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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.

Keywords: *Dolichos biflorus* agglutinin; Horse gram lectin; Allergen; Histamine release; Basophils; Mast cells

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 $CaCl_2$, 1 mM $MgCl_2$ 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 A_1 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 μL 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 C_{18} 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 μg/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 μg/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