International Journal of Biotechnology and Biochemistry
ISSN 0973-2691 Volume 5 Number 3 (2009) pp. 189-196
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https://dx.doi.org/10.37622/IJBB/5.3.2009.189-196

Immunological Characterization of Immunoglobulin G towards House Dust Allergens

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Abstract

Immunoglobulins are γ- globulin proteins secreted by B cells that are found in blood or other bodily fluids of vertebrates which have the basic structural units each with two large heavy chains and two small light chains. Five different immunoglobulin isotopes are known in mammals, which perform different roles, and help to direct the appropriate immune response. Dust mites and pollen which are common in house hold dust and industrial dust has the main role in asthmatic allergy. Majority of severe allergic reactions are thought to be immunological and mediated via IgE but high serum concentrations of some IgG subtypes have been measured which are associated with in vitro degranulation of basophils and mast cells, the activation of the complement cascade. The premise behind this testing is that high circulating levels of IgG antibodies are correlated with allergy due to house hold allergens.

Key words: IgG, Allergens, house dust, Ag-Ab interaction.

Introduction

Household dust [3, 6, 15, 24] is a general name for minute solid particles with diameters less than 200-500 micrometers. The allergic response to household dust [7, 15] is due to the presence of high amount of dust mite and pollen [7, 17, 18, 23]. House dust mites (HDM) [24, 25] are microscope bugs that primarily feed on organic detritus. They can cause allergic reactions [18] in asthmatics and others who are allergic to their feces. Dust mites can be transported airborne [18] by minor air currents generated from normal household activities.

Exposure to such allergens, can initiate an acute immune response in allergen-sensitive individuals that leads to airway inflammation [6] and a chronic respiratory

disorder characterized by the production of IgE [1, 17-21] antibodies. The immediate response is the early in which mast cells and basophils undergo degranulation to release histamine, and cysteinyl leukotrienes. These mediators cause smooth muscle contraction and bronchial constriction which are manifested by a shortness of breath, wheezing, and coughing.

The late phase reaction occurs several hours after the initial reaction and is characterized by excessive inflammation, infiltration of the airway by eosinophils and other cytokine-secreting leukocytes, and structural changes that lead to airway remodeling.

The recognition and processing of allergens by dendritic cells which drive naive T cells to differentiate into T helper type 2 cells (Th2). Th2 lineage commitment is established by STAT6-dependent expression of GATA-3 which induces the expression of Th2 cytokines, including IL (3, 4, 5, 9, and 13) and GM-CSF. Together these cytokines direct the inflammatory response to allergens [6]. The hallmark Th2 cytokine, IL-4 promotes clonal expansion and, along with IL-13 and specifc co-stimulatory molecules, induces B cells to produce allergen-specifc IgE antibodies. This antibodies bind to the Fce RI high affinity receptors found on mast cells, basophils, neutrophils, and eosinophils. Upon allergen re-exposure, allergen binding to the IgE-Fce RI complexes on mast cells and basophils leads to receptor cross-linking which triggers the release of mediators that cause immediate hypersensitivity.

IgE[19, 20] antibody to House hold dust (HHD)[3, 14] allergens induces early allergen specific mast cell degranulation and contributes to the late-phase reactions by chronic tissue damage via the down stream effect of mast cell mediators and by facilitating allergen presentation to T cells.

IgG [1, 2, 5, 13, 15, 17, 19- 21] antibody is almost exclusively produced by subjects with allergy. IgG [5, 13, 14, 17, 20] could therefore be considered to be a marker of allergenicity but conceivably could also regulate allergic responses by blocking mast cell degranulation or IgE [16, 20] antibody–facilitated allergen presentation [12, 18]. In contrast the major cat and mouse allergens induce IgG4 [2, 5, 15, 19, 22] in the absence of IgE in subjects without allergy in what has been proposed as High dose immune deviation, a phenomenon that could mediate or be a marker of protection from disease.

Since the prevalence of IgG [1, 5, 13, 17, 19, 20] in blood is more than that of IgE [16, 17]. In this work the IgG have been measured to investigate whether they are related to allergenicity and the possibility that minor allergens induce deviated responses.

Material and Methods

Isolation, purification and immunological test were performed through the kit provided by the GeNei^{TM} Bangalore. The assay buffer was diluted to 1X for every test.

Isolation and purification of immunoglobulin from blood

5 ml blood was taken with the help of syringe using venipunture from the body and it was subjected to cold centrifuged at 10,000 rpm for 15 min. The upper serum layer

was taken for the further use discarding the settle RBC and other components. The obtained serum was subjected to column chromatography for the purification of IgG. The isolated IgG was analyzed using the SDS-PAGE.

Collection of dust

The dust samples from two different sources were collected one from the corridor where the dust from environment has main effect and another from the laboratory in college where the dust came from the internal works which have relatively low environmental effect. The collected dust sample was made to varying concentration's using distilled water. From a stock solution of 1mg/ml varying concentrations of 150, 125, 100, 75 and 50 µg/ml are prepared. These varying concentrations are checked for antigenicity by various antigens – antibody reactions.

Test for antigen-antibody interaction

Different immunological test were performed for the test of antigen antibody interaction. These entire tests were done by following the standard protocol.

Quantitative precipitin assay

Quantitative precipitin assay (QPA**)** [8, 9, 10, 11] is based on the interaction of antibody and antigen to form a large protein complex that will result in precipitation.

In our test the antigen of different concentration was taken (50, 75, 100, 125 and 150) µg/ml in different test tubes with the equal concentration of sample (100 µl). These are allowed sufficient time for reaction then centrifuged for 15 min. in cooling centrifuge. The supernatant was removed without disturbing the pellet and process repeated for second time with the addition of 1X assay buffer. Finally the pellet obtained was dissolved in 1ml of 1X NaOH. The solution was read spectrophotometrically at 280nm (table-1).

Protein content in the sample was calculated and the graph was drawn between the protein content vs. antigen concentration (Fig-1). From graph tube with maximum precipitate was 178.57 µg and the amount of antigen added was 100 µg.

Radial immuno diffusion

Radial immunodiffusion (RID) [8, 9, 10, 11] is used extensively for the quantitative estimation of antigens. Ag is allowed to diffuse from wells cut in the gel in which the antiserum is uniformly distributed and the formed ring is measure to calculate the unknown concentration.

120 µl of antiserum was added to 6 ml of agarose solution and mixed with gentle swirling for uniform distribution of antibody and after solidification the wells were prepared. 20µl of standard antigen and test antigen was added to the wells. The gel plate was kept in a moist chamber (box containing wet cotton) and incubated for 18

hours at room temperature. The diameter of the ring formed due to antigen antibody reaction was measured (table-2). Graph was plotted between the diameters of ring vs concentration of antigen (fig-2).

The concentration of test antigen was 138µg/ml.

Rocket immunoelectrophoresis

Rocket Immunoelectrophoresis (RIEP) [8, 9, 10, 11] also known as electro-immuno diffusion is a simple, quick and reproducible method for determining the concentration of Ag in an unknown sample**.**

In our test, 1ml of antiserum was added to 6 ml of agarose solution and mixed uniformly and allowed for solidification. After solidification the 3mm wells were prepared. 10 µl of test and standard Ag was added to the wells along with Ezee blue dye and electrophoresis was started. After 30 min the electrophoresis was stopped and the height of the rocket was measured (table-3). Graph was plotted against the height of the rocket vs antigen concentration (fig.3).

Concentration of antigen in test sample 98µg/ml.

Immunoelectrophoresis

Antigen is loaded in trough to get dispersed in the gel by electro diffusion process and in prepared wells antiserum of IgG and total serum is added for diffusion which shows a difference in the zone of precipitation. This indicates the presence of IgG towards HDD.

Enzyme-Linked Immunosorbent Assay

ELISA (Enzyme-Linked Immunosorbent Assay) [24-25] has been used both qualitatively and quantitatively to measure antigen-antibody binding. Depending on what variation we use, it will detect antigen or antibody in body fluids. Being very sensitive we used ELISA to compare the results as well as to verify the reaction between IgG and HDD. From this test we concluded that 125µl and 150 µl of antigen concentration showed the positive result, 75 µl and 100 µl showed the moderately positive result but the 50 µl of antigen concentration shows no effect towards antiserum table(4).

Discussion

The allergic response towards HHD [3] also provided by the IgG [14, 20] along with IgE [20]. The initial response may be given by the IgE [4, 7] even the very low concentration of HHD but during the high concentration HHD IgG [4, 15] play a very important role. It has been shown that high level of HHD present in allergic response towards human. In our study we found that when the concentration of HHD is high (100, 138, 98) mg/ml, IgG response was observed**.**

Table 1: Quantitative precipitin assay.

Antigen in (µg)	O.D (A_{280})	Protein in (µg)
50	0.09	64.28
75	0.18	128.57
100	0.25	178.57
125	0.10	71.42
150	0.08	57.14

Table 2: Radial immunodiffusion.

Concentration (µg/ml)	Diameter of ring (mm)
50	0.5
75	1
100	7
125	12
150	17

Table 3: Rocket immunoelectrophoresis.

Antigen concentration (in mg/ml)	Rocket height (in mm)
50	1.6
75	3.9
100	5.3
125	7.4

Table 4: Reproducibility of allergen reactions by ELISA**.**

S.No	Conc. of the allergen (µl)	Antiserum coated (µl)	Number of times	Result	Remarks
1	50	50	3	0.275 ± 0.3	Negative
2	75	50	2	0.355 ± 0.5	Moderately Positive
3	100	50	5	0.456 ± 0.5	Moderately Positive
4	125	50	7	0.550 ± 0.5	Positive
5	150	50	7	0.502 ± 0.3	Positive

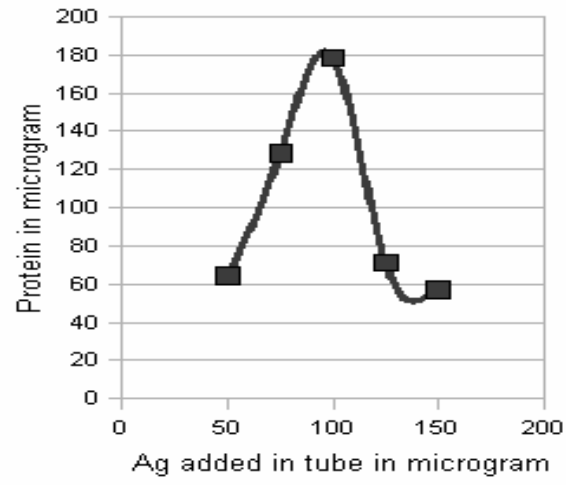

Figure 1: Quantitative precipitin assay.

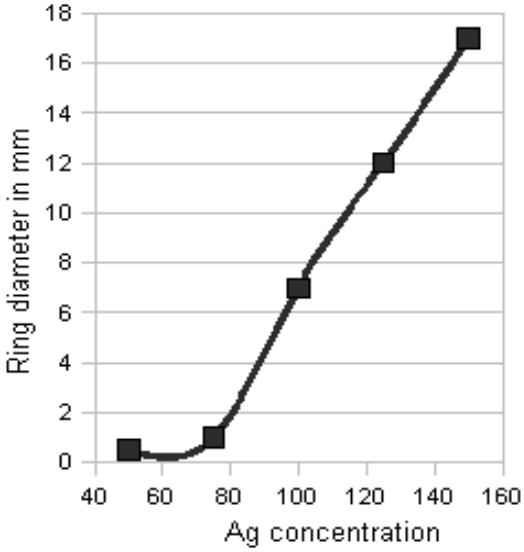

Figure 2: Radial immunodiffusion.

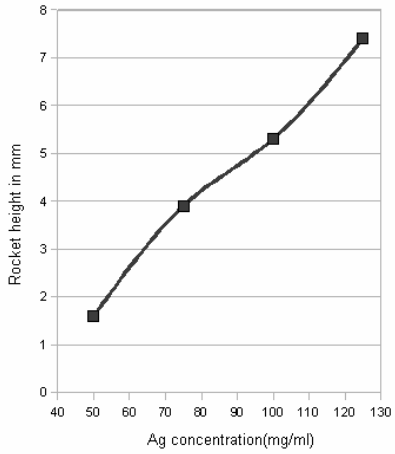

Figure 3: Rocket immunoassay.

Conclusion

The expression of allergens is becoming new insights of an important diagnosis and the therapy of allergies as well as molecular approaches to immunological and structural studies of allergens [12]. Mite allergens in the house dust which causes hypersensitivity reactions mainly in children [15], an immunoglobulin responsible for triggering immediate response has to be analyzed, although previous studies conformed that IgE has house dust mite allergen reactivity. The idea behind to work on IgG [2, 4, 22] is its concentration levels in serum and its isoforms. The antigenicity and allergenicity of IgG [14, 15, 20] towards house dust mite [3, 14] were almost same as that of IgE [16, 17, 20] with reactions. The expression of an enzymatically inactive and highly antigenic molecule IgG could be a suitable strategy for the development of vaccines as well as for specific immunotherapy.

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