Review Article

Immunoglobulin E specific to carbohydrate determinants and its relevance in legume allergic cross-reactivity

Abstract: A glycol-related Immunoglobulin E (IgE) reactivity has been demonstrated in most allergen sources, especially in the plant kingdom. Recent progress in glycobiology has allowed a clear classification of these glycan-epitopes. Unlike classical peptide chain-based epitopes, glycoepitopes can share significant structural homologies beyond the limits of protein families. These glycol epitopes are thus prone to extensive cross products as distinct as pollen and Hymenoptera venoms. Because a monovalent IgE-binding is reactivity. Many of these glycoepitopes behave as "pan-epitopes" leading to cross-reactivity between sufficient in serum-based assays, glycol epitopes, and cross-reactive carbohydrate (CCD) are classically considered as a potential source of positive *in-vitro* results without clinical significance. Reports demonstrated that glycol epitopes could induce a response at the cell level and suggested that they might play a role *in-vivo*. So, accurate measurement and specificity assessment of CCD-specific IgE is a critical factor to understand the allergenic cross-reactivity and clinical sensitivity mediated by CCD- specific IgE. The present review considers studies with *in-vitro* allergenic cross-reactivity due to IgE binding may not be a predictive measure of clinical symptoms and that is very true in the case of haptenic or glycan IgE binding of antigens. The CCD and related IgE binding have to be assessed and evaluated for clinical relevance for avoidance by sensitized subjects and also it is required for allergenic assessment before the functional proteins are labeled for allergenicity during the commercialization of food products

containing legume CCD-glycoproteins. The legume allergenic cross-reactivity and biological relevance of CCD-specific IgE will provide information for the clinician to review the diagnostic role of IgE to identify allergenicity. It promotes further research to evaluate the clinical relevance of CCD IgE in defining true allergic cross-reactivity among glycoproteins.

Keywords: Immunoglobulin E; Cross-reactive carbohydrate determinants CCD; glycoproteins, hyptenic antigen, clinical allergy; glycan-epitopes.

Abbreviations:

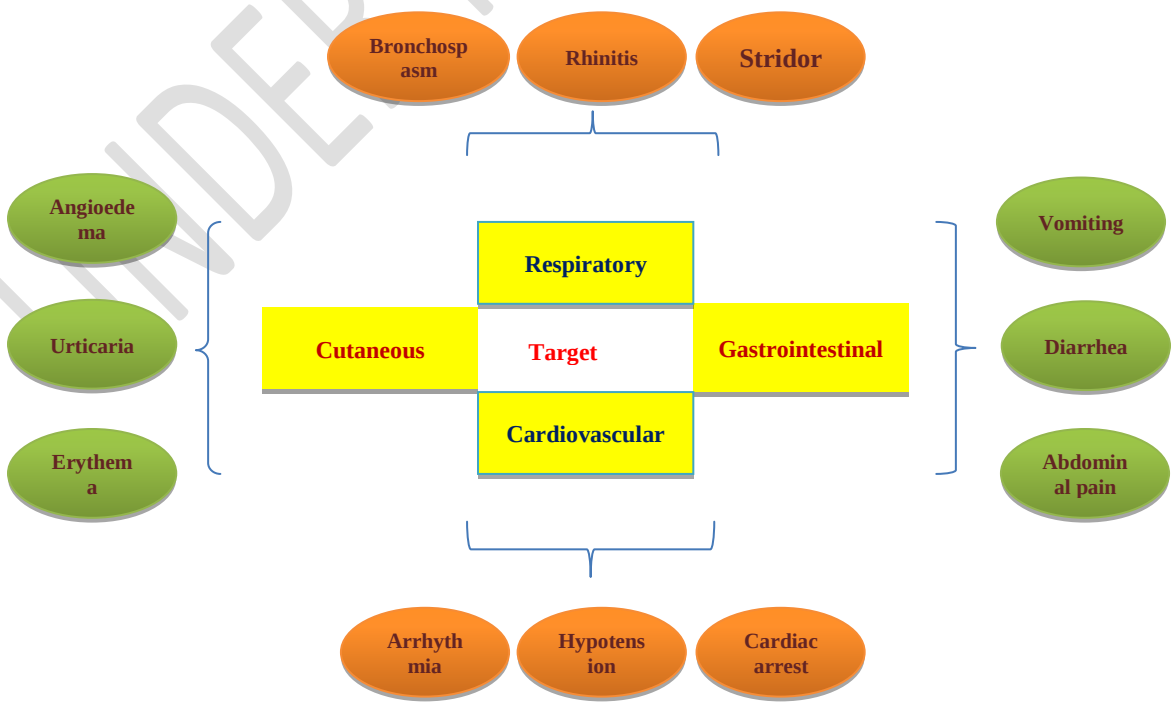
CCD: cross-reactive carbohydrate determinant; US: United States; EU: European Union; SPT: skin prick test; MMXF3: two mannose additional on N-glycans with β- 1,2 xylose and α-1,3 fucose residues; MUXF3: deletion of one mannose on N-glycans with β- 1,2 xylose and α-1,3 fucose residues; MMF3: two mannose additional on N-glycans with only α-1,3 fucose residues; MMF3F6: two mannose additional on N-glycans with α-1,3 fucose and α- 1,6 fucose residues deletion of β- 1,2 xylose residue; MMXF3F6: two mannose additional on N-glycans with β- 1,2 xylose, α- 1,3 fucose residues and α- 1,6 fucose residues; SEA: Schistome soluble Egg Antigen; CAP-RAST: Pharmacia CAP -Radioallergosorbent test; HRP: Horseradish peroxidase; RBL: Rat Basophill Leukemia.

1. Introduction

"Allergic diseases are responsible for serious health problems such as asthma, dermatitis, food allergy, bee sting allergy, conjunctivitis, and severe systemic anaphylaxis". [1] "These diseases affect a substantial portion of the general population. Asthma and atopic dermatitis, respectively, affect 10% and 15% of children in some countries. A considerable portion of the health care expenses has been directed at the treatment of allergic diseases. Considerable efforts have been made for identifying factors contributing to these diseases" [2]. "An allergic reaction is initiated

when an allergen interacts with an IgE antibody. Many allergens are proteins and the prediction of these allergen proteins facilitates the study of molecular mechanisms and risk assessment of allergic reactions in different individuals". [3] "Computational methods have been developed for predicting allergen proteins from sequence segments that show identity, homology, or motif match to a known allergen. These methods achieve good prediction accuracies but are less effective for novel proteins with no similarity to any known allergen. Common structural and physicochemical features have been explored for predicting allergenicity from protein sequences". [4] The US, EU, and many other countries have now implemented requirements for evaluating the potential allergenicity of proteins. The primary focus, either elaborated or implied, is on preventing the transfer of a known allergen, or a protein sufficiently similar to a known allergen that it may trigger allergic cross-reactions in food components. The severity of allergic reaction in different organs of human are shown in (Figure.1)

Figure.1 Allergy Consequences:



2.0. Allergenicity and risk assessment:

An appropriate risk assessment strategy for most novel proteins and glycoproteins includes sera IgE determination from allergic donors. [5] Based on only these *in-vitro* immunochemical methods it is hard to differentiate between proteins that are likely to cause harm from those likely to be safe. [6] Because the primary focus for most immunological and bioinformatics approaches used to assess the allergenicity of novel proteins will not rule out the non-specific i.e. false positive IgE binding with no relevance in clinical sensitivity and is more likely to result in wrong interpretation in assessing the protein allergenicity. [7] Since it is impossible to suspect based on biochemical assays, that the consumer with positive IgE reactivity to a novel protein might experience a clinically important allergenic reaction after consumption without any biological assays. There are many reports which show significant *in-vitro* IgE binding and pieces of evidence of cross-reactivity with no clinical symptoms for a particular antigen. [8] Even in assessing cross-reactivity for an allergen the consideration of biological relevance in IgE binding assays is essential to predict the possibility of its potential cross-reactivity with proteins or glycan chains of proteins of its relative classes which share more than 50% sequence homology.

3.0. Allergenic IgE cross-reactivity

"The introduction of novel proteins into foods carries a risk of eliciting allergic reactions in individuals sensitive to the introduced protein or of sensitizing susceptible individuals. IgE cross-reactivity between a novel protein and a known allergen is an important issue in the evaluation of the allergenic potential of novel proteins". [9] "To investigate the potential cross-reactivity among proteins, specific sera IgE screening studies are generally performed. However, most immunochemical methods are nonfunctional assays and do not provide information on the ability

of a protein to induce type I allergic reactions, as in the case of *in-vivo* tests". [10] However, such *in-vivo* tests, including the skin prick test (SPT) and intradermal testing have limitations regarding evaluating the allergenic potential of proteins in foods due to ethical concerns. To overcome these problems, *in-vitro* cell-based degranulation assays with measurement of histamine and beta-hexosaminidase mediator were employed for assessment of allergenicity. These can serve as a tool along with other immunochemical-based assays in determining the cross-reactivity and potential allergenicity of some novel proteins to clinical sensitivity. The functional *in-vitro* bioassay using cell lines was compared with immunochemical and basophil histamine release assays used for the analysis of allergen extracts and evaluated concerning its use in the assessment of biological reactivity of allergenic proteins. [11,71]

4.0. Legume allergenic cross-reactivity:

"Legumes are dicotyledonous plants belonging to the Fabales order. The main distinctive characteristic of which is their fruit legumes and seeds contained in pods. This botanical order is formed by three families: Mimosaceae, Caesalpiniaceae, and Papilionaceae (Fabaceae). The Papilionaceae family includes the most important allergenic species: *Lens culinaris* (lentil), *Cicer arietinum* (chick-pea), *Pisum sativum* (pea), *Arachis hipogea* (peanut), *Phaseolus vulgaris* (bean) y Glycine max (soy). Legumes are an important ingredient in the Mediterranean diet. Among children, sensitivity to legumes is the most prevalent dispute of food allergy. Peanut, lentil and chick-pea are the most frequent cause of allergic reactions to legumes". [12,70] "Legumes could be involved in severe allergic symptoms mentioned in Table 1. The different legumes have structurally homologous proteins, but they are not all equally allergenic, thus making it difficult to distinguish *in-vitro* and *in-vivo* cross-reactivity". [13]

"Peanut allergy can be associated with an allergy to lentils, chick-pea, and peas but less frequently. Contrarily, white beans and overall green beans and soy are well tolerated by children allergic to other legumes. In one study, 82% of the children allergic to legumes had sensitization to pollen. Pea and bean are the legumes with more *in-vitro* cross-reactivity with *Lolium perenne* (perennial ryegrass), *Olea europea* (olive), and *Betula Alba* (birch)". [14] "This cross-reactivity occurred because of common antigenic determinants or due to the coexistence of pollen and legume allergy. Pan-allergen's implication seems to be less probable". [15] "It is important to emphasize that despite an evident clinical and immunological cross-reactivity, diagnosis of legume allergy should not be based only on specific IgE tests. The decision to eliminate one legume from the diet should be based on a positive oral food challenge. The peanut, *Arachis hypogea*, is a member of the legume family and is related to beans and peas, immediate hypersensitivity reactions to peanuts are much more prevalent and severe than other legumes and tend to persist into adulthood". [13] The peanut allergic subjects are known to show *in-vitro* IgE binding to several other legume extracts, which are claimed to have cross-reactivity. Since many protein IgE reacting elements are glycoproteins and most of the legumes contain glycoproteins and are suspected to cause legume cross-reactivity. CCD-specific IgE is the key factor in recognizing the multiple IgE binding for other legume glycoproteins. [14] Clear evidence of demonstrating possible clinical reactivity of CCD-IgE in legume cross-reactivity is lacking in the literature. A list of some well-known legume allergens were listed below. (Table.1)

Table.1. Some well-known Legume allergens list

Sl. no	Legume source	Legume allergen	Molecular weight	Protein family	Protein super-family	References
1	Arachis hypogea	Ara h1	64	7S globulin- vicilin	Cupin	[41]
	Peanut	Ara h2	17	2S albumin- conglutin	Prolamin	[42]
		Ara h3	60	11S globulin- legumins	Cupin	[43]
		Ara h4	37	11S globulin- legumins	Cupin	[44]
		Ara h5	15	Bet v2 –Profilin	Profilin	[45]
		Ara h6	15	2S albumin- conglutin	Prolamin	[46]
		Ara h7	15	2S albumin- conglutin	Prolamin	[44]
		Ara h8	17	Bet v1-family	PR-proteins	[47]
		Ara h9	9.8	Non-specific lipid transfer protein1	Prolamin	[48]
		Ara h10	16	Oleosin- lipid storage bodies	Oleosin	[49]
		Ara h11	14	Oleosin- lipid storage bodies	Oleosin	[50]
2	Glycine max	Gly m1	7	Non-specific lipid transfer protein	Prolamin	[51]
	Soybean	Gly m2	8	Defensin	Scorpion toxin-Knottin	[52]
		Gly m3	14	Profilin	Profilin	[53]
		Gly m4	17	Bet v1-family	Bet v1	[54]
		Gly m Bd28k	28	7S globulin- vicilin	Cupin	[55]
		Gly m Bd30k	34	Thiol protease- papain	Cupin	[55]
		Gly m Lectin	14.5	Lectin-SBA agglutinin	Prolamin	[47]
		Gly m Bd60k	63-67	7S globulin- vicilin	Cupin	[56]

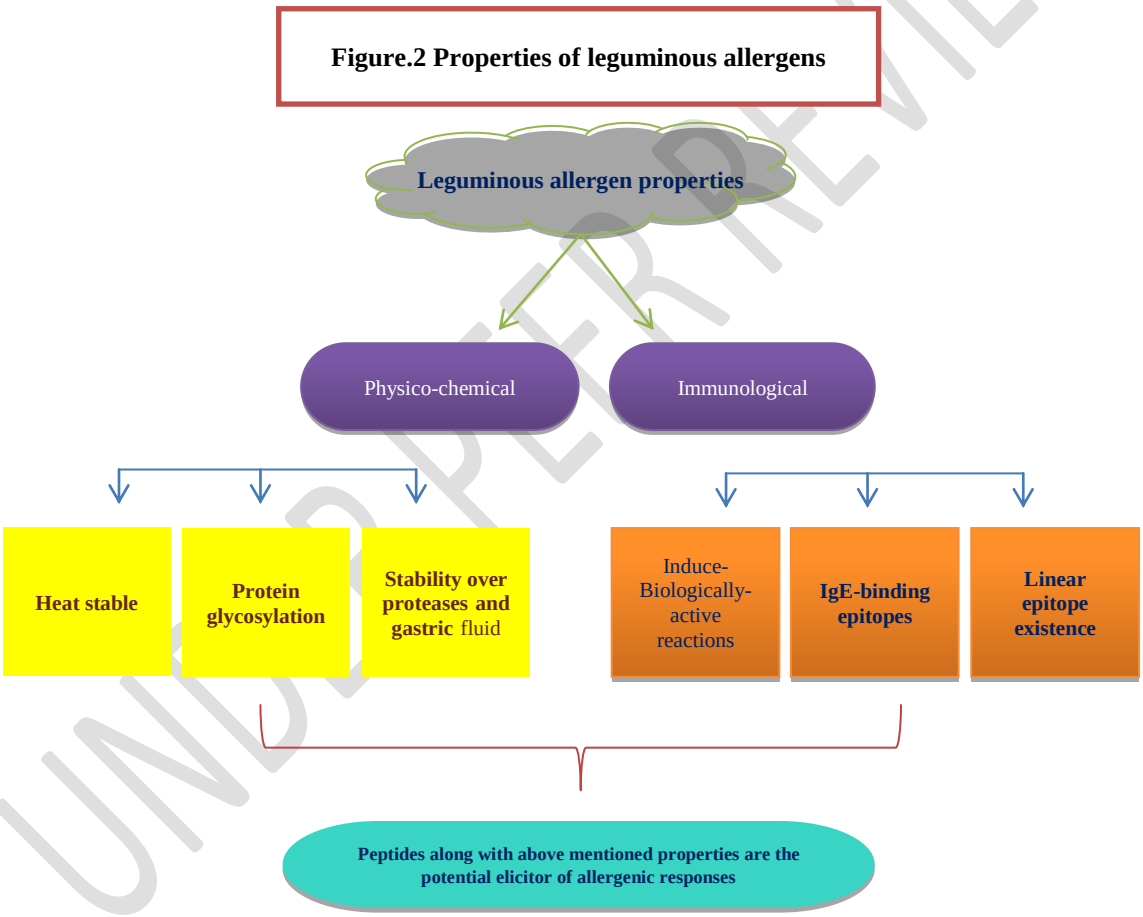
		Gly m glycinin G1	40	11S seed storage protein- Glycinin	Glyinin	[57]
		Gly m glycinin G2	22	11S seed storage protein- Glycinin	Glyinin	[57]
		Gly m glycinin G4	61.2	11S seed storage protein- Glycinin	Glyinin	[57]
		Gly m T1	20	Kunitz- trypsin inhibitor	-	[50]
3	Pisum sativum	Pis s1	44	7S globulin- vicilin	Cupin	[58]
	Pea	Pis s2	63	7S globulin- vicilin	Cupin	[58]
4	Cicer arietinum	Cic a 2S Albumin	10-12	2S albumin-Albumin prolamin	Prolamin	[59]
	Chickpea	Cic an 11S Globulin	46.5 and 39.8	11S Glycinin-legumin type	Cupin	[60]
5	Lens culinaris	Len c1	48	Vicilin- Gamma subunit	Cupin	[61]
6	Lentil	Len c2	66	Biotinylated protein- seed specific	-	[61]
		Len c3	9	Non-specific lipid transfer protein1	Prolamin	[62]
7	Lupinus angustifolius	Lup an1	55-61	Beta conglutin-vicilin	Cupin	[63]
	Lupin	Lup a vicilin	66	Vicilin	Cupin	[64]
		Lup a 11S globulin	20	11S globulin	Cupin	[65]
8	Phaseolus vulgaris	pha v3	8.8-9	Non-specific lipid transfer protein1	-	[66]
	Green bean	pha v3		Non-specific lipid transfer protein	Prolamin	[67]

			8.8-9			
9	Blackgram	Vig m	28	Homology- Rho-specific inhibitor	-	[68]

5.0. Leguminous Allergen properties:

Not one single characteristic alone is sufficient to recognize a certain protein as allergenic. Especial characteristics like physical-chemical and immunological properties to identify allergenic proteins which are capable of eliciting allergenic responses in Atopic people. Based on the sensitization pattern IgE-mediated food allergies are classified into two categories namely A) Class-1 food allergens: Stable in gastric fluid (sensitization occurs via gastrointestinal tract). [17] B) Class-2 food allergens: Heat sensitive and suspective to gastric digestion (sensitization does not exist via oral tract). They promote allergenic responses utilizing IgE-cross-reactivity in already sensitized people. For examples inhalant allergens like pollen-associated food allergies. [18] Physical-chemical and immunological properties of allergen based on the characteristic of abundance, stability, tertiary structure, modification, and glycosylation characteristics of the protein are essential to decide a protein as an allergen. Most of the allergens are glycoproteins of molecular weight 10-70 kDa, with greater stability over acid, heat as well as proteases treatment, and water-soluble. The majority of food allergens are stable to pepsin digestion (8-60 min) and survive in the gastrointestinal tract but the non -allergenic food proteins get easily digested in the gastrointestinal tract. [19] Protein glycosylation is an additional criterion in the enhancement of allergenicity of a protein, one such example- is major allergens from the legumes family namely Ara h1, Gly m Bd28K and Gly m Bd60K are extremely glycosylated. Previous reports further indicated that glycan moiety can also induce allergenicity by cross-linking with IgE. Immunological characteristics not only incorporate the binding of IgE but also include awareness

about B and T-cell epitopes. Epitopes with greater IgE-binding ability on allergens are accountable for provoking allergenic reactions. These IgE cross-linking epitopes occur in two forms namely linear or conformational form. During the digestion of the legume family, leguminous allergens lose their conformational form and appear as linear epitopes and sensitize individuals in the gastrointestinal tract. [20] Some of the physicochemical and immunological properties of allergens were shown. (Figure.2)



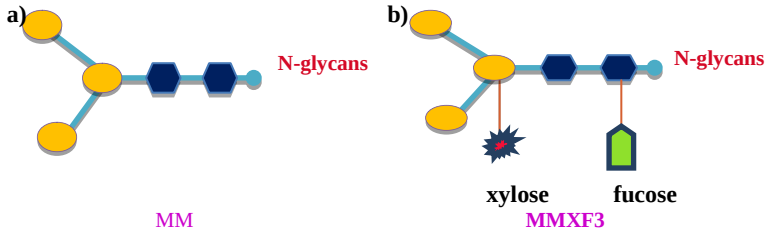
6.0. CCD cross-reactive antigen determinant

The N-linked carbohydrate groups of glycoproteins induce IgE, leading to cross-reactivity between several foods and pollen allergens. [21] These are called cross-reactive carbohydrate

determinants (CCDs) which are present in glycoproteins of vegetables such as peanut, celery, tomato, and potato. IgE binding to CCDs has been described in the literature for various allergenic extracts and there are several research studies have also been performed to understand and verify the presence, as well as the biological function of the CCD-IgE. [22] "Glycosylation has been described for many allergenic and non-allergenic molecules. The glycan structures have been investigated and a heterogeneous IgE reactivity has been reported". [23] "*In-vitro* demonstration of the glycan allergenic activity has been reported only for a native glycoprotein derivative and for synthetic neo-glycoproteins, whereas no in vivo activity of any purified allergenic glycoproteins has ever been reported. From an allergological perspective, the most important feature of CCD is the wide distribution among plant-derived proteins, though similar structures can also be present in non-plant glycoproteins. Monoclonal and polyclonal antibodies to CCD have been used to define reactive carbohydrate epitope distribution, whereas limited data are available on the prevalence and distribution of IgE reactive with CCD". [24] Some of the well-evidenced CCD-glycoprotein epitopes are short-listed from plants, insects, parasites and non-Human mammalian oligosaccharides are MMXF3, MUXF3, MMF3, MMF3F6, MMXF3F6, and alpha-gal. (Shown in Figure.3) MMXF3 (MM- indicates the presence of tri-mannose residues)-Horse radish peroxidase -HRP model glycoprotein frequently employed for the study of carbohydrate-based IgE cross-reactivity, MUXF3- (MU-indicates deletion of one Mannose residue) Bromelain commonly used research glycoproteins, these are equally common in pollens, plant foods and rubber latex allergens, MMF3F6, MMF3, MUF3F6(indicates deletion of xylose and addition α 1,6-fucose residue)-yellow jacket venom (Ves v2) [25] honey bee, vespid and Hymenoptera venom [26] MMXF3F6 (indicates the addition of α 1,6-fucose residue along with xylose and fucose) - Parasite-Schistome and Schistome soluble Egg Antigen (SEA), Alpha-gal-Non-human mammalian oligosaccharides. These are randomly picked up to rule out the leading task of α-1,3 fucose residue in the target of IgE antibody in allergenicity. [27] Preliminary researchers disclosed Xylose as a core element; ulteriorly studies on xylose summarized no IgE or intensively less antibody binding competence observed. [28] These days α-1, 3 fucoses surveyed all-important for IgE-reactivity at the standpoint of allergy. Structural comparison of plant, insect, parasite, and non-human mammalian glycoproteins was mentioned. (Figure.3)

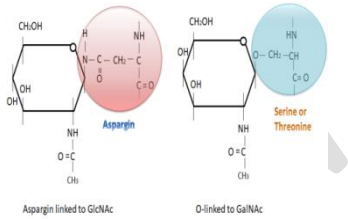
Figure.3 some of well published CCD structures from different sources

A: Capital Nub of N-glycans



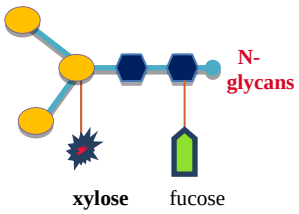
MM MMXF3

B: Core structures of N-linkage and O-linkage



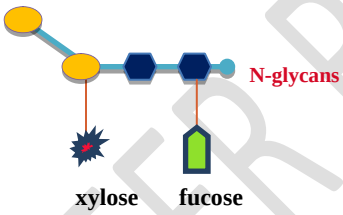
C: Some of Well-known structures of Oligosaccharides that are accepted as Targets of IgE :

a) Horse radish peroxidase (HRP)



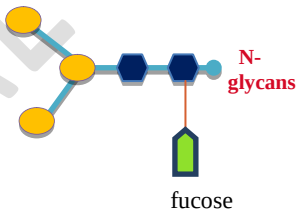
MMXF3

b) Bromelain (Brom)



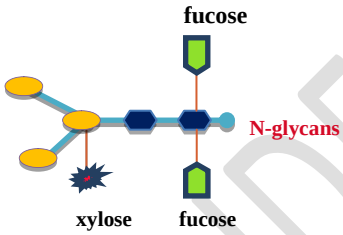
MUXF3

c) Bee Venom



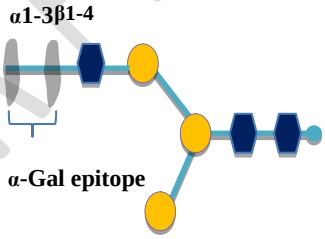
MMF3

c) Schistomes and Schistome soluble egg Antigen (SEA)



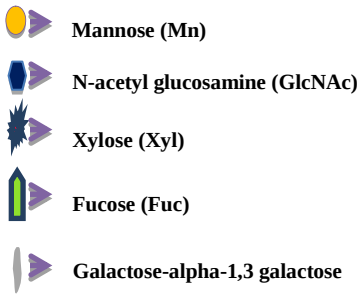
MMXF3F6

d) Non-Human Mammalian oligosaccharides



Alpha-Gal

D: Monosaccharides present in plant and human Oligosaccharides



7.0. CCD-IgE and clinical allergy

"The clinical relevance of IgE to cross-reactive carbohydrate determinants (CCDs) is still controversial, because of the widespread occurrence of structurally similar CCDs in plants. IgE to carbohydrates often leads to false positive diagnostic results in CAP-RAST, and immunoblot analysis, when only IgE binding to extracts or allergens concerning the initiated symptoms is considered".[7] "Many glycoproteins like bromelain, which carry only one IgE-binding glycan, are unable to cross-link IgE-bound receptors of mast cells and basophils, and clinical symptoms might not appear in patients whose IgE response is restricted to CCDs". [15] based on this view several authors regard anti-CCD IgE as specific antibodies without clinical relevance. However there are also many glycoproteins with more than one N-linked glycan, for example; HRP, beta fructofuranoside, polygalacturonase, and pectin esterase are potential plant allergens and have got the capability of cross-linking adjacent IgE, on mast cells and basophils resulting in clinical symptoms. [29] Until now, studies to prove whether natural multivalent glycoproteins might be able to induce histamine release or not and thus might also contribute to clinical symptoms of allergic patients are very rare. Most likely due to difficulties in the experimental design, the lack of suitable purified multivalent allergenic glycoproteins failed to report their effective role in inducing false positive results. Many reports describe the wide "CCD-IgE binding to clinically irrelevant allergenic extracts and the defined degree of interference of CCD-IgE in the detection. The presence of CCD-IgE directly correlates with a positive assay for several different allergenic extracts that are unable to trigger an allergic reaction in the skin prick test". [8] However some studies demonstrated that CCDs are the essential part of IgE epitope in some glycoproteins and show that glycoproteins carrying multiple glycan units can be biologically active in patients sensitized to CCD. [30] "Plant-derived glycoproteins are indeed the most reacting ones, but the

higher the concentration of CCD-IgE is more probable the chance to detect IgE for any SPT-negative allergenic extract. Moreover, the differences recorded in the sera reactivity, and the presence of sera with CCD-IgE but not reacting with certain extracts, suggest a possible heterogeneity of the CCD-IgE. The use of purified allergenic glycoproteins in comparative studies would certainly increase the information on the specificities of CCD-IgE. From an immunochemical point of view, extended inhibition studies are needed to confirm the statistical correlation of CCD-IgE reactivity". [31]

8.0. CCD glycan epitopes and IgE binding:

"The asparagine-linked carbohydrate moieties of plant glycoproteins are the most abundant environmental immune determinants. They are the structural basis of CCDs despite some structural variation". [32] "Two main motifs are xylose and the core 3-linked fucose, which form the essential part of two independent epitopes. Plants contain both epitopes whereas insect glycoproteins contain only fucose [33] about 20% or more of allergic patients generate specific anti-glycan IgE, which is often accompanied by IgG". The clinical relevance of IgE specific for glycan (CCD) has been a matter of controversy. Until now, no convincing experiments have been performed to test the biological significance of individual multivalent allergens that carry multiple carbohydrate epitopes. [7] We sought to contribute to understanding the role of CCD-specific IgE antibodies and to study whether CCD-specific IgE antibodies can activate basophils using different multivalent glycoproteins as allergens. Most glycoproteins in legumes are known to present in multimeric forms and if each subunit contains a single glycan chain, and as a multimer, it provides more than one interacting glycan chain on IgE recognition which can

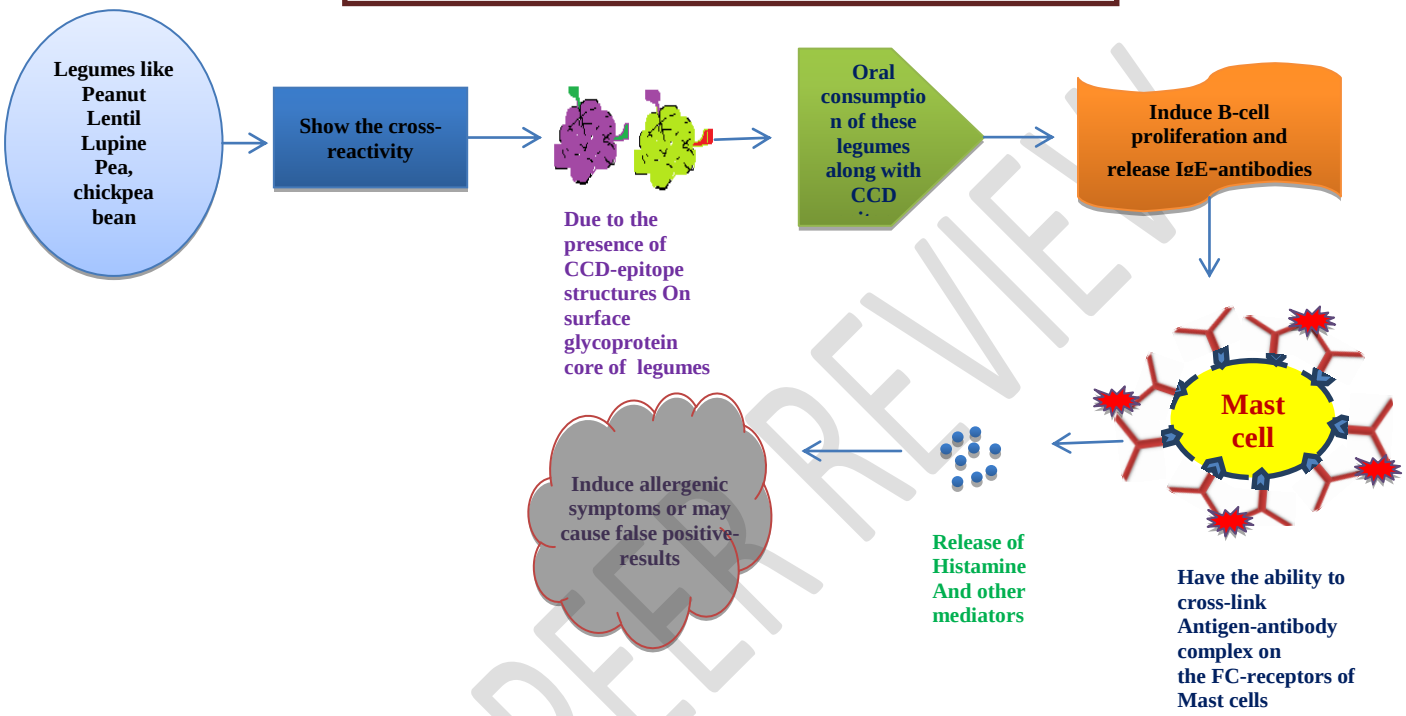
facilitate the trigger of degranulation. So the addressing of CCD-specific IgE against legume glycoproteins is important to predict the possible cross-reactivity among legume species.

9.0. Legume allergenic cross-reactivity, CCD-IgE, and its clinical relevance

Legumes are the major protein diet of the Indian subcontinent. All parts of India consume high amounts of legumes through various types of foods. Allergic reactions to legumes were reported in different regions of India. Recently some studies identify a few allergens from major legume species.[34] "Though there is a large number of clinical reports of avoidance of legumes by a sensitized population a thorough study on legume allergy is lacking in India. Significant cross-reactivity among legumes is natural because of the presence of structurally homologous proteins that share common epitopes. This has been demonstrated and observed to have pathological symptoms". [35] "In the majority of cases, patients were found to be sensitive to more than one legume. Previous reports have demonstrated a great degree of immunological cross-reactivity among legumes and reported that the prediction of immunological cross-reactivity should not be based only on allergen-specific IgE antibody determination". [34] However, "some reports have been published on cross-reactivity between certain members of the Leguminosae family due to N-glycosylation. Peanut allergy can also be associated with an allergy to lentils, chickpea, and peas, but is less frequently reported. Lentil, chickpea, and pea are widely consumed in the Mediterranean area and significant cross-reactivity exists among them whereas the low clinical significance of legume cross-reactions is reported in earlier reports. A glycan-related IgE reactivity has been demonstrated in most allergen sources, especially in the plant kingdom. Recent progress in glycobiology has allowed a clearer classification of these glycon-epitopes. Unlike classical peptide chain-based epitopes, glyco-epitopes can share significant structural

homologies beyond the limits of protein families. These glycoepitopes are thus prone to extensive cross reactivity". [36,69] So, accurate measurement and specificity assessment of CCD-specific IgE is a crucial and critical factor to understand the allergenic cross-reactivity and clinical sensitivity mediated by CCD-IgE. The work proposed in this study will be useful to evaluate and assess the possible involvement of CCD-IgE in cross-reactivity and clinical sensitivity as they are present in the novel and recombinant glycoproteins. We claim that several studies have demonstrated and proven the presence of specific IgE in the glycan portion of many dietary glycoproteins. Their clinical relevance is not fully understood yet and not many reports in the literature show their possible involvement in clinical cross-reactivity except in a few preliminary reports. Accurate measurement and specificity assessment of CCD-specific IgE is a crucial and critical factor to understand the allergenic cross-reactivity and clinical sensitivity mediated by CCD-IgE. In India, the studies relevant to food allergy were very limited and most focus has been given to pollen and dust allergy compared to food-induced hypersensitivity. Presently few groups are working on the characterization of legume allergy and few well-established reports were published with *in-vitro* legume cross-reactivity with very low clinical relevance. Many new legume allergens were characterized and reported which were different from the western world. There is variation in the sensitization and tolerance among the Indian population to the legume diet which is earmarked with a history of diet and genetic adaptations. The current review aims in understanding the CCD IgE in clinical relevancy which is more responsible for observed in vitro IgE cross-reactivity among similar legume allergens. (Figure.4) shows the overview of allergy occurrence to legume CCD factors.

Figure.4 Legume CCD cross-reactivity and Allergy:



10. Allergenic risk associated with CCD-IgE

The evaluation of allergenic potential is a key parameter in the safety assessment of novel proteins, including those expressed and used as functional proteins. The majority of allergic reactions to food proteins are immediate type food hypersensitivity reactions in which the principal biological effector is the IgE antibody. [37] The presence of IgE to CCD is well known in many plant extracts and are known to be common in the allergic population and binding to the skin-prick test negative allergenic extracts further state its poor biological activity and clinical

sensitivity. [38] CCD-IgE can be recorded for a wide range of allergenic unrelated extracts. Allergenic sera to be used in any *in vitro* assay employing native or recombinant glycoproteins should be carefully evaluated for the presence of CCD-IgE to assess its in vivo allergenic potential and cross reactivity. The accurate measurement and specificity assessment of CCD-specific IgE is a crucial and critical factor to understand the allergenic cross-reactivity and clinical sensitivity mediated by CCD-IgE. [39] So it induced degranulation in vitro. This method is also useful to evaluate and assess the possible involvement of CCD-IgE in cross-reactivity and clinical sensitivity as they are present in the novel and recombinant glycoproteins. The rat basophilic leukemia (RBL) cells expressed with human FcεRI which is selectively bind to human serum IgE antibodies and can be stimulated by cross-linking with protein allergens to degranulate, releasing granules containing various mediators. This method has been utilized extensively to study the mechanisms of IgE interactions with mast cell FcεR and for the measurement of degranulation-induced allergenic proteins mediated by IgE antibodies. The present proposal stands on the prediction of the possibility of IgE to cross-reactive carbohydrate epitope and assessment of their specificity concerning sensitivity resulting in clinical symptoms. Most glycoproteins showed IgE elicitation to its glycan portion was reported to have poor biological activity but still gives false prediction in allergic assessment using in vitro diagnostic methods. [40] The study of specificity and the design of evaluation methods to predict the CCD-specific IgE are essential. On the other hand, the question of its clinical relevance in terms of its ability to elicit symptoms and cross-linking of IgE on mast cells to induce degranulation. The present proposal is focused on the detailed study of CCD-IgE specificity to legume glycoproteins and prediction and assessment of the possible cross-reactivity among legumes due to CCD-IgE

and evaluation of the clinical significance of CCD-IgE by designing some *in vitro and in vivo* assessment methods should be incorporated.

10.1. Conclusion:

We claim that several studies have demonstrated and proven the presence of specific IgE in the glycan portion of many dietary glycoproteins. Their clinical relevance is not fully understood yet and not many reports were in the literature to show their possible involvement in clinical cross-reactivity except in a few preliminary reports. Accurate measurement and specificity assessment of CCD-specific IgE is a crucial and critical factor to understand the allergenic cross-reactivity and clinical sensitivity mediated by CCD-IgE. So the work proposed in this study will be useful to understand, evaluate and assess the possible involvement of CCD-IgE in cross-reactivity and clinical sensitivity as they are present in the novel and recombinant glycoproteins. The use of basophilic degranulation study using rat basophilic leukemia (RBL) cells will set a standard method to assess the possible clinical cross-reactivity among the closely related allergenic glycoproteins containing CCD-specific IgE in a sensitized population. This proposal also aims to elucidation of the possible molecular mechanism of CCD-induced degranulation of basophils/mast cells and also evaluates the potential of CCD-specific IgE to induce clinical symptoms as a function of sensitization to CCD and recognition of its cross-reactive glycoproteins of related families.

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