a major allergen (i.e., one to which more than 50% of individuals sensitive to that material respond in IgE-specific immunoassays) like Der p7 (Shen et al., 1995), FAO/WHO (2001) indicated that a minimum of eight relevant sera is required to achieve a 99% certainty that a new protein is not an allergen. FAO/WHO (2001) further indicated that a minimum of 24 relevant sera might be required to achieve a 99% certainty with regard to a minor allergen. Such quantities of well-characterized sera may not be available for testing purposes. Standard criteria are also needed for selection of allergic individuals (Eigenmann and Sampson, 1997). Detailed allergic histories should be conducted on potential serum donors by experienced clinical allergists. In addition, clinical allergists should also conduct any appropriate human or clinical studies (e.g., skin-prick testing; food challenge studies) and assess in vitro IgE sera screening study results. The importance of characterizing serum samples prior to conducting an IgE-binding study was demonstrated in our study. Initially, 20 sera from patients determined to have an allergy to dust mites based on an allergic history profile, elevated serum dust mite-specific IgE antibodies, as measured by the Pharmacia UniCAP or a positive SPT were obtained. However, upon subsequent immunoblot analysis, only 10 patients were found to have IgE antibody-specific for the Der p7 protein.

As suggested above, the assay procedure for evaluating IgE-binding needs to be standardized to determine its positive and negative predictive values (Bindslev-Jensen and Poulsen, 1997). Although in vitro IgE assays are commercially available for a much larger range of food allergens (Sampson and Ho, 1997; Sampson, 2001), in vitro methods have not been demonstrated to be useful for the diagnosis of food allergy except for a few of the major allergens. Typically, IgE-specific Western blots and enzyme-linked immunosorbent assays (ELISAs) have been used to identify allergenic proteins. It is imperative that during assay development, conditions be optimized (e.g., detection methods, blocking reagents) in order to establish assay sensitivity and specificity and to minimize false-positive IgE binding. Besides direct binding studies, inhibition Western blot assays and ELISAs should also be conducted to demonstrate the specificity of any identified IgE binding and to further minimize false-positive findings (Goodman and Leach, 2004). This is an important point, as the broad range of IgE binding that sometimes occurs may not be clinically relevant (Bindslev-Jensen and Poulsen, 1997; Pasini et al., 2000; Taylor and Heffte, 2000), particularly for cross-reactivity associated with carbohydrate determinants (Vieths et al., 2002).

Sequence homology searches comparing the amino acid sequence of proteins to known allergens are conducted using various algorithms such as the FASTA sequence alignment tool (Pearson and Lipman, 1988) to predict overall structural similarities. The FASTA algorithm, however, examines significant homology over large spans of the protein. Thus, there is some concern that the FASTA search might miss short regions (i.e., potential IgE-binding epitopes) within a protein that are identical or highly similar in sequence to an existing allergen and have the potential to bind IgE. Because IgE-binding epitopes have only been identified for a few allergens, it is currently not feasible to construct a comprehensive database of IgE-binding epitopes. For example, the IgE-binding epitopes for the Der p7-allergen have not yet been characterized. Although the epitopes for Der p7 have not been identified, there is no evidence that the 6 amino acid sequence observed between Cry 1F and Der p7 is either an antigenic or allergenic epitope of Der p7. If it was a major allergenic epitope in Der p7, we would have expected to see binding with the Cry1F protein since we observed strong binding to Der p7. In addition, IgE epitopes have been predominately characterized by in vitro mapping studies using sera from a limited number of allergic patients and without consideration of the affinity of the antibody or the clinical significance of the in vitro binding. Although the majority of identified IgE-linear epitopes were found to be 8 amino acids or longer (Chatchatee et al., 2001; Reese et al., 1999; Shin et al., 1998), a few IgE epitopes may be as short as 5 amino acids (Banerjee et al., 1999; Bezehold et al., 1999). Importantly, some data suggest that high affinity IgE binding requires 8 or more amino acids (Banerjee et al., 1999; Rabjohn et al., 1999).

In the absence of a comprehensive IgE epitope database, a stepwise contiguous identical amino acid segment search is performed between the query protein and known allergens to identify short amino acid sequences that may represent linear IgE-binding epitopes. This conservative approach is based on the theoretical, but somewhat
unlikely possibility that two non-homologous proteins could have common and potentially cross-reactive epitopes. When this approach was initially recommended, an eight contiguous amino acid match between a novel protein and a known allergen(s) was suggested to identify sequences that may represent linear epitopes (Metcalfe et al., 1996). In 2001, an FAO/WHO panel recommended that a six contiguous amino acid match be employed to identify potential linear IgE epitopes between a novel protein and a known allergen.

The use of a six contiguous amino acid match, however, does not appear to have utility in identifying potentially cross-reactive allergenic proteins. A contiguous match of 6 amino acids occurs too commonly between unrelated proteins and therefore, is not a reliable criterion for predicting allergenic potential (Hileman et al., 2002; Stadler and Stadler, 2003). Many random, false-positive matches that are very unlikely to indicate potential IgE epitopes are observed using such a short sequence. For example, Hileman et al. (2002) compared the sequences of 50 randomly selected corn proteins to a database of known or putative allergens for matches of identical contiguous amino acids. Of the 50 proteins evaluated, 41 of the 50 (i.e., 84%) contained at least one identical 6 amino acid sequence match with an allergen. In contrast, 6 of the 50 proteins contained at least one identical 8 amino acid sequence match with an allergen. Similarly, Stadler and Stadler (2003), using the criteria of six contiguous identical amino acids, identified more than two-third of the proteins in the Swiss-Prot database as allergens. These authors also reported high percentages of predicted allergens, 76 and 43%, respectively, for rice and human trGen sequences when using a 6 amino acid match.

In our study, the 6 amino acid match between the Cry1F and Der p7 proteins was also found to occur in a number of other proteins from several species, including humans. Nevertheless, the potential cross-reactivity identified by the single six contiguous identical amino acid match between the two proteins was further investigated. No IgE binding to the Cry1F protein was observed, indicating that the Cry1F and Der p7 proteins are not cross-reactive. This study provides in vitro IgE sera screening data, that when considered in the context of other bioinformatic data (Hileman et al., 2002; Stadler and Stadler, 2003), adds further evidence arguing against the use of a six contiguous identical amino acid search to identify potential cross-reactive allergens.

5. Conclusion

The Cry1F protein did not show significant similarity or a match of eight contiguous amino acids with any dermal, respiratory, or food allergens. The Cry1F protein is also heat labile, rapidly hydrolyzed in the in vitro pepsin resistance assay, and not glycosylated. In addition, there have been no reports of allergenicity to B. thuringiensis, including occupational allergy associated with manufacture of products containing B. thuringiensis over the last 40 years (EPA, 2000, 2005). To further investigate whether the Cry1F protein in maize grain was cross-reactive with the Der p7 protein based on the single six contiguous amino acid match identified between the two proteins, an IgE sera screening assay was conducted. There was no evidence of cross-reactivity between the Cry1F and Der p7 proteins. The six contiguous amino acid match that was observed between the Cry1F and Der p7 proteins was also found to occur in proteins from a number of other species, including humans. The IgE sera screening study results confirmed the conclusion based on the previous weight of the evidence data and assessment that Cry1F lacks allergenic potential.

References


