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Potato lectin activates basophils and mast cells of atopic subjects by its interaction with core chitobiose of cell-bound non-specific immunoglobulin E

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Summary

A major factor in non-allergic food hypersensitivity could be the interaction of dietary lectins with mast cells and basophils. Because immunoglobulin E (IgE) contains 10–12% carbohydrates, lectins can activate and degranulate these cells by cross-linking the glycans of cell-bound IgE. The present objective focuses on the effect of potato lectin (*Solanum tuberosum* agglutinin; STA) for its ability to release histamine from basophils *in vitro* and mast cells *in vivo* from non-atopic and atopic subjects. In this study, subjects were selected randomly based on case history and skin prick test responses with food, pollen and house dust mite extracts. Skin prick test (SPT) was performed with STA at 100 μg/ml concentration. Histamine release was performed using leucocytes from non-atopic and atopic subjects and rat peritoneal exudate cells. SPT on 110 atopic subjects using STA showed 39 subjects positive (35%); however, none showed STA-specific IgE; among 20 non-atopic subjects, none were positive by SPT. Maximal histamine release was found to be 65% in atopic subjects ($n = 7$) compared to 28% in non-atopic subjects ($n = 5$); the release was inhibited specifically by oligomers of *N*-acetylglucosamine and correlates well with serum total IgE levels ($R^2 = 0{\cdot}923$). Binding of STA to *N*-linked glycoproteins (horseradish peroxidase, avidin and IgG) was positive by dot blot and binding assay. As potato lectin activates and degranulates both mast cells and basophils by interacting with the chitobiose core of IgE glycans, higher intake of potato may increase the clinical symptoms as a result of non-allergic food hypersensitivity in atopic subjects.

Keywords: basophils; histamine release; mast cells; N-glycans; non-allergic food hypersensitivity; potato lectin

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Introduction

Bridging of immunoglobulin E (IgE) molecules on the cell surface by allergen or bivalent antibody against IgE is a necessary event for IgE-mediated basophil or mast cell degranulation [1–3]. As well, lectins can either interact with specific carbohydrates on cell-bound IgE or directly with the specific carbohydrates of cell surface glycoproteins/glycolipids [4–6]. Such interactions release histamine and other inflammatory mediators [1–3]. Alternatively, lectins or specific antibodies can cross-link adjacent IgE Fcε receptors present on mast cells and basophils. In all these cases, the final response is similar to the allergen-specific IgE interaction seen in IgE-mediated food allergy [7]. Therefore, it is important to address the role of plant food lectins in mediating non-allergic food hypersensitivity [8] (termed inappropriately false food allergy).

Non-allergic food hypersensitivity [8] denotes a special type of non-immunological reaction, in which a substance in the food triggers the mast cells/basophils directly or with the involvement of non-specific IgE antibodies. In this respect, lectins are probably the best-studied food components in triggering mast cells and basophils [7]. The activation of these cells has been studied mainly with concanavalin A (Con A) [9–11], KM^+ (mannose-specific lectin from jackfruit seeds) [6] and other mannose-specific lectins [4,5]; these lectins cross-link the abundant mannose residues on *N*-glycans of IgE [12].

Lectins can also bind to the lining of the gut wall and render it more leaky [13], so that more undigested lectins

(most lectins are resistant to heat and proteases) enter into the bloodstream [7]. There is also some evidence that, in atopic individuals, certain lectins stimulate the body to produce IgE in preference to other antibodies [3,5]. It has been demonstrated that basophils rapidly release interleukin (IL)-4 upon antigen-specific or non-antigen-specific stimuli (certain lectins). This effect makes lectins interesting candidates for inducing a T helper 2 (Th2) response and IgE-mediated allergy in non-sensitized individuals [3,7,14]. All these different effects of lectins could contribute to adverse reactions to foods.

Because lectins are an important constituent of dietary proteins in many plant foods [15], they can interact with specific sugars of *N*-glycans of cell-bound IgE. Con A was the first lectin used to study the activation of basophils and mast cells *in vitro* [9–11]. In the present study we have chosen a lectin from a widely consumed food source, namely potato. Potato lectin (*Solanum tuberosum* agglutinin; STA) [16] is classified as a chitin-binding lectin; although its optimal specificity is for chitotriose and chitotetraose [$(GlcNAc)_3$ and $(GlcNAc)_4$, respectively], it binds with lower affinity to other oligomers of *N*-acetyl-D-glucosamine (GlcNAc). It also possesses the ability to bind to poly-*N*-acetyllactosamine [β-D-Gal-(1→4)-D-GlcNAc; LacNAc] moieties present in complex-type *N*-linked glycoproteins and glycosphingolipids [17,18].

Although the major biochemical component in potato is starch, it contains 1·6 g protein per 100 g tuber; its lectin content is ~ 6·5 mg per 100 g, which represents 0·4% of total protein [19]. In view of the ubiquitous dietary importance of potato, it appeared interesting to study the effect of purified potato lectin on the degranulation of mast cells and basophils from non-atopic as well as atopic subjects.

Materials and methods

This study was undertaken after clearance by the Institutional Human Ethics Committee; informed consent was obtained from all atopic and non-atopic subjects aged 15–60 years (for subjects below 18 years of age, consent was obtained from their parents or legal guardian).

STA was isolated from potato tubers, as described recently [20], and found to be homogeneous by reverse-phase high-performance liquid chromatography (HPLC) (95% purity; specific haemagglutination activity: 3900 units/mg). Compound 48/80, chitosan, pepsin, avidin, Con A, *Lycopersicon esculentum* agglutinin (LEA; tomato lectin), sheep anti-mouse IgG (whole molecule)-alkaline phosphatase (AP) conjugate and murine anti-human IgE (monoclonal)-AP conjugate were products from Sigma-Aldrich Co. (St Louis, MO, USA). Lysozyme, ovalbumin (OVA), bovine serum albumin (BSA), horseradish peroxidase (HRP) and avidin-AP were purchased from Bangalore Genei, Bangalore, India. Flat-bottomed 96-well microtitre plates (Microlon) were purchased from Greiner Bio-One GmbH (Frickenhausen, Germany). All other chemicals/reagents used in this study were of analytical grade.

Identification of atopic and non-atopic subjects

Atopic and non-atopic subjects were identified based on their case history (atopic subjects chosen at random who had clinical symptoms of at least one allergic condition among the following: allergic rhinitis, asthma and food allergy) and skin prick test (SPT) results with commercial extracts of house dust mite (HDM) and pollens. The following allergenic extracts were used for SPT for confirming the allergic status: grass pollen mix 1 (Southern grass pollen mix no. 1651; Bayer Corp., Spokane, WA, USA), house dust mite (*D. farinae*, 10 000 AU/ml), weed pollen mix and grass pollen mix 2 (no. P28; Greer Laboratories, Lenoir, NC, USA).

Serum total IgE and histamine levels

Murine monoclonal anti-human IgE antibody (murine IgG2a, κ; hybridoma cell line ATCC HB-121, designation E5BB3IIA2) was purified from hybridoma cell culture supernatant on protein A-agarose. This cell line was obtained from the National Centre for Cell Science (Ganeshkhind, Pune, India). Serum total IgE (expressed as IU/ml) was quantified [21] using this antibody. Following trichloroacetic acid (TCA) precipitation of serum, histamine was extracted, determined by fluorometry [22] and expressed as ng/ml serum. Eosinophil count was carried out on whole blood and expressed as numbers per μl of blood; the normal reference value is 40–400 [23]. The eosinophil count was found to be 240–350 in non-atopics and 430–860 in atopics.

SPT

In allergological studies, purified allergens (natural or recombinant) have been used for SPT in the concentration range 20 μg/ml–1 mg/ml. Most whole extracts are used for SPT at a maximum concentration of 10 mg/ml. Potato lectin represents 0·4% of total proteins in the tuber [19]; based on the maximum concentration of potato extract that can be used in SPT, the lectin represents 40 μg/ml. Hence, we selected an approximate concentration of 100 μg/ml for SPT so that positive results for detection of potato lectin-sensitized subjects were not missed in the present study. STA (100 μg/ml) was prepared in 50% glycerinated phosphate-buffered saline (PBS). Glycerinated PBS was used as negative control and histamine base (1 mg/ml) was used as positive reference standard. SPT was carried out as described previously [24]. After 20 min, the wheal/flare diameters were measured; a wheal diameter of > 3 mm was considered as positive.

Enzyme-linked immunosorbent assay (ELISA) for detection of STA-specific IgE

STA-specific IgE was detected by indirect ELISA [21]. Briefly, microtitre wells were coated with 30 μg of STA at pH 9·6 at 4°C overnight. After the blocking step, the wells were incubated with subjects' sera at 1 : 3 dilution in PBS containing 1% BSA/0·05% Tween-20 at 4°C overnight. Next, it was incubated with murine monoclonal anti-human IgE-AP conjugate at 1 : 1500 dilution at 37°C for 2 h, followed by colour development.

Isolation of leucocytes containing basophils

The buffy coat (leucocyte layer containing basophils) was isolated from 10 ml of heparinized venous blood as described previously [25] using 6% dextran T 700 gradient. The buffy coat was washed four to five times with isotonic PBS and resuspended in 10 mM Tris-HCl buffer, pH 7·4, containing 1 mM $CaCl_2$, 1 mM $MgCl_2$ and 0·03% BSA (Tris-CAM). The isolated leucocytes were counted using crystal violet. Percentage viability of leucocytes in the buffy coat was determined by Trypan blue dye exclusion.

Isolation of rat peritoneal exudate cells (PEC)

PECs were isolated from male Wistar rats (adult, 4 weeks old; weighing ~250–300 g) following the standard procedure [11] using Tyrode buffer, pH 7·4, containing 0·1% BSA. After injecting the peritoneal cavity, the fluid containing PECs was collected after 5 min, washed, and finally resuspended in Tris-CAM. PECs were stained for mast cells using toluidine blue and viability was assessed by Trypan blue dye exclusion. The PEC preparation contained 15–20% mast cells.

Histamine release (HR) assay [25,26]

Cells and reagents (STA or other proteins) in Tris-CAM were added to polystyrene tubes (final volume: 1 ml) in an ice bath. Each tube containing 2×10^6 cells/ml was incubated at 37°C for 45 min. In each experiment, addition of 3% perchloric acid or boiling at 100°C for 10 min for one set of tubes was performed to obtain the total histamine content of cells (Pc). Blank tubes containing only cells and buffer were used for non-specific release (Ps) during the reaction (generally < 10%). After 45 min, the tubes were transferred to an ice bath and centrifuged at 275 ***g*** at 4°C for 20 min. The supernatants were assayed for histamine content (Pt).

The released histamine was quantified by a fluorometric assay [25]; the fluorescence intensity was measured using 360 nm for λ_{ex}, and 450 nm for λ_{em}. The formula for the calculation of percentage HR is $[(Pt - Ps) \div (Pc - Ps)] \times 100$, where Pt = test release, Ps = spontaneous release and Pc = complete release.

Preparation of chitosan oligomers [27]

Chitosan oligomers were obtained by digestion of 500 mg chitosan with 5 mg pepsin at pH 5, 45°C for 6 h, followed by neutralization, centrifugation and lyophilization of the supernatant [27]. The composition of chitosan oligomers by HPLC analysis (aminopropyl column) was found to be 78·2% chitotetraose, 1·6% chitotriose, 0·9% chitobiose, 18·2% GlcNAc and 1·1% GlcN (D-glucosamine).

Dot blot for glycoprotein binding

Dot blot [28] was carried out using STA or LEA (another lectin having similar specificity as STA), which was applied as a spot on the nitrocellulose (NC) membrane along with control proteins (Con A and BSA). After the spots were air-dried, the membrane was blocked as described for ELISA, incubated with 0·1 mg/ml HRP at 37°C for 2 h, and colour development was carried out using TMB/H_2O_2 substrate. The dot blot was performed similarly using avidin-AP (1 : 2000 dilution), and developed using BCIP/NBT.

Preparation of raw and heat-processed potato extracts

For raw potato extract (RPE), 50 g of peeled potato tuber was taken and blended with 50 ml of phosphate-buffered saline, pH 7·4. The extract was passed through a muslin cloth and then filtered using Whatman no. 1 filter. The extract was then centrifuged in the cold at 5200 ***g*** for 15 min.

For heat-processed potato extract (HPPE), 50 g of peeled potato tuber was taken and suspended in 50 ml of PBS, pH 7·4. The contents were boiled over a hot-plate for 20 min. After cooling, 1 ml of the supernatant was taken for analysis (HPPE supernatant), and later the remaining contents were blended at 25°C. The extract was passed through a muslin cloth and then filtered using Whatman no. 1 filter. The extract was then centrifuged in the cold at 5200 ***g*** for 15 min.

Haemagglutination (HA) activity of raw and heat-processed potato extracts was carried out using trypsinized human erythrocytes as described previously [20]. One unit of HA activity is the concentration of the protein at the highest dilution required for agglutination. The specific activity is given as the number of HA units per mg of protein.

Glycoprotein binding assay [20]

Microtitre wells were coated with 10 μg purified STA or LEA at pH 9·6 at 4°C overnight. After the blocking step using 3% gelatin in PBS, the microtitre wells were incubated with HRP (100 μl of 0·1 mg/ml), or avidin-AP conjugate (100 μl of 1 : 2000 dilution) or sheep anti-mouse IgG-AP conjugate (100 μl of 1 : 10 dilution) in PBS containing 1% BSA/0·05%

Table 1. Demography of the selection of non-atopic and atopic subjects for the study.

Parameters	Subjects' status	
	Non-atopic	Atopic*
Number of subjects (M/F)	20(11/9)	110 (53/57)
Wheal/flare diameter for allergen extracts (mm)¶	0–1/0 (–)	3–5/10–15 (1+ to 2+)
Wheal/flare diameter to HDM (mm)	0–2/0–5 (–)	3–7/10–30 (1+ to 3+)
Serum total IgE (IU/ml)†	35–44	59–330
Plasma histamine (ng/ml)§	1·2–2·4	8·6–13·4
Serum histamine (ng/ml)‡	21–32	110–215

*Number of subjects, based on case history with: pollen (71), house dust mite (HDM) (76), food (73) allergy. ¶Allergen extracts include commercial pollen and HDM extracts; wheal/flare diameter (range): histamine base, 5–6/20–25 mm; glycerinated phosphate-buffered saline, 0–1/0 mm; skin prick test grading: 1+, 3–4/5–10 mm; 2+, 4–5/10–20 mm; 3+ , > 5/20–30 mm. †Reference normal value for serum total IgE = < 120 IU/ml [21]. §Reference normal value for plasma histamine = 0·5–2 ng/ml [22]. ‡Reported range for serum histamine (normal subjects) = 5–27 ng/ml [22].

Tween-20 at 37°C for 2 h. Following addition of the respective substrate, the absorbance was measured.

Statistical analysis

Each datum represents the arithmetic mean and standard deviation (s.d.) of the different experiments under identical conditions. Student's *t*-test was used to make a statistical comparison between the paired and unpaired groups. The correlation between histamine release values and serum total IgE was analysed to find the correlation coefficient. All the data were analysed using a computer program (Statistical Analysis System).

Results

Selection of atopic and non-atopic subjects for the study

Subjects classified as non-atopic had no history of any allergic diathesis and had negative SPTs and normal IgE levels. Subjects classified as atopic had one SPT positive for any of the inhalants with a clinical history and examination suggestive of allergic rhinitis, asthma or both. Evaluation of atopic cases, including those avoiding potato ingestion, was carried out by complete clinical case history in the form of questionnaire. The atopic condition exhibiting allergic rhinitis and asthma was confirmed by physical examination, clinical symptoms and spirometry. Based upon the above criteria, the allergic status of the subjects was classified arbitrarily as non-atopic or atopic.

Among the atopic subjects who had symptoms of allergic rhinitis or asthma (and who had reported the avoidance of potato in their diet), descriptive case history along with clinical interrogation of these subjects were evaluated carefully; these subjects reported that the consumption of potato (approximately 50–100 g in a meal) in the symptomatic state increased their symptoms of asthma after 30 min to 2 h. They also experienced some kind of associated gastrointestinal symptoms such as stomach cramping, uneasiness in the stomach along with vomiting sensation, regurgitation and hypermotility of the intestines. At the time of the study, this subgroup of atopic subjects was under medication with decreased symptoms of asthma, but still avoided eating potato-containing foods.

The SPT data for allergenic extracts/HDM, total IgE and histamine levels are summarized in Table 1. The total IgE was found to be significantly higher in atopic subjects, and represents a two- to eightfold increase over the value for non-atopic subjects. However, in non-atopic subjects, the values were in the reference range of normal. The serum and plasma histamine levels were found to be approximately five- to eightfold higher in atopic subjects compared to the levels for non-atopic subjects.

SPT of STA on atopic and non-atopic subjects

Table 2 shows the results of SPT with STA (100 μg/ml) tested on 110 atopic subjects who had generalized symptoms characteristic of allergic conditions and 20 non-atopic subjects. SPT reactions as assessed by the mean values of wheal/flare diameter were mildly positive (3·5/5 mm) or moderately positive (4·5/10 mm). STA showed positive SPT in 39 of 110 atopic subjects (35·5%). Almost 49% of these subjects avoided consumption of potato in their diets. Based on the clinical symptoms, the atopic subjects are subgrouped as representing allergic rhinitis, asthma or both. Again in the subgroups, almost 42–56% of subjects who tested positive to STA by SPT were found to avoid potato.

Total IgE and STA-specific IgE in atopic and non-atopic subjects

Serum samples of non-atopic ($n = 10$) and atopic ($n = 10$) subjects were checked for the presence of STA-specific IgE. Con A (Man/Glc-specific lectin) and BSA (non-lectin protein) were used as negative controls. STA-specific IgE values for moderately STA-sensitive atopic subjects were very similar to those seen for mildly STA-sensitive atopic and non-atopic subjects (Table 3). However, the total IgE level of

Table 2. Results of skin prick test (SPT) to *Solanum tuberosum* agglutinin (STA) in non-atopic and atopic subjects.

Subjects' status	No. of subjects¶	Subjects + to STA¶	% positive	Avoidance to potato (*n*)†	Avoidance to potato (%)
Non-atopic§	20 (M11, F9)	0	0	n.a.‡	n.a.
Atopic (total)*	110	39 (M18, F21)	35·5	19/39	48·7
Allergic rhinitis	33	09 (M3, F6)	27·3	05/09	55·6
Asthma	42	18 (M8, F10)	42·9	09/18	50·0
Allergic rhinitis with asthma	35	12 (M7, F5)	34·3	05/12	41·7

¶M, male; F, female. †Number of subjects avoiding potato consumption/number of subjects positive to STA by SPT; avoidance is based on self-reported assessment by the subjects. §Healthy subjects (non-atopic) with no clinical symptoms of allergy. *Atopic subjects are selected for SPT based on their case history and SPT results to allergenic extracts including house dust mite, and classified into subgroups based on their clinical symptoms. ‡n.a., not applicable.

moderately STA-sensitive subjects is approximately twofold higher than that of mildly STA-sensitive subjects, and approximately 3·5-fold higher than that of non-atopic subjects who are not sensitive to STA.

HR from non-atopic and atopic subjects using STA

In the case of atopic subjects ($n = 3$), the HR assay was performed initially in the concentration range of 0·0001–20 μg per ml STA or positive/negative control proteins. The positive reference control, Con A, was found to induce HR in the range of 0·01–20 μg/ml, and maximum release of 55% was observed at ~1 μg/ml (Fig. 1a), whereas STA showed a maximum HR of 53% at the same concentration. The non-lectin control, OVA, shows no HR.

The HR from non-atopic and atopic subjects as a function of STA, Con A and OVA in the narrower concentration range of 0·5–3 μg/ml is shown in Fig. 1b. The maximum HR was seen at 2 μg/ml in the case of both STA and Con A. STA shows HR of ~28% in non-atopic ($n = 5$) and 67% in atopic subjects ($n = 7$), and the difference in HR between these two groups is significant ($P < 0·001$). Con A shows a typical bell-shaped curve; HR was ~34·5% in non-atopic subjects and ~73% in atopic subjects. OVA shows HR of only 4% (non-atopic subjects) and 6% (atopic subjects); similar values were observed with BSA and lysozyme.

Correlation of HR with total IgE

Ten subjects in the non-atopic group and 20 subjects in the atopic group were analysed for HR and total IgE levels. The results are shown in Fig. 2a. The percentage HR was found to have a strong correlation with the serum total IgE levels ($R^2 = 0·923$, $n = 30$). The mean value of percentage HR as well as the range of serum total IgE values for both non-atopic and atopic (including its subgroups) groups are shown in Table 3.

Inhibition of HR by chitosan and chitosan oligomers

As the specificity of STA is for GlcNAc oligomers, we studied the inhibition of HR from non-atopic and atopic subjects at 2 μg/ml STA in the presence of chitosan or chitosan oligomers. There is remarkable inhibition of HR in

Table 3. Summary of *in vivo* and *in vitro* diagnostic tests in the subgroup of atopic subjects positive to *Solanum tuberosum* agglutinin (STA) by skin prick test (SPT).

Subjects' status	Subjects positive to STA	Wheal/flare diameter (mm)†	Total IgE range (A_{492})*	STA-specific IgE (A_{405}) mean ± s.d.*¶	Histamine release (%) mean ± s.d.*
Not sensitive to STA (non-atopic)	0	0–1/0	0·248–0·314	0·045 ± 0·012	27·3 ± 2·2
Not sensitive to STA (atopic)	0	2–3/0–5	0·360–0·435	0·056 ± 0·009	31·6 ± 4·5
Mildly sensitive to STA (atopic)	24	3·5/5	0·372–0·647	0·078 ± 0·010	46·6 ± 6·1
Moderately sensitive to STA (atopic)	15	4·5/10	0·844–1·558	0·080 ± 0·013	70·5 ± 6·4

†Mean value; positive control: 1 mg/ml histamine base (5–6/20–25 mm). *$n = 10$ in each group, percentage histamine release values are significant for mildly STA-sensitive atopic subjects at $P \leq 0·005$ and moderately STA-sensitive atopic subjects at $P \leq 0·001$ compared to non-atopic and atopic (not STA-sensitive) subjects. ¶Specific IgE for lectin control concanavalin A (Con A), $n = 3$: non-atopic, 0·056; atopic, 0·099. Specific IgE for non-lectin control [bovine serum albumin (BSA)], $n = 3$: non-atopic, 0·018; atopic, 0·020. STA-specific IgE values are not significant for mildly/moderately sensitive atopic subjects compared to non-atopic and atopic (not STA-sensitive) subjects at $P \leq 0·005$.

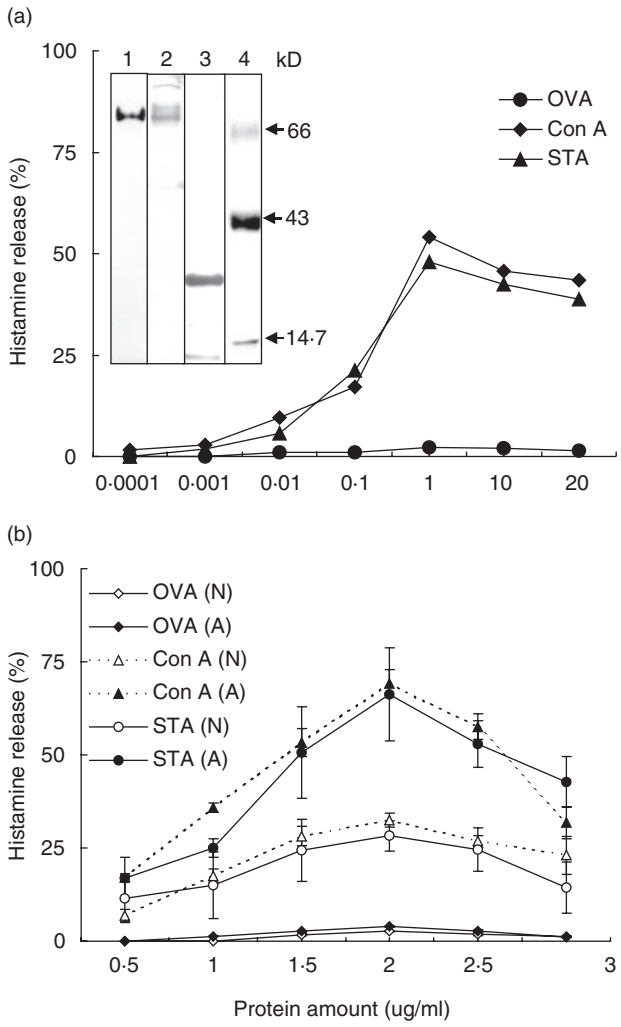

Fig. 1. Histamine release from leucocytes. (a) Histamine release (HR) (from atopic subjects) as a function of protein concentration. Inset: sodium dodecyl sulphate-polyacrylamide gel electrophoresis (12%, reducing) analyses of *Solanum tuberosum* agglutinin (STA) (lane 1), *Lycopersicon esculentum* agglutinin (lane 2) and concanavalin A (Con A) (lane 3). Lane 4: mol. wt. markers [bovine serum albumin (BSA), ovalbumin (OVA), lysozyme]. (b) HR from non-atopic (N; $n = 5$) and atopic (A; $n = 7$) subjects as a function of STA concentration. OVA: negative control; Con A: positive control.

both the groups (Fig. 2b); however, among the inhibitors tested, chitosan oligomers appear to be more potent. This inhibition is significant at $P \leq 0{\cdot}001$. On the other hand, GlcNAc shows only weak inhibition of HR by STA (not significant at $P \leq 0{\cdot}005$). Similar results were seen in the case of LEA.

Dot blot for glycoprotein binding

The inhibition of haemagglutination produced by STA (at 2 μg) was studied using the glycoproteins HRP and avidin. At 0·1 mg/ml, they were found to inhibit haemagglutination of rabbit erythrocytes at 6·25 μg and 12·5 μg, respectively (data not shown).

Figure 3a shows the binding of HRP and avidin-AP to STA, LEA and Con A. The binding of HRP and avidin-AP to both STA and LEA was inhibited strongly by chitosan oligomers, and weakly by GlcNAc when used at 0·1 mg/ml concentration.

Glycoprotein binding assay for STA

In order to confirm the binding of STA to core GlcNAc of *N*-linked glycoproteins, we used three glycoproteins

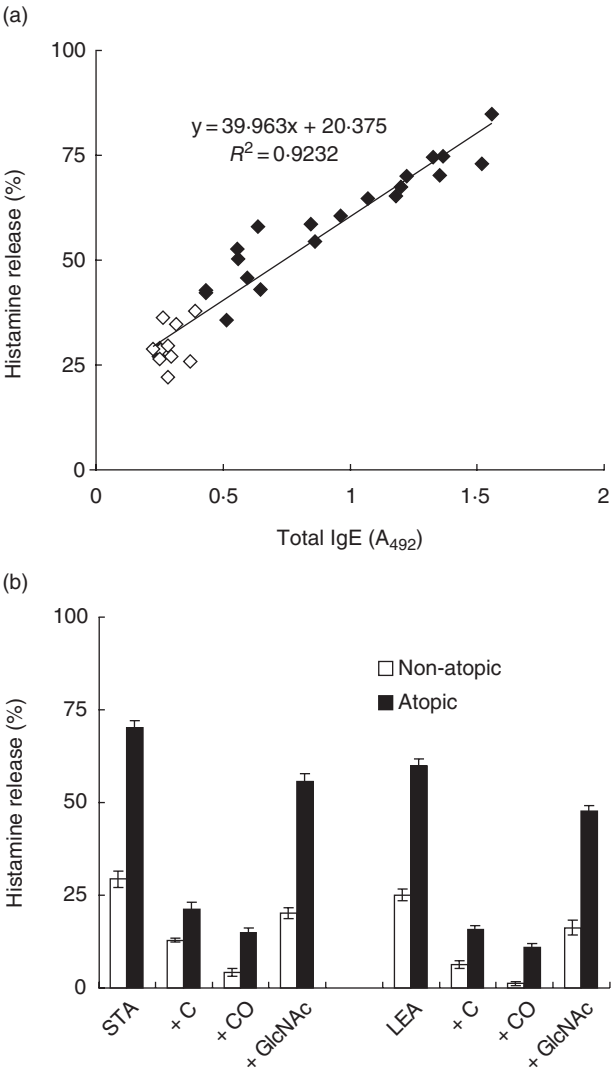

Fig. 2. (a) Correlation of leucocyte histamine release to serum total IgE (enzyme-linked immunosorbent assay units at A_{492}), $n = 30$. (◇), non-atopic subjects ($n = 10$); (◆), atopic subjects ($n = 20$). (b) Histamine release (HR) from leucocytes of non-atopic and atopic subjects at 2 μg/ml *Solanum tuberosum* agglutinin or *Lycopersicon esculentum* agglutinin and its inhibition by chitosan (C), chitosan oligomers (CO) or GlcNAc at 50 μg/ml.

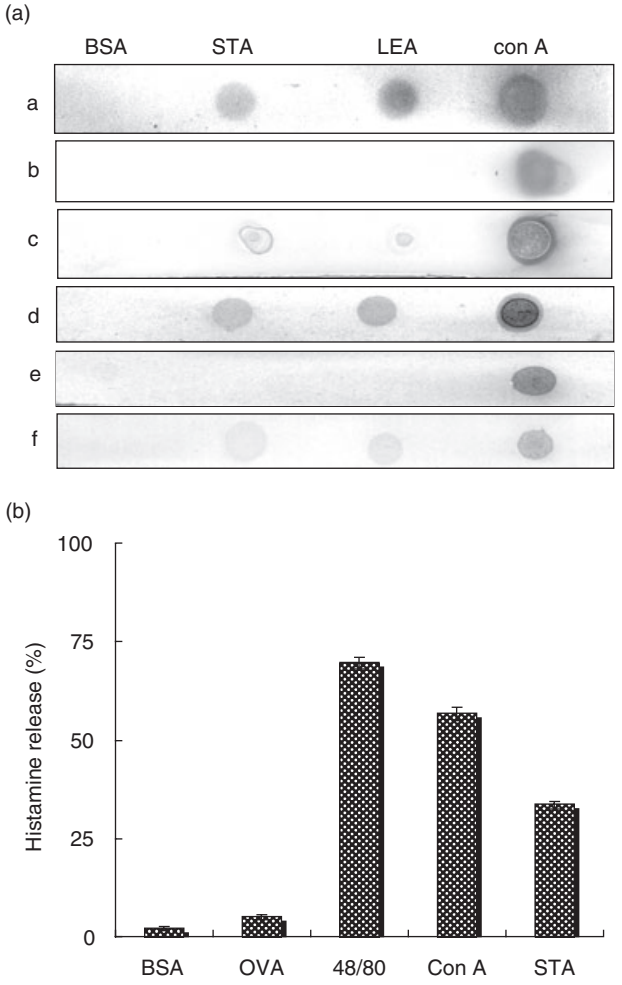

Fig. 3. (a) Binding of *Solanum tuberosum* agglutinin (STA) and *Lycopersicon esculentum* agglutinin (5 μg/spot on NC) to *N*-linked glycoproteins by dot blot [rows a–c: horseradish peroxidase (HRP); rows d–f: avidin-alkaline phosphatase (AP); bovine serum albumin (BSA): non-lectin control; concanavalin A (Con A): lectin control]. (a) HRP alone; (b) HRP + 50 μg/ml chitosan oligomers; (c) HRP + 50 μg/ml GlcNAc; (d) avidin-AP alone; (e) avidin-AP + 50 μg/ml chitosan oligomers; (f) avidin-AP + 50 μg/ml GlcNAc. (b) Histamine release (HR) from rat peritoneal exudates cells by *Solanum tuberosum* agglutinin and other proteins, at 4 μg/ml. Compound 48/80 (10 μg/ml) was used as a positive control for HR from mast cells present in peritoneal exudate cells.

differing in glycosylation. Remarkable binding of HRP (21% glycans) and avidin (13% glycans)-AP was seen in the case of both STA and LEA compared to the non-lectin BSA (Table 4). Both chitosan and chitosan oligomers inhibited this binding significantly. The binding of STA and LEA to an IgG antibody (2–3% glycans)–enzyme conjugate was also observed modestly when sheep anti-mouse IgG-AP conjugate was used at a very low dilution of 1 : 10. On the contrary, Con A showed a similar magnitude of binding for the all three glycoproteins, indicating that mannose is abundant compared to GlcNAc in the glycan portion. It should be noted that AP is a non-glycoprotein.

Analyses of raw and heat-processed potato extracts

Analysis of heat-processed potato shows that only 43% protein is recovered upon extraction in comparison to raw potato (Table 5). HPPE retains only 55% of biological activity as assessed by haemagglutination assay on a per mg protein basis, indicating that approximately half of the biological activity of STA is not lost upon heat-processing. As an attempt to isolate STA from HPPE was not successful due to the gelation of starch during heat processing, we determined the STA content in HPPE based on glycoprotein binding assay (avidin-AP) using a known amount of purified STA. Approximately 39% of STA was present in HPPE in comparison to RPE.

HR from rat peritoneal exudate cells

The HR from rat PECs using STA was studied in the concentration range of 1–5 μg/ml; the release was found to be maximum (36%) at 4 μg/ml concentration (Fig. 3b). Con A shows the characteristic bell-shaped curve (data not shown) with a maximum HR of 57% at 3 μg/ml, whereas OVA showed a maximum release of only 9% at 4 μg/ml. Compound 48/80, a known mast cell secretagogue, was used as a positive control for mast cell activation, and maximal release was observed at 10 μg/ml.

Discussion

Lectins are an important constituent of dietary proteins in many foods, especially legumes [7,15]. They have been implicated to play a role in non-allergic food hypersensitivity [7]. Although not a major protein, potato lectin is present in amounts of 5·2–7·8 mg per 100 g raw tubers, and ~0·5 mg per 100 g cooked material [19]. The present study was undertaken to investigate the effect of a dietary lectin having specificity to GlcNAc oligomers on mast cells and basophils of non-atopic and atopic subjects; we chose potato lectin as a prototype. This class of lectins includes the Solanaceae lectins (STA [16] from potato, LEA [29] from tomato and *Datura stramonium* agglutinin or DSA [15] from datura) and wheat germ agglutinin (WGA) [15] from wheat.

SPT of atopic subjects using STA revealed that ~35% of atopic subjects showed a positive reaction. However, considering that 20–25% of the general population is atopic [30], the SPT results for STA translates to 7–9% of the general population. The SPT results are unusually high for a food protein compared to the incidence of 3–4% for food allergy in adults [1,7]. The sensitization rate for a major food is 0·5–1% for peanut [31] and ~1·2% for potato in the general population. This sensitization rate for potato is calculated based on the value of 5·7% for the 45 kDa major allergen in

Table 4. Glycoprotein binding assay for potato lectin and its inhibition by chitosan or chitosan oligomers.

	Glycoprotein binding assay (A_{492} or A_{405}[a])		
Protein coated (10 μg)	HRP (0·1 mg/ml)	Avidin-AP (1 : 2000)	IgG-AP[b] (1 : 10)
BSA	0·020	0·013	0·036
STA	0·419	0·411	0·418
+50 μg chitosan[c]	0·198	0·260	n.d.
+50 μg chitosan oligomers[d]	0·137	0·071	n.d.
LEA	0·413	0·395	0·375
Con A	0·474	0·526	0·527

[a]Mean of triplicates; [b]sheep anti-mouse IgG (whole molecule)-AP conjugate (0·84 mg/ml); [c]chitosan (1 mg/ml); [d]chitosan oligomers (prepared as given in Materials and methods). n.d., not done. HRP: horseradish peroxidase; BSA: bovine serum albumin; STA: *Solanum tuberosum* agglutinin; LEA: *Lycopersicon esculentum* agglutinin; Con A: concanavalin A; AP: alkaline phosphatase.

potato (patatin; Sola t 1 [32,33]) in a study of 1886 Korean patients with various allergic disorders [34]. Therefore, we tried to assess the number of subjects who may have been sensitized to potato lectin by analysis of STA-specific IgE. Including this study, potato lectin has not so far been identified as an allergen. It should be noted that in the study by Lee *et al*. [34], the potato extract concentration used for SPT is 1 : 20 (w/v), which is equivalent to 5%. On a protein basis, this extract contains 0·8 mg protein/ml; patatin (30–40% of total protein [35]) and STA (0·4% of total protein [19]) concentrations in this extract amount to 0·24–0·32 mg/ml and 3·2 μg/ml, respectively. Thus, STA is present at 75–100-fold lower concentration compared to patatin in a given potato extract.

Interestingly, it has been reported that potato lectin induces a mainly IgG (IgG1 subclass) response, and failed to induce a vigorous IgE response in BALB/c mice [36]; the authors conclude that potato lectin lacks allergenicity. Only a few lectins have been reported previously as minor allergens in four plant foods; namely, peanut (Ara h agglutinin) [37], soybean (Gly m lectin) [38], wheat (Tri a 18; WGA) [39] and horse gram (Dol b lectin; DBA) [40]. The positive and negative predictive SPT values for potato lectin is in concordance with the observed positive SPT results in atopic subjects.

It has been shown that STA reacts selectively with mast cells in human connective tissue cells and epithelial cells [41]. Because cell-bound IgE is a glycoprotein rich in oligosaccharides (~12%) in its Fc portion (both oligomannose and complex bi-antennary types), we examined the composition and structures of the *N*-linked glycans on the heavy chain of IgE [42]. Among the glycans of human IgE, ~86% of glycans terminate in galactose or sialic acid [12], which represent complex bi-antennary type glycans [42]. Although the total IgE level was two- to eightfold higher in atopic subjects (who are positive to STA by SPT) compared to non-atopics the STA-specific IgE was found to be very similar in both atopic and non-atopic subjects, confirming that none of the atopic subjects were truly allergic to potato lectin.

Although potato lectin has optimal specificity for $(GlcNAc)_3$ and $(GlcNAc)_4$ [16] it appears that it may also bind to the core $(GlcNAc)_2$ units found in *N*-linked glycoproteins, including IgE [43] (Fig. 4). This has been confirmed in the present study by inhibition of STA-mediated haemagglutination by *N*-linked glycoproteins such as horseradish peroxidase, avidin and IgG, as well as inhibition of glycoprotein binding to STA by chitosan oligomers. Tomato lectin behaves similarly to potato lectin in glycoprotein-binding assay, based on their structural homology and identical sugar specificity [29].

In a recent study, LEA (and also STA) showed similar specificity for *N*-glycans based on the preference of high mannose-type glycans [44]. Both LEA and STA consist of two chitin-binding modules that are connected by a hydroxyproline rich glycoprotein domain; this feature helps to adopt an elongated structure [16,29], which enables them to approach the binding sites; namely, chitobiose core [44]. Our observations on the binding of STA and LEA to *N*-linked

Table 5. *Solanum tuberosum* agglutinin (STA) content in raw and heat-processed potato extracts based on haemagglutination (HA) and glycoprotein-binding activities.

Sample	Protein (mg/g)	HA activity (units/mg)	HA activity (%)	Avidin-AP binding (A_{405})[a]	STA content (%)[b]
RPE[c]	5·50	116·4	100·0	1·237	100·0
HPPE[d]	2·35	64·1	55·1	0·475	38·4
HPPE supernatant	0·29	n.d.	0·0	0·033	2·7

[a]Mean of triplicate values; protein amount, 100 μg; avidin-AP, 1 : 1500 dilution [b]calculated based on A_{405} value obtained for a known amount of purified STA; [c]raw potato extract, [d]heat-processed potato extract. AP: alkaline phosphatase. n.d.: not detectable.

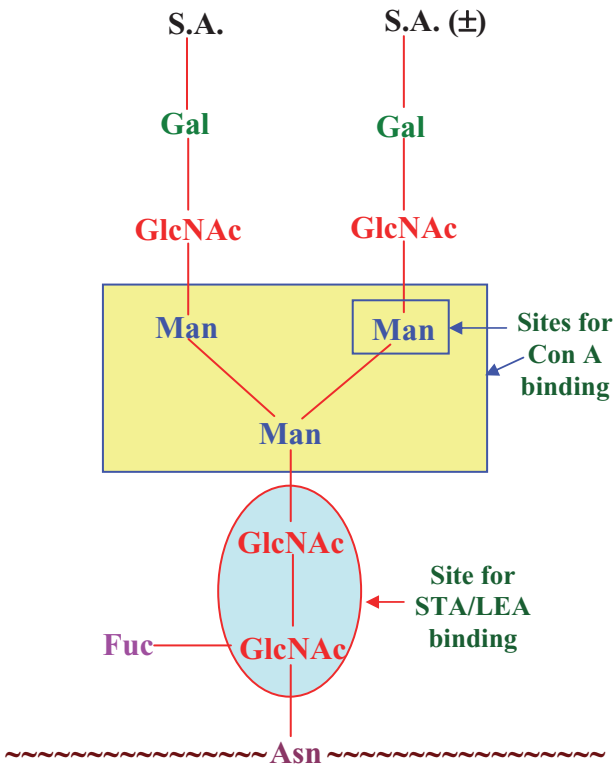

Fig. 4. Structure of complex bi-antennary type *N*-glycan of IgE [42,43] showing the binding site for concanavalin A (Con A) [specificity for mannose shown as Man (shown in a smaller box), and for trimannosidic core (shown in a larger box)], and *Solanum tuberosum* agglutinin (specificity for GlcNAc oligomers shown as circled chitobiose). Fucose (Fuc) is absent in avidin. Horseradish peroxidase has high-mannose type, IgE and IgG have complex bi-antennary type, and avidin has hybrid type *N*-glycans [12,42,43]. One of the two branches of the IgE glycans may or may not contain sialic acid (SA).

glycoproteins indicate strongly that these Solanaceae lectins can bind to human IgE on mast cells and basophils and cross-link adjacent non-specific cell-bound IgE. Therefore, we hypothesize that STA and LEA may induce symptoms similar to those seen in IgE-mediated food allergy in some atopic individuals; however, this is far from proved.

HR from the leucocytes of non-atopic and atopic subjects by STA was found to be dependent upon serum total IgE levels; the release shows a strong correlation to total IgE levels ($R^2 = 0{\cdot}923$). This indicates clearly that the effect of STA depends on the basophil IgE density for its interaction in causing non-specific activation. This is strikingly similar to the effect of Con A, wherein the HR is higher than spontaneous release in non-atopic subjects, and comparatively more so in atopic subjects [45]. It is interesting to note that a clear correlation between serum IgE and expression of FcεRI on basophils has been observed previously in allergic diseases [46]. Con A-induced HR has been shown to be dependent upon the density of IgE present on basophils [9,10,45]. Con A binds to terminal and internal mannose on IgE glycans (Fig. 4) and cross-links cell-bound non-specific IgE molecules [4,5,10], leading to degranulation.

In addition to binding to the chitobiose core of IgE, STA can also bind to the chitobiose core of the *N*-glycans of α-chain of human FcεRI, the high-affinity IgE receptor on mast cells and basophils [1,47]. The extracellular domain of the α-chain is heavily glycosylated (38–42% *N*-linked and 4% *O*-linked glycans of the molecular mass of α-chain) [48]. The expression of FcεRI is dependent upon serum IgE; as the receptor number is certainly greater in the case of atopics [45,46], STA can possibly cross-link two adjacent free cell surface FcεRI through the α-chain and cause activation.

The stability of STA, which is resistant to heat, base and acid, appears to be due to its high content of carbohydrate residues and disulphide bonds [49]. This appears to be true in the case of STA present in heat-processed potato extract, wherein ~40–50% of its biological activity (as measured by haemagglutination and glycoprotein binding assays) is retained. The observations made in the present study on the activation of basophils *in vitro* may also occur *in vivo*, although to a lesser extent, in view of the retention of biological activity in cooked potato. It is important to note here that tomato lectin, which is structurally and functionally similar to STA, has been shown to resist digestion in the mammalian gastrointestinal tract [50].

STA was found to release histamine from rat peritoneal mast cells similar to, but not to the same extent as, Con A [11]. This could be due to the heterogeneity of the mast cell population. It has been shown that dermal and subepidermal mast cells in the rat and mouse, and mucosal and dermal human mast cells showed very similar lectin-binding properties to each other [41]. Based on the HR from rat PECs and positive SPT to STA in ~35% of atopic subjects (or 7–9% of general population), we conclude that the binding of STA to basophils and mast cells is primarily through its interaction with the chitobiose core of *N*-glycans of cell-bound non-specific IgE. This may explain why certain atopic subjects (suffering from allergic rhinitis, asthma or both) experience adverse reactions upon consumption of foods prepared with potato and avoid eating potato-based foods, although they are not truly allergic to potato. A similar, but biologically relevant, clinical scenario may be occurring in the case of consumption of tomato, as tomato lectin behaves in an identical fashion to potato lectin, and that tomato is consumed to a greater extent than potato in raw form. An important caveat of our study conclusions regarding biological relevance of STA upon potato consumption is the lack of objective data in clinical studies on atopic subjects upon food challenge using various amounts at STA in potato-based foods.

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