Chapter-1

INTRODUCTION

1. Introduction

Allergenic rhinitis or hayfever is a kind of allergy which has been found to affect a large number of people (Fireman, 2002). More than 10- 30% of the people in Western countries suffer from hay fever (Dykewicz and Hamilos, 2010). It becomes the most common allergenic diseases affecting approximately 400 million of people and is associated with "a reduced quality of life of the patients, affecting productivity and performance, involving a lot of investment in medical treatment" (Bousquet et al., 1994; Cockburn et al., 1999; Malone et al., 1997; Pawankar, 2009; Strachan et al., 1997). According to Wheatley and Togias (2015), hay fever is largely prevalent in people in their twenties and forties. It is triggered by allergens such as pollen, mould, fungal spores, dust, mites, pet hair, etc which are a part of the environment constituents. They induce our immune system to overreact to these allergens in the air resulting in a type of inflammation in the nose. Inherited genetics and environmental exposures are the two main factors which induce the symptoms of these allergies.

1.1 Role of pollen in allergenic rhinitis

Pollen's role in the etiology of nasobronchial allergy is now very well established. Unlike other aeroallergens, "the global distribution of pollen" from several varieties of grasses, weeds, herbs, shrubs and trees, preclude any realistic possibility for pollen-allergic individuals to avoid these aeroallergens. Previously, all the various clinical manifestations caused by the pollen or other bio aerosols were considered as separate entities by physicians until in 1966, when Ishizaka discovered the immunoglobulin E (IgE) and established it to be a common link responsible for causing all these conditions and thus opened a new perspective in immunological research and the study of allergy got onto a more sound footing. About 20-30% of the general population are atopic i.e. genetically predisposed individuals having increased level of IgE antibodies. In normal individuals, IgE comprises lower than 0.001% of total immunoglobulins while in patients with respiratory allergic diseases it has been found that 25-50% of the whole IgE is made up by pollen-specific IgE (Chanda, 1996). The discovery regarding the increased level of IgE has led to an eruptive increase in our knowledge about the generation of IgE and its responsibility in allergic disorders by operating eosinophil, basophil and mast cell mediated inflammatory responses. The most important and significant inflammatory responses are the generation of chemokines, cytokines, immunological adhesion molecules associated with the Th2

profile, the mast cell degranulation along with the signaling of a variety of newly generated preformed mediators and the activation and special recruitment of secondary effector cells particularly basophils and eosinophils.

Pollen proteins are water soluble possessing molecular weights within the range of 5,000-70,000 Daltons (Mathiesen et al., 1991). Immediate manifestation of allergic symptoms suggests that the allergens are readily available on the surface or subsurface of the wall of pollen grains and diffuse out quickly on coming in contact with the moist eye surface and upper respiratory tract (Chanda, 1996). Once the pollen enters the noses or eyes and lands on mucous membrane tissue of the upper or lower respiratory tracts, the mucous liquid solubilizes the pollen allergens which penetrate the mucous tissue. An allergen stimulates the synthesis of IgE antibodies by the plasma cells and lymphocytes. When an allergen induces the synthesis of IgE antibodies, these antibodies possess a strong propensity to adhere to mast cells, which are at that time present in the mucous tissue and basophils. IgE antibodies bind to cell surface receptor with the help of the Fc components of their heavy chains. Upon re-exposed to the specific allergen, the mast cell or basophil equipped with cytophilic IgE antibodies releases a extended variety of vasoactive mediators and a series of powerful reactions is being taken place. The first reaction in this series includes the binding of the antigen (allergen) - antibody molecule (IgE antibodies of previously attached mast cell). In this process, adjacent IgE antibodies crosslink with each other as antibodies bound to them are multivalent in nature inducing the crosslinking reactions. This crosslinking leads to perturbations of the IgE- Fc receptors and initiates two independently occurring parallel reactions. One is mast cell degranulation. During this degranulation process, mast cells release preformed primary mediators like histamines, ECF-1, eosinophils and a distinct neutrophil chemotactic factor and the other reaction, involving the synthesis and release of leukotrienes, a secondary mediators (Robbins et al., 1984). The primary mediators like histamines induce the allergic symptoms, i.e. contraction of bronchial and nasal muscles, endema of mucous membrane, constriction of nasal or bronchial passages, dilation of blood capillaries and hypersecretion of nasal fluids (Stanley and Linskens, 1974). The T-cells and leukotrienes in term control the production of IgE antibodies by the T-lymphocytes (Bapat and Bapat, 1994). These reactions together constitute Type 1 hypersensitivity reaction, which is mediated by the IgE antibodies.

1.2 Symptoms

Allergic rhinitis and common cold both have very similar symptoms and are manifested within minutes after successive exposure to the allergens, affecting sleep or working ability in the patient. The allergic rhinitis has characteristically important manifestations such as nasal blockage and obstruction, rhinorrhea (excess nasal secretion), itching, sneezing fits (Sur and Scandale, 2010). Characteristic physical symptoms include conjunctival swelling and erythema, venous stasis of the lower eyelids or "allergic shiners") and swelling, swollen nasal concha, and building up of fluids in middle ear (Valet and Fahrenholz, 2009). However, the symptoms differ from common cold in that they do not include a fever and often be prolonged for more than two weeks. In case of allergic diseases induced by pollens, the symptoms are manifested during particular time of the year. According to Wheatley and Togias (2015), patients with allergic rhinitis often have symptoms of asthma, allergic conjunctivitis, or atopic dermatitis. The manifestation of allergic rhinitis is noted right from early childhood with the symptoms aggravating in the upcoming stages of life up to the age of forty but a late onset of this disease is also noted in infancy or in adults later on. It has been observed that multiple members of the family are often affected like other atopic diseases. Although this has been attributed to genetic determinants of specific allergen sensitization and total IgE levels but neither the atopic disorders nor specific allergen sensitivities are considered as simple heritable genetic traits. There is also increasing evidence supporting the fact that the impact of prenatal and early life exposures is also a reason for the expansion of allergic diseases, which has been found to affect the gene regulation and expression causing a permanent effect through epigenetic regulation (North and Ellis, 2011).

Clinical symptoms of pollen allergy generally include the irritation of respiratory tract particularly the upper part of the tract due to the large size of pollen grains which prevent them from penetrating deep into the respiratory system. However, occasionally lower parts of the lungs are also affected resulting in asthmatic reaction which is caused by sub-micronic size particles and diffused pollen proteins (Mygind, 1986). According to the current definition, seasonal rhinitis is spotted when a patient has four basic complaints including nasal itch, sneezing-particularly more than five times in a series, typically abundant watery or mucous rhinorrhea and periodic nasal blockage (Buczylko and Wnuk, 1979; Lund, 1994; Obtulowicz, 1939; Obtulowicz et

al., 1991; Wiithrich et al,, 1995). According to Buczylko (1996), extensive examination of patients have shown that besides the symptoms mentioned in the above definition, frequently complaints concerning other organs are revealed (Bonini et al., 1989; Buczylko, 1992; Davies, 1989; Persson, 1995; Rogala and Rogala, 1993; Wiithrich et al., 1995). The other symptoms involved in pollinosis include: irritation, anxiety (central nervous system), facial pain, thickening of mucosa, lingua crenata, slight itching of the papules on the palate, hawking, itching and burning sensation of the throat, hoarseness voice fatigue of larynx, hypocusis, deformed light reflex otoscopy and tympanometric changes of ear, barking cough and irritation of trachea, cough and dyspnea of bronchi, cough without ausculatatory changes of small bronchi, colicky pain during bowel ejaculation, flatulence- particularly when pollen is ingested along with swallowed nasal mucous, pruritus, exematous or urticarial dermatitis on areas of skin exposed to pollen [Buczylko, 1996]. Thus, allergic rhinitis or hay fever involves multi-organ manifestation and treatment must not be restricted to local therapy based only on the symptoms, or even random therapy within one or two organs.

1.3 Allergenic reactions

The allergic reactions are generally classified into two phases - early or immediate phase and late phase. The early allergic reaction occurs after exposure of a person to a particular allergen within a few minutes and lasts for about 2-3 h. This is largely attributed due to degranulation of mast cells, which are present abundantly in the epithelial lining of mucosa of the nasal pathway in the sensitized individual. Upon re-exposed to the same allergens, allergen specific IgE antibody adheres to the mast cell and crosslinks with the specific allergen. This reaction implements the mast cell degranulation which in turn liberates a variety of mediators like histamine, leukotrienes, prostaglandins, etc.“Histamine induces sneezing and mucous is secreted from the mucous gland (rhinorrhea)”. This is brought about by the triggering of the trigeminal nerve i.e. the Vth cranial nerve. Histamines, prostaglandins and leukotrienes affects the blood vessels causing nasal congestion” (Naclerio, 1991).

The late phase reaction starts after 4-6 h of antigen stimulation. This phase is characterized by prolonged symptoms of sneezing and rhinorrhea but predominantly there is long lasting sustainable nasal congestion lasting for about 18-24 hrs. The

influx of different cells like the eosinophils, the basophils and the T lymphocytes takes place due to inflammatory reactions in this phase (Naclerio, 1991). A variety of mediators such as kinins, leukotrienes, histamines are secreted by these cells. As a result the symptoms of rhinorrhea persist for long time.

1.4 Types of allergenic rhinitis

Allergic rhinitis either may be seasonal occurring in particular season of pollen or perennial happening throughout the year. Younger children are commonly affected by perennial allergic rhinitis. Further, depending upon the time of manifestation of symptoms, allergic rhinitis may be "Mild-Intermittent, Mild-Moderate-Severe intermittent and Moderate-Severe Persistent". In case of Intermittent types the symptoms persist for 4 days or a week and continuous for more than 4 weeks, while in case of Persistent type the symptoms occur more than 4 days in a week and greater than 4 consecutive weeks. Symptoms can be mild and not troublesome when there is no disturbance of sleep or daily activities like school, work, household functions. On the other hand severe symptoms results when there is disturbance of sleep and impairment of daily life, school or work (Bousquet et al., 2008).

Allergic rhinitis perhaps is confused with Local allergic rhinitis. In the latter case allergic reaction occurs in the nose when exposed to a particular allergen without systemic allergies. So in this case skin-prick test, other blood tests for allergy and Intradermal skin tests may all give negative results (Rondón et al., 2010). Symptoms are similar to that of allergic rhinitis which also affects the eye as in case of allergic rhinitis and may be either seasonal or perennial, and symptoms may be mild or moderate or may be severe. Rondon et al.(2010 & 2012), by his study reported that 25% of people had local allergic rhinitis and more than 40% of the people were identified to be suffering from non-allergic rhinitis actually have local allergic rhinitis.

1.5 Diagnosis

There are several tests to discover a specific allergen to which a human is sensitive Sometimes skin prick test, an intradermal scratch or other test like intradermal or intracutaneous injection are also employed to detect if a particular allergen is responsible for allergenic rhinitis. In the intradermal test, an aqueous allergen extract

is injected superficially in the skin, while during the skin prick test, a droplet of glycerinated allergen is settled on the skin after making a superficial incision with a lancet which produces rapid wheal and flare reactions within 15-20 minutes, and delayed reactions in 4-8 hours. The positive control is histamine while dilutents are used as the negative control (Mygind, 1986). An intradermal skin test is however more sensitive and reliable, while the prick test is more convenient and has the least chance of systemic reactions (Hejjaoui et al., 1992; Lin et al., 1993). Another test which is less common includes dissolving of the suspected allergen and dropping this suspension onto the lower eyelid. This test however can be harmful if done improperly and requires the expertise of a physician. There are other methods like inhalation, nasal or bronchial mucosa-provocation tests or the histamine release test (Stanley and Linskens, 1974). There are also certain immunoassay methods to measure the total and specific serum IgE. These are the radio-allergo-sorbent-test (RAST) developed by Wide et al. (1967) and ELISA or Enzyme Linked Immuno Sorbent Assay developed by Engvall and Pearlman (1971). In case of patients who cannot be subjected to skin testing to determine specific allergic sensitivity RAST blood test may be useful. In this regard differential leukocyte count shows peripheral eosinophilia. However the major imperfection of allergy testing is that it may often exhibits allergies that do not responsible for causing the symptoms or fails to show the allergies that actually do cause symptoms. In this regard, intradermal allergy test has been found to be efficient, sensitive and reliable than the normal skin prick test.

1.6 Treatment for allergic rhinitis

- The primary aim of treatment of rhinitis is to minimize the symptoms of tissue inflammation of affected parts caused by allergies. The best remedy is to **avoid the allergen (**Sur and Scandale, 2010). Medications such as Intranasal corticosteroids is the primary method to cure the rhinitis. If the condition is persistant other options may be used such as airfilters, mite-proof covers and restraining certain foods in childhood. But these methods do not have any evidences supporting their effectiveness (Sur and Scandale, 2010).
- In case of local rhinitis, oral antihistamines and **steroid nasal sprays** are useful (Rondón et al., 2010). Itching, sneezing, conjunctivitis and rhinorrhea

are among the main symptoms of allergenic rhinitis, which can be cured by oral or nasal antihistamine drugs. It is useful and advised to take Antihistamine medication like azelastine prior to exposure, especially in case of seasonal allergic rhinitis. Symptoms can be resolved within 15 minutes after medication.

- Intranasal antihistamines are used for rhinorrhea, sneezing and nasal pruritis while ophthalmic forms or ophthalmic antihistamines (such as azelastine in eye drop form and ketotifen) are prescribed largely for conjunctivitis (May and Smith, 2008). Nasal sprays which are steroid in nature are successfully used in allergenic reactions to cure the problems without additional use of oral antihistamines. Intranasal corticosteroids are effectively used to combat the symptoms like sneezing, nasal congestion, itching, rhinorrhea, etc. But all these remedies are "time taking" to cure the problems and so the medication has to be continued for several weeks as their therapeutic effects take time to be started.
- Other measures including decongestants, receptor antagonists cromolyn, nonpharmacologic therapies such as nasal irrigation, and leukotriene may be used as second line (Sur and Scandale, 2010). For nocturnal symptoms, intranasal corticosteroids can be combined with nightly oxymetazoline, an adrenergic alpha-agonist, or an antihistamine nasal spray without the hazard of 'rhinitis medicamentosa" (Baroody et al., 2011).
- Allergy Specific Imunotherapy Treatment or AIT is also termed as desensitization. It's a kind of treatment in which doses of allergen are administered which are harmless like pollen, spores, mites to accustom the body to those substances. The treatment of AIT involves administering allergens by subcutaneous injections under skin or orally in form of sublingual tablets or drops.

1.7 Undesirable side effects

- Antihistamine drugs like oral antihistamine tablets may cause undesirable side effects particularly drowsiness. First-generation antihistamine drugs such as diphenhydramine cause drowsiness, but second- and third-generation

antihistamines such as cetrizine and loratadine are less likely to show any side effects (May and Smith, 2008).

- To reduce nasal inflammation systemic steroids like prednisone tablets and glucocorticoids (such as betamethasone) injection or intramuscular triamcinolone acetonide are effectively used but their uses are limited for their undesirable side effects and side effects for prolonged steroid therapy (Ohlander et al. 2013).
- Tropical decongestants are also used to reduce symptoms but should be restriained from being used for a long time as withdrawal after prolonged use can result in radiate nasal congestion called rhinitis medicamentosa.

1.8 Cross reactivity in allergic rhinitis

Cross-reactivity occurs when the allergenic proteins of two different allergens (typically pollen) match each other. As the consequences, immune system can't recognize them differentially. According to Knox and Suphioglu (1996) and Grote et al. (2000, 2005), allergens of pollen are generally soluble in water and are generally proteins or glycoproteins. The molecular weight ranges from 5 and 80 kDa). From sequence comparison it was found that they correspond to only 29 out of 2651 protein families of seed plants which include expansins, calcium binding proteins, pathogen related proteins and profilins (Radauer & Breiteneder, 2006; Chapman et al., 2007). As a consequence, remarkable sequence similarities have been found in many allergens which cause the phenomenon of cross-reactivity.

Hence although a person had not been exposed to a particular allergen earlier, he or she might show the allergic reaction because of cross-reactivity to a similar IgE epitope of a similar protein to which the patient had earlier been exposed.

1.9 Significance of the study:

1.9.1 Pollen of *Datura* sp.

Datura is an annual herbaceous leafy bush distributed in mostly warmer regions of the world like India. It grows mainly in wild and is often found to be cultivated for its ornamental and medicinal properties. *Datura* is a herb raising along the road sides, in wastelands, open fields or even in gardens. It has long, straight and trumpet shaped

pleasantly scented flowers. The colours of it may be purple, white, blue and pink. The plants can be easily propagated by seeds which are born within large fruits with spiny capsules. It splits to release seed which causes the propagation very easy. Hence this plant has been considered as weed in certain wastelands. As they propagate often quickly so they may appear as the invasive plants in fields, wastelands and open area. In Hindu religion the flowers of *Datura* are widely used in the worship of Lord Shiva by Hindu Women. So, the women are constantly exposed to the pollen of this plant which are extensively sold along the roadside flower stalls.

1.9.2 Role of the pollen of *Datura* in allergenic rhinitis

Inspite of playing the main role in allergenic rhinitis *Datura* pollen has always been ignored as a major allergenic hazard may be due to its anemophilous nature of taxa (Parui and Mandal, 1998). Since the possibility of the occurrence of anemophilous pollen in the atmosphere is more than entomophilous because of the huge pollen production in the former, most aerobiologists have ignored or neglected the entomophilous pollen in the airspora during their aeropalynological survey (Agashe, 1989; Agashe et al., 1983; Atluri et al., 1992; Singh and Babu, 1982; Singh and Devi, 1992; etc.) Further pollen grains tends to be distributed densely near their sources and hence local in occurrence (Gregory, 1961). This is more applicable to entomophilous pollens because of their tendency to distribute in higher concentration near their sources (Durham, 1947).The presence of pollen of *Datura* in air has been documented (Santra et al., 1991; Jain et al., 1992). The potentiality in causing allergy of the pollen of *Datura* had also been established by several researchers (Santra et al., 1991; Jain et al., 1992; Parui and Mandal, 1998). However it is also true that even being proven as allergenic the pollen of this plant can potentially cause allergy in certain individuals but not in all. An important factor for sensitive patients is that how common and widespread these plants are around the place where they live. Moreover it seems impossible for sensitive patients to stay away or protect themselves from this allergen as different species of *Datura* are grown dominantly in different localities. The same case prevails in West Bengal as well. An earlier study on skin sensitivity test was executed by Parui and Mondal in 1999. This study was experimented with people between 9-56 years of age with the pollen extracts of *Datura metel*. It was found that both women and men are equally affected belonging to the age group of 31-40 years.

Allegenic incidence were also found to be common among housewives who used to use this flower regularly for the worship of God and the incidence of allergenicity was also common among people who grow this plant either for ornamental or ritual purpose in their garden. Gardeners ignore whatever physical discomfort they face and this does not stop them from planting this ornamental species. The severity of the manifestations or the occurrence of discomfort varies during the life span of a patient and there can be period when he or she has no symptoms. Since the different species of *Datura* generally flower throughout the year hence there is less possibility for *Datura* sensitive patients to avoid getting exposed to this allergen.

1.9.3 Pollen morphology

Two main aspects of aerobiological studies are to properly diagnose the allergy and to determine the source of aeroallergens. Because of the vastness of Indian flora and for the presence of huge number of airborne pollens it would become a tedious task for aerobiologists to identify the source materials in relation to their process of releasing in time and space. The prevalent pollens of an area are generally the members of plants growing in that region. The abundance of pollens in atmosphere synchronizes with the flowering of the particular plants on that very special season. Therefore allergy should not be considered as "all or none" phenomenon as in chronic allergic disorder total allergic load rather than a single allergen causes various symptoms. So, allergy in varying degrees is not an exception rather than a rule. In such cases, an attempt to find out an allergen as the cause of symptoms of a patient becomes an exercise in futility (Bapat and Bapat, 1994).

Most of the time, pollen morphology determines a plant's taxa i.e. the mother plant to which a pollen belongs to. Hence it helps in identification of a plant taxonomic group. The outermost part of pollen i.e. exine reveals the main morphological characters of a pollen grain. There is still a lacunae prevailing in the knowledge in the field of palynology of present day plants which leads to the erroneous diagnosis of symptoms of allergies and thus wrong identification of the airborne pollen. Based on morphological characters of native flora important atlases of pollen have been published. The basic pollen grains' structure of pollen grains is well known as many publications give a detailed description of it. However detailed descriptions and

proper magnified photographs or images of the specimens analyzed in such accounts are often lacking leading to error in identification of the pollen allergen.

Secondly many investigators have used different palynological terms to describe their results, which create a lot of confusion. The exoaperture and endoaperture are the highly determined constituent parts of pollen grains, containing colpi, costae and fastigia (Kosonko and Sventorzhetskaya, 1999; Larsen and Barrett, 2000). Generally pollen grains possess a double wall - intine or endospore and exine or exospore. Spines are often present on the exine or it may bear various ornamentations or sculpturing giving the characteristic difference between different species often termed as echinae or microechinae. But this exine structures are point of variations and contradictory terms because some investigators have used scabrae in place of microehinae. Colpi appears to be surrounded by halo like structure under LM because of a space like girdle or band that separates the colpi margins from the other parts of the mesocolpium (Harley and Baker, 2001; Hesse, 2002). These apertures are such a route through which allergens are being emitted via cytoplasmic expulsion upon contact of pollen grains with water by rupturing the pollen walls (Bacsi et. al. 2006; El-Ghazaly et. al., 1999; Grote et. al., 2000; Taylor et. al., 2004). So for the palynological study and for better understanding of the allergenicity of pollen, it is necessary to investigate not only the morphological structure of pollen but also pollen class, pollen outline, polar equatorial measurements, P/E ratio, ectoaperture and endoaperture structures, to find out if there are any differences among the examined pollen species and to investigate if allergenicity will be relied on for such differentiation (Moore and Web, 1978; Victor and Van Wyk, 2000).

Thirdly, in case of very closely related or similar species their taxonomic relationship and circumscription is often very controversial. Exine ornamentation (sculpture), differences in the structure of pollen grain wall and other many characters are considered to be very important to distinguish between such species. The present study will provide insight into pollen systematic determinations and allergenic analysis but also in studies related to aerobiology, melissopalynology and reproductive biology.

1.9.4 Importance of epitope mapping in Immunotherapy

The most successful treatment for allergic rhinitis is immunotherapy, also called hypo-sensitization. This therapy involves repeated subcutaneous injection of the allergen responsible for allergic symptoms in gradually increasing concentrations. Controlled studies have established that patients are partially comforted from their difficulties by such treatments applied over a period of time or may be years. Unfortunately, allergen specific immunotherapy (ASIT) as practiced today has not only proved to be cumbersome and expensive, but is also associated with the risk of fatal side reactions. This is because commercial antigenic extracts used for immunotherapy consists of heterogeneous (crude) pollen extracts (complex mixture of several complex carbohydrates, enzymes, proteins, lectins, lipids, nucleic acids etc.).These extracts may contain many contaminants irrelevant to the allergen to which a person may have been sensitized and relevant allergen might be present in minute irreproducible amounts. In such cases it might lead to 100 X variation in allergenic potency. Pollen antigens generally comprise 0.5 to 1.0% of the total pollen proteins extractable. Further only limited areas in the antigen take part in the forming of immune complexes. These sites are known as antigenic determinants or epitopes. Therefore, the contaminating components to which the patients was not sensitized previously can lead to the induction or synthesis of IgE antibodies with new specificities and this can lead to untoward effects including anaphylaxis. Thus identification of the actual allergenic components of pollen, their characterization and epitope mapping of these allergenic fractions is very essential for preparation of vaccines for successful immunotherapy.

1.10 Aims and objectives of the study

The present study was undertaken to identify the allergenic protein fractions of the pollen of three related species of *Datura* (*Datura metel, Datura inoxia* and *Datura stramonium*) and Epitope mapping of the allergenic proteins extracted from the pollen of *Datura* sp. to identify and characterize the binding sites of antibodies. This will enable the development of new therapeutic vaccines for successful immunotherapy. Thus the overall study involved the following:

- Ultrastructure study of the pollen by Light microscopy, SEM and TEM for proper identification of the airborne pollen to which a patient is exposed and to study any specific features of pollen morphology and the ultrastructure that might have the potential to influence its allergenicity.
- Extract, quantify and study the soluble protein profile of the pollen of the three related species of *Datura* using standard methods, gel electrophoresis and partial characterization of the proteins (determining the molecular weights).
- Study the variation in protein profile with maturity of pollen (before and after anthesis).
- Isolation of the individual protein fractions by gel filtration.
- Identification of the allergenic protein fractions by performing Ouchterlony Immunodiffusion and ELISA using blood plasma of *Datura* sensitive patients.
- Development of antibodies in Male LOBUND –Wistar rats and study cross reactivity among the 3 species of *Datura* using Ouchterlony Immunodiffusion and ELISA to identify the common proteins fractions.
- Crosslinking coupled Mass Spectrometry for epitope mapping. Detection and identification of antigen-antibody binding location with high mass MALDI detection (high resolution mass spectrometry or MS/MS techniques) and amino acid sequencing of the epitopes.