

Effect of horse gram lectin (*Dolichos biflorus* agglutinin) on degranulation of mast cells and basophils of atopic subjects: Identification as an allergen

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Abstract

Horse gram (*Dolichos biflorus*) is widely consumed in the tropical south Asian countries including rural areas of India. Since *D. biflorus* agglutinin (DBA) is an important dietary lectin in horse gram, we have studied its effect on the degranulation of mast cells and basophils of atopic subjects. Allergy to horse gram lectin has not been reported so far. Skin prick test (SPT) was performed with 100 µg/mL of DBA. DBA-specific IgE was detected by dot-blot, and ELISA. Histamine release (HR) assay was carried out using leukocytes from non-atopic and atopic subjects, and rat peritoneal exudate cells. Among the atopic group, 10 of 48 subjects (21%) were found to be positive for DBA by SPT, and none were positive in the non-atopic group ($n=20$). Two subjects out of the ten who tested positive for DBA by SPT were found to be sensitized to DBA as revealed by the presence of specific IgE by ELISA and dot-blot. The HR was found to be 2- to 3-fold higher in DBA-allergic subjects than in non-atopic and atopic subjects. Basophil HR by DBA was found to be similar in both non-atopic and atopic subjects. However, DBA induces activation of mast cells *in vivo* in a sub-population (21%) of atopic subjects. Two subjects have been identified as having food allergy to horse gram based on the presence of DBA-specific IgE with a positive correlation to basophil HR. This is the first report of food allergy to horse gram, and DBA has been identified as an allergen.

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1. Introduction

Immediate hypersensitivity is the basis of acute allergic reactions caused by the activation of basophils and mast cells when an allergen interacts with membrane-

bound IgE [1]. The complex of allergen, IgE, and FcεRI on the surface of these cells triggers the release of histamine and other biological mediators [2,3]. Collectively, these mediators are responsible for the clinical symptoms seen in a variety of allergic disorders [1–3]. Another mode of activation occurs when some plant lectins cross-link two adjacent IgE molecules by binding to the carbohydrates on the Fc portion of IgE [4,5]. Con A, which has specificity for mannose/glucose, was the first lectin shown to activate basophils and mast cells [4,6,7]. Other lectins studied in this regard are mostly mannose- and GlcNAc oligomer-specific lectins [8,9]. Lectins are

Abbreviations: con A, concanavalin A; DBA, *Dolichos biflorus* agglutinin; GalNAc, *N*-acetyl-D-galactosamine; GlcNAc, *N*-acetyl-D-glucosamine; HR, histamine release; OVA, ovalbumin; PEC, peritoneal exudate cells; SPT, skin prick test; Tris-CAM buffer, 10 mM Tris buffer, pH 7.4 containing 1 mM CaCl₂, 1 mM MgCl₂ and 0.03% BSA.

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an important component of total dietary proteins in foods, especially in the case of legumes where they are present in amounts of up to 25% [10,11].

Horse gram (*Dolichos biflorus*) is a native of India and grown throughout the tropical regions as a dry crop [12]. The cultivated crop is usually a mixture of several varieties differing in seed coat color and the period of maturity [12]. Horse gram is a major pulse in south India and is consumed more widely in the rural sector than the urban. The seeds are germinated and used in preparation of foodstuffs like curry and soup; they are also eaten whole, unlike other pulses that are consumed after splitting [13]. The horse gram including the split husk is also extensively used as feed for cattle and horses. Horse gram seeds contain a very abundant seed lectin representing up to 10% of the total soluble seed protein [8,10,11].

Horse gram lectin (*D. biflorus* agglutinin; DBA) agglutinates type A₁ erythrocytes and has specificity for terminal α -linked *N*-acetyl-D-galactosamine (GalNAc) [10,11]. It is a heterotetrameric glycoprotein found in two forms, A and B: form A (113 kDa) and form B (109 kDa). The predominant form A is composed of four similar subunits, which are grouped into two subunits, IA (27.7 kDa) and IIA (27.3 kDa). There are two GalNAc-binding sites per lectin molecule, and both are present on subunit IA [10,11].

Apart from mannose- and GlcNAc oligomer-specific lectins [4,7–9], lectins with other sugar specificities have not been studied with respect to their effects on basophils and mast cells. It appears that some dietary lectins are responsible for non-allergic food hypersensitivity reactions that exactly mimic the symptoms of immediate or type I (IgE-mediated) hypersensitivity reactions [9]. In view of the extensive consumption of horse gram in the Indian sub-continent, it appeared interesting to study the effect of purified horse gram lectin on the degranulation of mast cells *in vivo* and basophils *in vitro* from non-atopic and atopic subjects.

2. Materials and methods

This study was undertaken after clearance by the Institutional Ethics Committee; informed consent was obtained from all atopic and non-atopic subjects in the age range of 15–60 years. Male Wistar rats housed in the animal house facility of our institute was used for the preparation of peritoneal exudate cells (PEC) as per standard operating procedures, after obtaining approval from the Institutional Animal Ethics Committee (IAEC).

2.1. Identification of atopic and non-atopic subjects

These subjects were identified based on case history (atopic subjects are chosen at random who had symptoms of at least one allergic condition such as allergic rhinitis, atopic dermatitis,

asthma, food allergy, and allergic conjunctivitis) and skin prick tests (SPT) of certain commercial pollen and food extracts. Most of the subjects examined in this study were from the rural areas.

2.2. Eosinophil count, serum total IgE and histamine levels

Eosinophil counts was carried out on whole blood and expressed as numbers per μ L blood [14]. Serum and plasma histamine were analyzed by fluorometry using *o*-phthalaldehyde (OPT) [15], which is described later under Histamine release (HR) assay, and expressed as ng/mL serum or plasma [16].

Murine monoclonal anti-human IgE antibody (murine IgG2a, κ ; hybridoma cell line ATCC HB-121, designation E5BB3IIA2, obtained from National Centre for Cell Science (NCCS), Ganeshkhind, Pune, India) was purified from hybridoma cell culture supernatant on protein A-agarose. Serum total IgE was quantitated using this antibody for coating as per the sandwich ELISA protocol [17]; alkaline phosphatase (AP)-conjugated murine monoclonal anti-human IgE (Sigma-Aldrich Co., St. Louis, MO, USA) was used as the detection antibody at 1:1500 dilution. Results are expressed as IU/mL.

2.3. Assessment of purity of DBA

SDS-PAGE (12%, reducing) [18] was performed to assess the purity of DBA (Sigma-Aldrich Co., St. Louis, MO, USA). RP-HPLC analysis was carried out on C₁₈ column using Shimadzu LC-10A HPLC system (Shimadzu Corp., Kyoto, Japan).

2.4. Skin prick test (SPT)

Most purified allergens (natural or recombinant) have been used for SPT in the concentration range of 20 μ g/mL to 1 mg/mL. Since DBA represents 10% of the total proteins in horse gram, we have arbitrarily chosen the DBA concentration for SPT at 100 μ g/mL (prepared in 50% glycerinated PBS). Glycerinated PBS and histamine base at 1 mg/mL were used as negative and positive controls, respectively. SPT was carried out as per the standard protocol [19]. After 20 min, the wheal and flare diameters were measured; a wheal diameter of 3 mm greater than that of the negative control was considered as positive.

2.5. Case history of subjects allergic to horse gram

2.5.1. Case 1

A 49-year-old female (N.T.) had urticaria and wheezing. She avoids eating banana, sapodilla, tomato and citrus fruits. However, SPT was found to be negative to extracts of these fruits. She also strictly avoids horse gram in her diet as she has observed an increase in wheezing within a few minutes after ingestion of any food containing horse gram. She shows moderate SPT for grass pollen mix. The family history for allergy is negative.

2.5.2. Case 2

A 55-year-old male (S.D.) has complaints of urticaria and wheezing as his major health problems. He has observed an increase in wheezing, and also itching in some parts of his body

after eating a diet containing horse gram. Currently, he strictly avoids horse gram in his diet. He has also made similar observations with eggplant and was found to be moderately positive to eggplant by SPT. He avoids eating most of the common fruits and raw vegetables; however, he was negative to these extracts by SPT. The family history of allergy is positive.

2.6. ELISA and dot immunoblot to detect DBA-specific IgE antibody

DBA-specific IgE was detected by indirect ELISA [20]. Briefly, microtiter wells (Maxisorp; Nunc, Roskilde, Denmark) were coated with 15 µg of DBA at pH 9.6 at 4 °C overnight. After blocking with 3% gelatin, the microtiter plate was incubated with subjects' sera at 1:3 dilution at 4 °C overnight. Next, it was incubated with AP-conjugated murine monoclonal anti-human IgE at 1:1500 dilution at 37 °C for 2 h, followed by color development.

Dot immunoblot [20] was carried out using 15 µg of DBA which was applied as a spot on the nitrocellulose (NC) membrane along with concanavalin A (con A) and ovalbumin (OVA) as reference controls. After air-drying, the membrane was blocked and incubated with non-atopic or DBA-sensitized subject's serum (1:3 dilution) at 4 °C overnight. Murine monoclonal anti-human IgE-AP conjugate (1:1500 dilution) was used as the secondary antibody. The membrane was developed using BCIP-NBT substrate (Bangalore Genei, Bangalore, India) at 1:3 dilution. A positive spot appears as blue against a white background.

2.7. Isolation of leukocytes containing basophils

Leukocytes (buffy coat containing basophils) were isolated from 10 mL of heparinized venous blood as described [21] using 6% dextran T 700 gradient (Hi-Media Laboratories, Mumbai, India). The leukocyte layer was washed 4–5 times with isotonic PBS and resuspended in Tris-CAM buffer. The isolated leukocytes were counted using crystal violet; cell viability, as determined by Trypan blue dye exclusion, was 95%.

2.8. Isolation of rat peritoneal exudate cells (PEC)

PECs were isolated from male Wistar rats (4-week-old adults; weight: ~300 g) following the standard procedure [22]. Five minutes following the injection of Tyrode buffer, pH 7.4 containing 0.1% BSA, the fluid containing PECs was collected. The residual erythrocytes were removed by treatment with 150 mM NH₄Cl buffer. Next, the cells were pelleted, washed, and resuspended in Tris-CAM buffer. PECs were stained for mast cells using toluidine blue; their viability, as assessed by Trypan blue dye exclusion, was 92%. The PEC preparation was found to contain 15–20% mast cells.

2.9. Histamine release (HR) assay [23]

Cells and reagents (DBA or other proteins) in Tris-CAM buffer were added to polystyrene tubes at a final volume of 1 mL in an ice bath. Each tube containing ~2 × 10⁶ cells/mL was

incubated at 37 °C for 45 min. In each experiment, 3% (final concentration) perchloric acid was added to one set of samples (or, alternatively boiled at 100 °C for 10 min), to obtain the total histamine content of cells (Pc). Blank tubes containing only cells and buffer were used as controls for non-specific or spontaneous release (Ps), which was generally in the range of 6% to 8%. After 45 min, the tubes were transferred to an ice bath to stop the reaction and centrifuged at 275 × g at 4 °C for 20 min. The supernatants were assayed for histamine content of test samples (Pt).

The released histamine was quantitated by a fluorometric assay [15]. Briefly, the histamine in the supernatant was extracted initially into *n*-butanol, and then HCl. After neutralization and derivatization using OPT, the reaction was arrested with phosphoric acid. The fluorescence intensity was measured at λ_{EX}=360 nm, and λ_{EM}=450 nm. The formula for the calculation of percent HR is [(Pt – Ps) ÷ (Pc – Ps)] × 100.

2.10. Statistical analysis

Each datum represents the mean and standard error of the mean (S.E.M.) of the different experiments under identical conditions. Student's *t*-test was used to make a statistical comparison between the groups.

3. Results

3.1. Selection of atopic and non-atopic subjects for the study

Atopic and non-atopic subjects were selected based on detailed case history and clinical symptoms (Table 1, footnote). Among these groups, the atopic or non-atopic status was confirmed in a representative number of subjects (*n*=12 for atopics, and *n*=10 for non-atopics) based on the eosinophil count, serum total IgE and plasma/serum histamine levels. The results are summarized in Table 2. The serum total IgE was found to be significantly higher in atopic subjects, and represents approximately a 5- to 7-fold increase over the value for non-atopic subjects. In atopic subjects, the eosinophil counts were increased by ~2.6-fold over the mean value of non-atopic

Table 1
Skin prick test of DBA on atopic and non-atopic subjects

Subjects	Number of subjects tested	Number of subjects positive	Percent positive	Average wheal/flare diameter (mm)
Non-atopic ^a	20	0	0.0	0.5/0
Atopic ^b	48	10 ^c	20.8	3.5/8

^a Healthy subjects without any clinical symptoms of allergy (age range: 15–60 years).

^b Subjects displaying characteristic symptoms from any one of the following: asthma, allergic rhinitis, urticaria or food allergy (age range: 15–60 years).

^c Includes 2 subjects sensitized to DBA whose individual SPT data are shown in Table 4.

Table 2

Eosinophil counts, serum total IgE and serum histamine in non-atopic and atopic subjects

Subjects	Eosinophil	Total IgE		Histamine level	
	Mean $\pm$ S.E.M. (counts/ μ L) ^a	Mean $\pm$ S.E.M. (A ₄₉₂)	IgE (IU/mL) ^b [Range]	Serum mean $\pm$ S.E.M. (ng/mL) ^c	Plasma mean $\pm$ S.E.M. (ng/mL) ^d
Non-atopic (n=10)	302 $\pm$ 11	0.267 $\pm$ 0.010	34.7–43.9	28.2 $\pm$ 3.6	1.5 $\pm$ 1.2
Atopic (n=12)	776 $\pm$ 18	1.205 $\pm$ 0.120	177.3–330.1	184.2 $\pm$ 10.1	11.6 $\pm$ 1.0

^a Reference normal value for eosinophil counts=40–400 cells/ μ L [14]; $p \leq 0.001$ ($t=38.2$).^b Reference normal value for serum total IgE=<120 IU/mL [17]; $p \leq 0.001$ ($t=13.40$).^c Value for non-atopic subjects is 5–27 ng/mL [16]; $p \leq 0.001$ ($t=15.74$).^d Value for non-atopic subjects is 0.5 to 2 ng/mL [16]; $p \leq 0.001$ ($t=10.64$).

subjects (302 cells/ μ L). The serum and plasma histamine levels were found to be significantly higher in atopic subjects (~6- to 8-fold) as compared to the mean value for non-atopics.

3.2. Analyses of DBA for purity

Commercial DBA appears as a single homogeneous band in 12% reducing SDS-PAGE (Fig. 1, panel A); careful examination revealed two closely spaced bands around 27 kDa, in agreement with the literature [10,11]. DBA appears as a single peak in RP-HPLC analysis with a retention time of 37.47 min (Fig. 1, panel B).

3.3. Skin prick test, allergen-specific IgE, and dot immunoblot using DBA

Table 1 shows the results of SPT with DBA tested on 48 atopic subjects who have generalized symptoms characteristic of allergic conditions (based on case history and SPT), and on 20 non-atopic subjects who do not exhibit any allergic symptoms. DBA showed a positive SPT in 10 out of 48 allergic subjects (21%). SPT reactions as assessed by means of wheal/flare diameter were barely positive (designated as +; 3–3.5/5 mm) or moderately positive (designated as 2+; 4–4.5/10–15 mm) compared to the positive control, histamine base (6/25 mm). None of the non-atopic subjects gave a positive SPT (wheal/flare diameter of 0–1/0 mm). The characteristics of the 10 subjects who were positive for DBA by SPT, and their SPT and DBA-specific IgE results are given in Table 3. Only 2 subjects (subjects NT and SD) showed an SPT grading of 2+, and their DBA-specific IgE was 3-fold higher than the value for the other 8 subjects who showed an SPT grading of +.

The two DBA-sensitized subjects showed a significant wheal/flare diameter, and the results are shown in Table 4. The case histories of subjects NT and SD are given under Materials and methods. Serum samples of non-atopic ($n=6$) and atopic ($n=6$) subjects were checked for the presence of DBA-specific IgE antibodies. Con A (lectin control) and BSA (non-lectin protein) were used as negative controls; their ELISA values for non-atopic and atopic subjects are given as footnotes in Table 4. The ELISA value for DBA-specific IgE for atopic

subjects was very similar to that seen for non-atopic subjects, although the total IgE level of atopic subjects is approximately 3.5-fold higher than that of non-atopic subjects (Table 4). However,

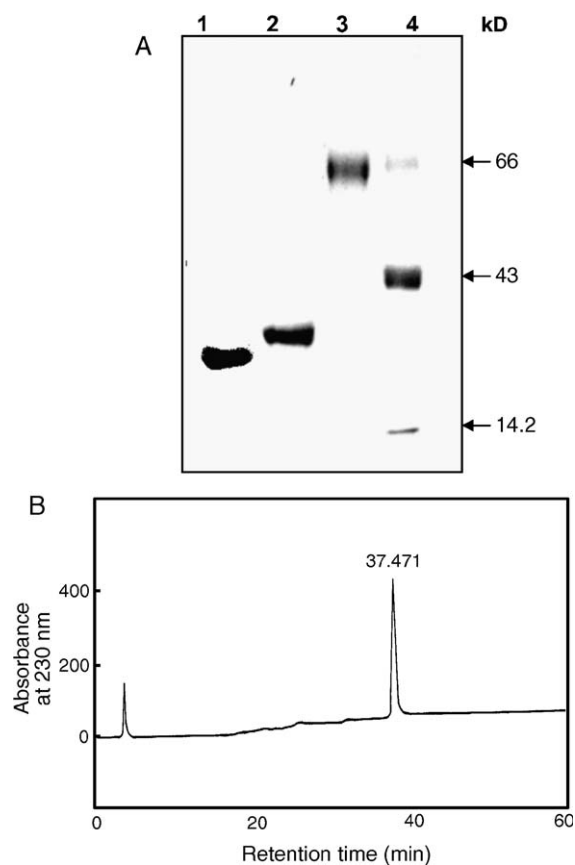


Fig. 1. Assessment of purity of *Dolichos biflorus* agglutinin. (A) SDS-PAGE (12%, reducing). Coomassie stained gel: lane 1, Con A; lane 2, DBA; lane 3, BSA; lane 4 (from top to bottom), BSA, OVA, and lysozyme. Protein load: 10 μ g. (B) RP-HPLC. Column: C₁₈ (4.5 $\times$ 250 mm; particle size 5 μ m). Elution: binary gradient of solvent A (0.1% TFA) and solvent B (80% acetonitrile in 0.05% TFA) at a flow rate of 1 mL/min. Protein detection: 230 nm.

Table 3

Characteristics of atopic subjects who are positive for *Dolichos biflorus* agglutinin^a (DBA) by skin prick test

Subjects	Sex/ age (years)	Allergic history (symptoms) ^b	SPT to DBA (grade) ^c	DBA-specific IgE ($A_{405} \pm S.D.$) ^d
YT	M/17	AR, N, Wh	+	0.066 $\pm$ 0.003
S	F/29	U	+	0.078 $\pm$ 0.002
NT	F/49	U, Wh	2+	0.227 $\pm$ 0.005
CMN ^c	F/46	P, U, Wh	+	0.059 $\pm$ 0.001
SD ^c	M/55	P, U, Wh	2+	0.248 $\pm$ 0.003
MG	M/60	P, U, Wh	+	0.057 $\pm$ 0.001
VK	M/16	N, Wh	+	0.089 $\pm$ 0.003
SR ^c	F/40	N, Wh, U	+	0.045 $\pm$ 0.002
BV	F/65	AR, N, P, Wh	+	0.063 $\pm$ 0.001
GT ^c	F/26	AR, N Wh, U	+	0.075 $\pm$ 0.002

^a Tested at 100 μ g/mL concentration in 50% glycerinated PBS.

^b AR: allergic rhinitis; N: nasal; P: pharyngeal; U: urticaria; Wh: wheezing.

^c SPT grading based on wheal/flare intensity: +, one-third of histamine control; 2+, two-thirds of histamine control.

^d DBA-specific IgE in the case of non-atopic subjects ($n=10$) is 0.066.

^e Family history of allergy is positive.

the DBA-sensitized subjects NT and SD, who were found to have a positive case history for the ingestion of horse gram, showed significantly higher ELISA values (0.227 for NT, and 0.248 for SD), for DBA-specific IgE (Tables 3 and 4). These two subjects also showed an increase of 3.2-fold and 4.5-fold, respectively, in the values for total IgE compared to non-atopic subjects.

In the case of the DBA-sensitized subjects NT and SD, IgE-dot immunoblot for DBA was found to be positive to DBA and negative to OVA and con A which were used as control proteins (Fig. 2, panel A). The dot immunoblot was, as expected, negative for the serum of non-atopic subject (N). This clearly reveals the presence of DBA-specific IgE in two

Table 4

Skin prick test, DBA-specific IgE, total IgE analysis and percent histamine release of non-atopic and atopic subjects

Samples or subjects	Wheal/ flare diameter (mm)	Specific IgE ELISA units ^a (A_{405})	Total IgE ELISA units (A_{492})	Histamine release ^b (%)
Negative control	0–1/0	n.a. ^c	n.a.	n.a.
Positive control	6.0/20	n.a.	n.a.	n.a.
Case 1 (N.T.)	4.0/10	0.227	0.844	68
Case 2 (S.D.)	4.5/15	0.248	1.181	71
Non-atopic subjects ^d	0.5/0	0.066	0.267	25 $\pm$ 1.3
Atopic subjects ^d	3.5/5	0.083	0.933	28 $\pm$ 1.1

^a Value for non-lectin control (BSA) ($n=6$): 0.018 (non-atopic); 0.020 (atopic). Value for lectin control (Con A) ($n=6$): 0.056 (non-atopic); 0.099 (atopic).

^b Measured at 2 μ g/mL DBA concentration.

^c n.a.=not applicable.

^d Mean value of different parameters for these subjects are shown ($n=10$).

cases that have positive case history (to the ingestion of horse gram), and were positive by SPT and allergen-specific IgE. However, these two subjects were unavailable for follow-up by double-blind placebo-controlled food challenge.

3.4. Histamine release (HR) from DBA-sensitized subjects

The leukocytes containing basophils were obtained from the venous blood of DBA-sensitized subjects (subjects NT and SD) for performing HR assay. The HR was found to be maximum at 2 μ g/mL DBA (Fig. 2, panel B); the values were found to be 68% for subject NT, and 71% for subject SD. There is an approximately 3-fold increase in HR compared to a non-atopic subject who showed 25% HR under identical conditions.

3.5. HR from non-atopic and atopic subjects using DBA

In order to find out the DBA concentration, which gives maximum HR in the case of atopic subjects ($n=3$), the HR assay

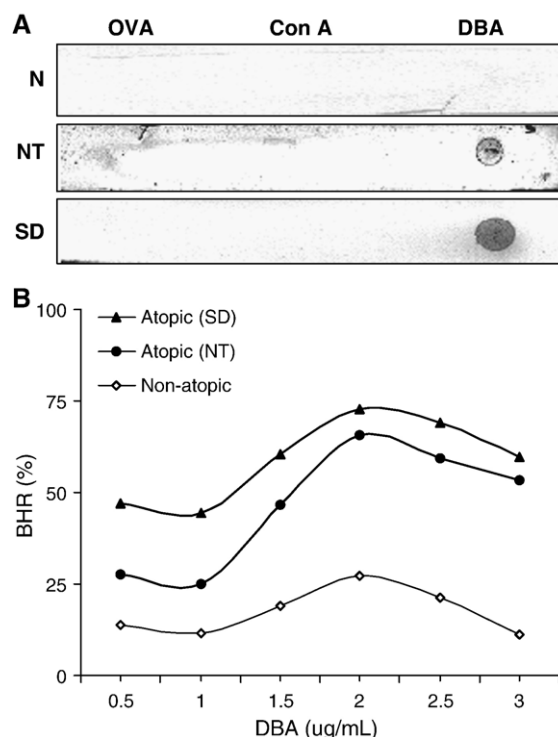


Fig. 2. Immunoanalysis of DBA-sensitized subjects using serum and leukocytes. (A) Dot immunoblot of DBA using non-atopic and atopic subjects' sera (subjects positive to DBA by skin prick test). N: non-atopic subject; NT: DBA-sensitized atopic subject NT (positive to DBA by SPT); SD: DBA-sensitized atopic subject SD (positive to DBA by SPT). (B) Histamine release from the leukocytes of two DBA-sensitized subjects (subjects NT and SD) and non-atopic subject, as a function of DBA concentration. Leukocyte concentration: 2×10^6 cells/mL. Following extraction and derivatization with OPT, the released histamine was determined by fluorometry. Details are given under Materials and methods.

was performed in the concentration range of 0.0001–10 $\mu\text{g/mL}$. The results are shown in Fig. 3 (panel A). The non-lectin control, OVA, does not show any HR. The positive reference control, con A, was found to induce HR in the range of 0.01–10 $\mu\text{g/mL}$, and maximum release of 55% was observed at approximately 1 $\mu\text{g/mL}$ in the concentration range of 0.1–10 $\mu\text{g/mL}$, whereas DBA showed a maximum HR of 22% at the same concentration.

The HR from non-atopic and atopic subjects as a function of DBA, con A, and OVA in the narrow concentration range of 0.5–3 $\mu\text{g/mL}$ is shown in Fig. 3 (panel B). Maximum HR was seen at 2 $\mu\text{g/mL}$ in the case of DBA and con A. DBA shows an HR of $\sim 25\%$ and 28% in non-atopic ($n=4$) and atopic ($n=5$) subjects, respectively, indicating that there is no significant difference in HR from these two groups. Con A shows a typical

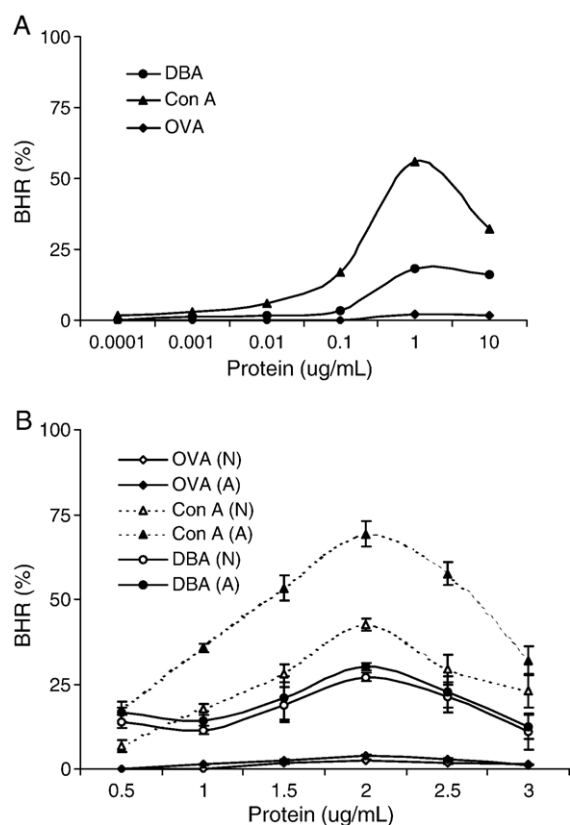


Fig. 3. Histamine release from leukocytes of non-atopic and atopic subjects. (A) HR from the leukocytes of human atopic subjects ($n=3$) as a function of DBA concentration in the range of 0.0001 to 10 $\mu\text{g/mL}$. Con A and OVA were used as lectin and non-lectin controls, respectively. Other details are as given in Fig. 2 (panel B). (B) HR from the leukocytes of human non-atopic and atopic subjects (subjects selected from these groups are described in Table 2) as a function of protein concentration (OVA, non-lectin control; con A, lectin control; DBA, *Dolichos biflorus* agglutinin). N: non-atopic subjects ($n=4$; open symbols); A: atopic subjects ($n=5$; closed symbols). In the case of con A, the mean values of HR is significant at $p \leq 0.001$ ($t=13.66$); for DBA, the mean values of HR is not significant at $p \leq 0.001$ ($t=1.75$; $p=0.1177$). Other details are as given in Fig. 2 (panel B).

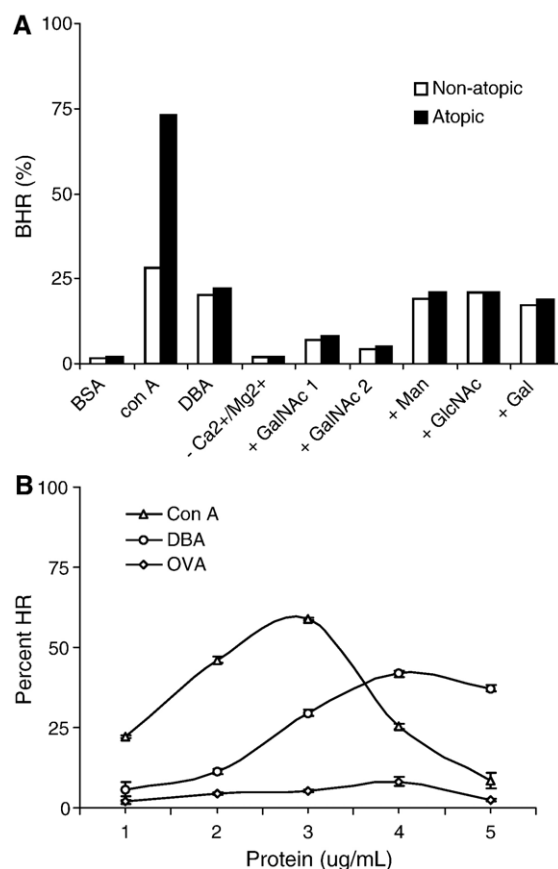


Fig. 4. Histamine release and its inhibition by various sugars. (A) HR from atopic ($n=2$) and non-atopic ($n=2$) leukocytes (2×10^6 cells/mL) using DBA at 2 $\mu\text{g/mL}$ concentration. Con A and BSA are lectin and non-lectin controls, respectively. The concentration of sugars used for inhibition of HR induced by DBA is 100 $\mu\text{g/mL}$, except GalNAc 1 (50 $\mu\text{g/mL}$). HR by DBA in the absence of Ca²⁺/Mg²⁺ (Tris-CAM buffer without metal ions) is also shown. (B) HR from rat peritoneal exudate cells as a function of protein concentration. PEC concentration: 2×10^6 cells/mL. The results represent mean of three analyses. Released histamine was determined by fluorometry as given under Materials and methods.

bell-shaped curve for HR with a maximum at 2 $\mu\text{g/mL}$. The HR was $\sim 40\%$ in non-atopic subjects and $\sim 73\%$ in atopic subjects. OVA (negative control) shows an HR of only 4% in the case of non-atopic subjects and 6% in the case of atopic subjects. Other non-lectin proteins (BSA, lysozyme) showed even lesser HR than OVA (data not shown).

3.6. Inhibition of DBA-induced HR by sugars

The effect of various sugars on DBA-induced HR (22%) at 2 $\mu\text{g/mL}$ is shown in Fig. 4 (panel A). D-Mannose, D-galactose, and GlcNAc (all at 100 $\mu\text{g/mL}$) do not cause any inhibition of HR. However, GalNAc at 50 $\mu\text{g/mL}$ was found to inhibit the HR by ~ 4 -fold (6% HR); this effect was more pronounced at 100 $\mu\text{g/mL}$ (2% HR). In the absence of Ca²⁺/Mg²⁺ from Tris-

CAM buffer, the HR was very low, and is comparable to the release by a non-lectin protein (BSA).

3.7. HR from rat peritoneal exudate cells using DBA

The HR from rat PECs using DBA in the concentration range of 1–5 µg/mL is shown in Fig. 4 (panel B); the release was found to be maximum (48%) at 4 µg/mL. Con A shows the characteristic bell-shaped curve with a maximum HR of 57% at 3 µg/mL, whereas OVA showed a maximum release of only 9% at 4 µg/mL.

4. Discussion

Horse gram, consumed widely in India, is a valuable protein supplement containing 22% protein [12]. The lectin is one of the major proteins as is the case with many legume lectins [8,10,11]. The present study deals with the effect of horse gram lectin on mast cells/basophils of non-atopic and atopic subjects, and to investigate whether a common dietary lectin (DBA) mediates non-allergic food hypersensitivity.

SPT of DBA revealed that approximately one-fifth of the atopic subjects showed a positive reaction, whereas there was no reaction in the case of non-atopic subjects. A positive SPT of 21% to a purified protein (DBA) from horse gram appears to be unusual for food allergy, since the incidence of food allergy in adults is generally 2–4% [20]. This may be due to the non-specific interaction of DBA with the mast cells *in vivo*. It has been shown by Hormia et al., [24] that DBA reacts selectively with mast cells in human connective tissue cells and epithelial cells. In the case of 10 atopic subjects who showed a positive SPT for DBA, the total IgE level was, in general, 3- to 5-fold higher than in non-atopic subjects. Despite this increased total IgE level, the magnitude of HR from atopic subjects is similar to that seen from non-atopic subjects.

The DBA-specific IgE was found to be absent in atopic subjects who were positive for DBA by SPT (with the exception of 2 subjects). The two subjects (subjects NT and SD), who are allergic to horse gram by case history, were identified as sensitized to DBA as confirmed by the presence of DBA-specific IgE by ELISA and dot immunoblot. While the total IgE levels of these two subjects were similar to those of other atopic subjects (~4-fold higher compared to non-atopic subjects), DBA-specific IgE is about 3-fold higher in these two subjects as compared to atopic subjects. These results correlate very well with the case history of the subjects wherein they have described the allergic symptoms experienced following ingestion of foods prepared with horse gram. The *in vitro* basophil activation using the leukocytes of DBA-

sensitized subjects show a significant increase in HR upon incubation with DBA indicating the degranulation of basophils via allergen-IgE (binding site) interactions.

Allergy to ingestion of horse gram has not been reported so far. The description of the two subjects in this article appears to be the first report of food allergy to horse gram; DBA has been identified as the allergen, and can be named Dol b Agglutinin. Lectins have been reported earlier as minor allergens [25–28] in only three plant foods, namely peanut (Ara h Agglutinin)[25], soybean (Gly m Lectin) [26], and wheat (Tri a 18; wheat germ agglutinin or WGA) [27]. Lectins have been ranked 9th in their assignment as plant food allergen families in Pfam database [28], and are considered as minor allergens.

HR from the leukocytes of non-atopic and atopic subjects in the presence of DBA are similar in pattern. This clearly indicates that DBA does not depend on the basophil IgE density to interact and cause activation to release biological mediators. Since mast cell-bound IgE is a glycoprotein rich in complex type glycans (~12%), the composition of the N-linked glycans on its ϵ -chains was examined. The glycans comprise of core GlcNAc, mannose, fucose, galactose, and sialic acid; notably, GalNAc was not present [29,30]. The lower magnitude of HR (20–25%) by DBA may be due to its interaction with some other glycoproteins/glycolipids containing terminal GalNAc residues on the surface of basophils; the low level of HR induced by DBA can be inhibited using the specific saccharide, GalNAc. This is in contrast to con A wherein the HR is higher in non-atopic subjects, and comparatively more so in atopic subjects. Con A-induced HR is dependent on the density of IgE present on basophils [4,6,7,31]. Con A cross-links the glycans of two adjacent IgE [4,6,7] which provides the basis for degranulation, and the effects are identical to that of allergen-mediated immediate hypersensitivity reactions [1–3].

DBA was found to release histamine from rat peritoneal mast cells similar to, but not to the same extent as, con A and KM+, a mannose-binding lectin from the nutritious seeds of jackfruit (*Artocarpus integrifolia*) [22]. Roberts et al. [32], found that dermal and subepidermal mast cells in the rat and mouse, and both mucosal as well as dermal human mast cells showed very similar lectin-binding properties to each other. It may be speculated here that the HR from rat PECs and positive SPT to DBA in 21% of atopic subjects may be due to the binding of DBA to similar GalNAc-containing glycoproteins on mast cells. Alternatively, the glycosylation pattern of mast cells in a sub-population of atopic subjects may be different in terms of O-glycosylation. In eukaryotes, GalNAc has been described as a glycan component in O-linked glycoproteins such as mucin, fetuin, human gonadotrophins,

antifreeze glycoproteins, and Tamm-Horsfall mucoprotein [33].

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