

Allergen Specific Immunotherapy as an Effective Therapy for Seasonal and Perennial Allergic Rhinitis Patients

Jonnagadala Latha¹, Vadde Ramakrishna*², George L Moses³, and Rallapalli Ramanjaneyulu¹

¹Department of Microbiology, Sri Krishnadevaraya University, Anantapur - 515 003, (A.P) India.

²Department of Biotechnology and Bioinformatics, Yogi Vemana University, Kadapa - 516 003. India.

³Good Samaritan Kilpauk Lab Services, Kilpauk, Chennai - 600 010, India.

Allergen immunotherapy continues to be used worldwide in the management of allergic rhinitis and asthma. In the present study five aeroallergens (house dust, pollen, Parthenium, Aspergillus, cotton) were used in allergen specific immunotherapy and evaluated their sensitivity in 50 diagnosed allergic rhinitis patients. The levels of serum total IgE, specific IgE, IgG and eosinophil were measured in susceptible individuals before and after immunotherapy during 3 year study. Intradermal test revealed the *house dust*, *pollens* and *cotton* are important aeroallergens sensitizing population, since half of the patients were shown positive skin test to these aeroallergens. Elevated levels of eosinophil and total IgE were observed before immunotherapy in both sexes of all age groups. However, immunotherapy with above aeroallergens the eosinophil and total IgE levels significantly lowered. Conversely, the specific IgE antibodies formed after immunotherapy was significantly higher compared to control subjects ($P < 0.05$). The 3 year successive allergen immunotherapy induced the IgG antibodies in patients which block the binding of IgE mast cells and down regulate allergic reactions. From the data it is clearly indicating that the highest sensitivity was noticed to *House dust* and *Pollens* in majority of the individuals. The present study showed a very good response to immunotherapy with significantly lowered allergic symptoms. Study suggests the allergen immunotherapy is an effective and well tolerated in the treatment of allergic rhinitis.

Keywords: Aeroallergens - allergen specific immunotherapy - allergic rhinitis.

INTRODUCTION

Allergy is a significant health problem worldwide and its prevalence is continuously increasing and becoming modern epidemic. Today, more than 25% of the inhabitants of industrialized countries are suffering from allergic rhinitis, conjunctivitis, asthma, dermatitis or food allergies (Nathan et al., 1997; Norman, 2004; Bjorsten and ISAAC, 2008; Niederberger, 2009; Rolland et al., 2009). Allergic rhinitis is an inflammation of the membranes lining the nose and characterized by nasal

congestion, rhinorrhea, sneezing, itching of the nose and/or postnasal drainage (Merk et al., 1998). The inflammation is caused by excessive degranulation of mast cells and it is classified as Gell and Coombs Type I immediate hypersensitivity. Inhalative allergens lead to Type I allergic reactions that affect approximately 30% of adults and up to 40% of children in western countries (Skoner, 2001). It is very important to note that allergic rhinitis and asthma are co-morbidities and recent knowledge tells us that allergic rhinitis is an important risk factor for asthma (Malone et al., 1997; Skoner, 2001). Allergic symptoms can be alleviated using corticosteroids, antihistamines, cromones, decongestants, antimuscarinics or leukotriene

Table 1. Study Design-General Data for Patients

SN	Parameter		Females		Males	
			Control	Patients	Control	Patients
1.	Number		8	24	12	26
2.	Age in years	1 - 20	2	9	4	9
		20 - 40	4	12	4	12
		40 - 60	2	3	4	5
3.	BMI (kg/m ²)		17.6	17.2	18.8	17.9
4.	Asthma		-	21	-	22
5.	Serum globulins (gm/dl)		3.02±0.42	2.72±0.52	3.10±0.72	2.82±0.45
6.	Hemoglobin (gm/dl)		13±2	12.8±2.4	15±2.2	14.5±1.8

antagonists. However, only immunotherapy is able to change the course of allergic disease and to lead to a long-term improvement which is sustained years after the discontinuation of this treatment (Larche et al., 2006). In the currently employed immunotherapy, administration of increasing doses of allergen extracts to allergic patients with the goal to induce a state of allergen-specific non-responsiveness (Bousquet et al., 1998; 2001). For a long time the mechanisms underlying immunotherapy remained elusive, and is still not completely understood today. Three models for the immunological mechanisms underlying this treatment have been proposed - shifting of Th2-dominated allergen-specific immune responses towards a Th1 response, induces allergen-specific T cell tolerance and vaccination (Larche et al., 2006; Akdis and Akdis, 2007). Although immunotherapy with allergen extracts has proven to be a very useful treatment, it is also associated with a number of problems and to overcome these problems, various strategies have been enforced (Valenta and Niederberger, 2007; Niederberger, 2009). The objective of allergen immunotherapy is to reduce responses to allergic triggers that precipitate symptoms in the short term and to decrease inflammatory response and prevent development of persistent disease in the long term.

For the past 80 years, immunotherapy has been used to treat allergic diseases caused by inhalant allergens (Bousquet et al., 1998; Joint Task Force on Practice Parameters, 2007; Jacobsen et al., 2007; Rolland et al., 2009; Pipet et al., 2009). Keeping facts in a view, the present study was carried out immunochemical studies on inhalant allergens in the development of allergic rhinitis and so far no detailed attempt has been made in this aspect. The main objective of this study is to determine the role of aeroallergen sensitivity in allergic rhinitis patients. The present studies mainly focused on the evaluation of allergen specific immunotherapy in a total of 50 cases between 1-60 years who were diagnosed as allergic rhinitis by history and clinical examination.

MATERIALS AND METHODS

Study population

Fifty new patients ranging in age groups of 1-20, 21-40, 41-60 years (Male and Females) from Good Samaritan Kilpauk Lab Services, Kilpauk, Chennai, were chosen and tested for studying allergic rhinitis, where patients have not undergone any previous treatment. A significant number of age and gender matched healthy women and men were included in this study as control group.

Study design

This study was conducted after approval from the institutional review board and individual consent by the patients. All patients were interviewed to obtain information on their clinical history which includes their name, age, sex and allergic symptoms, exposure to aeroallergens, detailed environmental survey, history of previous therapy, past medical history, family medical history, etc. The patients were informed about their allergic rhinitis and consent was obtained for further diagnosis, investigations and analysis. After obtaining informed consent, patients were carefully screened for allergic rhinitis with a focused history, focused physical examination and intradermal testing. Six symptoms of allergic rhinitis i.e., nasal congestion or running nose, itchy or watery eyes, sneezing, snoring or breathing through the mouth, drainage down the throat, frequent yellow or green nasal discharge were evaluated. Patients were considered to have a positive history if they reported at least 2 of the 6 symptoms. Patients were excluded if they had a history of adenoidal hypertrophy, current or recent upper respiratory tract infection, anatomic nasal defects, nasal foreign body, or suspected immunodeficiency. The total characteristics of normal healthy subjects and allergic rhinitis patients ranging in different age groups were tabulated (Table 1).

Allergen extracts

Allergen extracts of house dust, pollens of trees, grasses and weeds, *Aspergillus*, *Parthenium* and cotton were prepared by grinding, defatting, extraction, clarification, dialysis and sterilization (Sheldon et al., 1967). Generally, the potency of allergenic extracts will be expressed in terms of total nitrogen units. The extracts were standardized as weight per volume (w/v) and consist of 0.26 mg / ml of total nitrogen.

Table 2. Variation in eosinophil count before and after Immunotherapy

Age Group years	Females				Males			
	Control		Diseased		Control		Diseased	
	Before	After	Before	After	Before	After	Before	After
1 – 20	3.4 ± 1	3.5 ± 1	5.1 ^a ± 1.16	3.6 ± 1.9	3.6 ± 0.4	3.5 ± 0.7	5.2 ^a ± 1.9	3.8 ± 1.0
21- 40	3.35 ± 0.3	3.25 ± 0.5	5.8 ^a ± 3.5	3.8 ± 0.7	3.1 ± 0.8	3 ± 0.8	4.5 ^a ± 1.6	3.2 ± 0.9
41- 60	3.1 ± 0.76	3 ± 0.81	7.3 ± 6.6	3.3 ± 0.9	3.4 ± 0.8	3.5 ± 0.7	3.4 ± 0.89	3.2 ± 0.5

Individual blood samples were collected and eosinophil count (% of cells) was measured.

a – Significantly different from controls ($p < 0.05$).

Immunotherapy Treatment (IT)

Treatment has been carried out by using extracts obtained under sterile conditions and serial 10-fold dilutions of the extract are used for the building up phase of immunotherapy. The concentration of the full strength extract may vary, depending on the degree of atopy and other patient specific factors. Keeping the careful documentation of immunotherapy in a view, information has been noted in the medical record includes the concentration range, the volume of extract, patient's arm used for the injection, peak expiratory flow before and, when indicated, 20 to 30 minutes after injection for high-risk patients, a history of reactions from previous shots, treatment of any reactions that occurred, and any adjustments from the standard schedule. Injections were given subcutaneously in the posterior aspect of the middle third of the upper arm. During the buildup phase, when several dilutions are used, a redundant system to indicate concentration may help avoid errors.

Phases of therapy

The patients received weekly injections of single or combined extracts of allergens. Each treatment consisted of a single injection containing the allergens to which the patient was sensitive. There are two phases of immunotherapy administration: the initial buildup phase, when the dose and concentration of extract are slowly increased, and the maintenance phase, when the patient receives an optimal immunizing dose over a period of time. Typically, patients begin treatment with 1:100,000, 1:10000, 1:1000, 1:100, 1:50 diluted allergens were given in 0.5 ml. Before treatment the blood analysis was performed. Most patients require weekly twice injections for a period of 24 weeks to reach a monthly maintenance dose of 0.5 ml (1:50). Most patients receive 2 shots per visit from 2 different vials, each containing approximately 2 or 3 different aeroallergens. The maintenance dose of 0.5 ml of 1:50 dilution is administered monthly or bimonthly intervals for 3 years (Portnoy, 2001). After 3 years of successful immunotherapy with aeroallergens which induces prolonged clinical remission and the patient's seasonal symptoms are lowered. Blood analysis was performed after successful immunotherapy. Patients who develop hives, breathing difficulties, or light headedness are immediately evaluated and given epinephrine and other treatment under supervision of medical expert. Blood samples were collected before and after immunotherapy and used for measurement of hemoglobin concentration by Drabkin's cyanmethemoglobin methods (Cook, 1985), eosinophil count (Holfman et al., 2005) and Albumin: Globulin ratio (Spencer and Price, 1977). Total IgE (Savant et al., 1997), specific IgE (Pound, 1998), and IgG (Friedman and Young, 1997) in serum sample were measured by ELISA methods.

Statistical analysis

The statistical methodology was applied for expressing the significance of immunochemical studies on inhalant allergens in the development of allergic rhinitis and validity of results. The data for investigation was expressed as mean ± SD; t-test was applied to determine the significance of various parameters among allergic rhinitis patients and controls. P values < 0.05 were considered significant.

RESULTS AND DISCUSSION

The results of intradermal test on 50 patients revealed the total percentage of allergic rhinitis in both sexes for five aeroallergens was 96 % for house dust, 70% for pollen, 44% for Parthenium, 34% for Aspergillus and for cotton-76%. However, the house dust, pollens and cotton are important aeroallergens sensitizing population, since half of the patient's skin test positive to *house dust*, *pollens* and *cotton*. Based on intradermal skin tests the patients were selected for the study and subjected to respected specific allergen immunotherapy. Blood samples were collected before and after immunotherapy.

Eosinophils play an important role in the pathophysiology of asthma and other allergic diseases. In allergic conditions, the level of the eosinophils in the peripheral blood is 3-7% and the count ranges from 12-20%, an infectious factor is suggested, particularly in asthmatic subjects. This elevation of eosinophils may occur in a patient and leads to the enhancement of white cell count. A higher percentage of eosinophils count always suggests the possibility of an additional diagnosis in detection of allergic rhinitis and asthma (Kay, 2001). In the present investigation, there was a significant increase in the eosinophils in females (5.1 and 5.8%, $P < 0.05$) and males (4.5%, $P < 0.05$) in the age group of 1-40 years. No significant difference was noticed in 41-60 years male and female group (Table 2). After immunotherapy with specific aeroallergens, the eosinophil level was significantly reduced and comes more or less to control subjects. During subcutaneous immunotherapy against grass pollen allergy, an inhibition of eosinophil migration

Table 3. Variation in A:G ratio before and after Immunotherapy

Age Group years	Females				Males			
	Control		Diseased		Control		Diseased	
	Before	After	Before	After	Before	After	Before	After
1 – 20	1.29:1 ± 0.08:0	1.31:1 ± 0.13:0	1.05:1 ± 0.71:0	1.21:0.9 ± 0.16:0	1.3:1 ± 0.04:0	1.32:1± 0.03:0	0.81 ^a :1± 0.34:0	1.15:1 ± 0.21:0
21- 40	1.53:1 ± 0.34:0	1.56:1 ± 0.22:0	1.03 ^a :1± 0.44:0	1.01:0.9 ± 0.07:0	1.39:1 ± 0.07:0	1.38:1± 0.08:0	1.2:1 ± 0.37:0	1.21:1 ± 0.33:0
41- 60	1.43:1± 0.1:0	1.46:1 ± 0.15:0	1.16:1 ± 0.63:0	1.2 :1 ± 0.4:0	1.39:1± 0.22:0	1.4:1 ± 0.035:0	1.03:1 ± 0.43:0	1.01:1 ± 0.39:0

Blood sample collected before and after immunotherapy from control and diseased groups. Total protein (gm/dl) and albumin (gm/dl) were measured and globulin levels will be calculated by subtracting the albumin from total proteins. a – Significantly different from controls (p < 0.05).

Table 4. Variation levels of total IgE before and after Immunotherapy

Age Group years	Females				Males			
	Control		Diseased		Control		Diseased	
	Before	After	Before	After	Before	After	Before	After
1 – 20	99.8 ± 35	106.2 ± 31.9	742.2 ^a ± 230	198.2 ± 32.3	98.5 ± 32.3	102.5 ± 95.4	870.5 ^a ± 283.9	210.5 ± 54.7
21- 40	102.5 ± 21.4	115 ± 61.9	659.5 ^a ± 306.7	172.5 ± 47.5	103.5 ± 26.3	110 ± 73.3	439.5 ^a ± 165.3	185.7 ± 39.8
41- 60	109.6 ± 48.5	116.2 ± 60.4	503.3 ^a ± 212.2	125.5 ± 56.4	116.8 ± 45.3	125 ± 35.3	587 ^a ± 222.3	175.9 ± 47.3

Blood samples collected before and after immunotherapy from control and diseased groups and determined the levels of total IgE. Expressed in IU/ml. a – Significantly different from controls (p < 0.05).

into the nasal mucosa after challenge was demonstrated by a decrease in intra-epithelial eosinophil (Durham et al., 1996; Wilson et al., 2004; Hammad et al., 2004; Lorenzo et al., 2009).

The range of globulin in normal individuals varies from 7.0-8.0 g/dl. The A:G ratio in normal subjects is 1.5:1. In allergic rhinitis patients the levels of globulin increases and the levels of albumin decreases (Biewenga et al. 1995). In the present investigation the levels of A:G ratio was estimated in normal subjects as well as in allergic rhinitis patients. The data indicates that there was a significant difference in the levels of A:G ratio (P< 0.05) in the age group of 1-20 years males and 21-40 years females compared to controls

(Table 3).

Elevated levels of IgE indicate an increased probability of an IgE mediated hypersensitivity, responsible for allergic reactions. The IgE concentration in serum is dependent on both the extent of allergic reaction and the number of different allergens to which the individual is sensitized. Variation in the levels of total IgE in allergic rhinitis patients has been tabulated (Table 4) and observed that there was a significant increase in the levels of IgE in males and females of all age groups before immunotherapy. However, after allergen immunotherapy the total IgE levels are significantly lowered. Patients with allergic diseases such as atopic asthma, allergic

rhinitis have been shown to exhibit increased total IgE (Persson et al., 2008; Lorenzo et al., 2009) and after immunotherapy these IgE levels were reduced (Smith et al., 1998; Niederberger, 2009).

Allergen specific IgE antibody has been considered to play an important role in the pathogenesis of allergic rhinitis and asthma (Passalacqua and Durham, 2007). Estimation of specific IgE antibody in the serum is helpful for the identification of relevant aeroallergens and the allergic reaction depends on the levels of specific IgE. Specific IgE antibody level may be 100 fold different in different individuals and may change overtime in response to increase or decrease exposure to antigen. ELISA assays were

Table 5. Variation in the levels of specific IgE

	Age group (1 – 20 year)				Age group (21 – 40 year)				Age group (41 – 60 year)			
	Females		Males		Females		Males		Females		Males	
	Control	Patients	Control	Patients	Control	Patients	Control	Patients	Control	Patients	Control	Patients
House dust	23.3 ± 23	215.5 ^a ± 127	25 ± 7	213.3 ^a ± 115.7	22.5 ± 17.6	126.2 ^a ± 76.2	35 ± 21.7	125.8 ^a ± 61.1	36.2 ± 21.3	85 ^a ± 18	25 ± 7	53 ± 33.8
Pollen	26.6 ± 20.8	80.7 ^a ± 26.2	40 ± 0	146.25 ± 117.5	25 ± 13.2	65.5 ^a ± 49.2	36.6 ± 20.8	68.1 ± 48	30 ± 14.1	22.5 ± 3.5	15 ± 7	70 ± 14.1
Parthenium	10 ± 0	57.5 ± 53	10 ± 0	107.5 ± 56.9	20 ± 0	52.5 ^a ± 18.3	23.3 ± 15.2	54.1 ± 46.5	23.3 ± 5.7	0 ± 0	0 ± 0	46.6 ^a ± 23
Aspergillus	0 ± 0	94 ± 103.8	0 ± 0	78 ± 61.8	26.25 ± 17	40 ± 32.5	25 ± 7	42.5 ± 3.5	22.5 ± 3.5	172.5 ^a ± 130.8	20 ± 14.1	36.6 ± 28.8
Cotton	15 ± 7.07	81.6 ± 77.8	15 ± 7	118.5 ± 112.6	20 ± 14.1	78.8 ^a ± 69.8	15 ± 8.6	54 ^a ± 28.7	25 ± 18	160 ± 197.9	0 ± 0	95 ± 63.6

Blood samples collected from control and diseased groups and determined the levels of specific IgE. Expressed in IU/ml. a - Significantly different from controls (p < 0.05).

performed for the detection of specific IgE antibody in allergic rhinitis patients. The obtained results revealed the specific IgE antibodies to *house dust*, *pollens*, *Parthenium*, *Aspergillus* and *cotton* were significantly higher in females and males (P<0.05) (table 5). From the data it is clearly evident that the highest sensitivity to *House dust* and *Pollens* was observed in majority of the individuals, whereas in older patients (41-60 years) there was no significant difference in the sensitivity of allergens.

Allergy is currently considered as a peculiar case of an inflammatory reaction, in which the antigen is an allergen, and the host an allergic subject. Because it is an allergen penetrating into an allergic organism, the antigen presentation results in a Th2 differentiation of specific T cells. Schematically Th2 cells, by producing IL-4 and IL-13, trigger the IgE synthesis, while by producing IL-5 they attract and activate eosinophil polymorphonuclear cells. Then IgEs bind their high affinity receptor (Fcε RI) on effector cells

such as mast cells and in case of continuous exposure or re-exposure induce mast cell degranulation, histamine and leukotriene release and the early phase of the allergic reaction. In parallel, in the presence of allergens, IgE bind to B cells and other antigen presenting cells through the low affinity receptor of IgE, CD23. Eosinophils, through their production of basic proteins, injure epithelia, organize the late phase response and the chronic allergic reaction. Allergen specific immunotherapy has been widely used for many years and is an effective treatment for allergic rhinitis and asthma. It is the only therapeutic modality that can affect the natural cause of allergic diseases and may prevent the development of asthma in patients with allergic rhinitis. This immunotherapy acts at each step of the allergic reaction: IgE and IgG productions, mast cell and eosinophil homing, T cell activation, and antigen presentation (Rolland et al., 2009; Pipat et al., 2009; Niederberger, 2009).

Immunotherapy results in increase in IgG

antibodies, a gradual decline in antiallergen IgE antibodies and reduced number of nasal and bronchial mast cells, eosinophils and IL-4 (Magnan et al., 2000; Wilson et al., 2001; Akdis and Akdis, 2007; Niederberger, 2009). In aeroallergen immunotherapy, typically patients begin treatment with 1:100,000 (w/v) and progress through 1:10,000, 1:1000, 1:100 and 1:50 vials. The first dose from each vial is 0.05 ml and the last dose from each vial is 0.5 ml. A total of 23 weekly injections required to reach a monthly maintenance dose of 0.5 ml (1:50). There is a considerable variation in the level of IgG in allergic rhinitis patients after immunotherapy (Table 6). A significant increase of IgG levels was noticed in the age group 1 – 60 years (P<0.05). Changes in the levels of IgG in allergic rhinitis patients after Immunotherapy (in both females and males) were presented in table 6. The results also showed 37% of the allergic rhinitis patients showed a very good response to immunotherapy. The change in symptoms was significantly improved between

Table 6. Variation in the levels of IgG before and after Immunotherapy

Age Group years	Females				Males			
	Control		Diseased		Control		Diseased	
	Before	After	Before	After	Before	After	Before	After
1 – 20	792 ± 37	798 ±12	725 ± 15	911 ^a ± 101	750 ± 98	758±80	695±15	1264 ^a ±140
21- 40	835±40	820 ±20	785 ±20	1329 ^a ±149	807 ± 19	803±20	798±19	1119 ^a ±170
41- 60	781±83	785±20	715±30	1956 ^a ±131	810 ± 42	815±32	725±25	1614 ^a ±243

Blood samples collected before and after immunotherapy from control and diseased groups and determined the levels of total IgG. Expressed in ng/ml. a – Significantly different from controls ($p < 0.05$).

immunotherapy and the control group during the pollen season of the first year and later in subsequent 2nd and 3rd year the symptoms had become significantly lower in patients compared to control group.

As the models proposed on immunological mechanisms underlying in the immunotherapy treatment (Larche et al., 2006) are (i). Allergen-specific immunotherapy leads to a shift of Th2-dominated allergen-specific immune responses towards a Th1 response, (ii) immunotherapy induces allergen-specific T cell tolerance and (iii) immunotherapy has vaccination character and that injection of adjuvant-bound allergens induces a new type of allergen-specific immunity. This immune response is dominated by high levels of allergen-specific IgG1 and IgG4 antibodies which antagonize the effect of IgE. Therapy-induced IgG antibodies are able to block IgE binding and are thus able to inhibit allergen-induced mast cell and basophil degranulation, thereby down regulating immediate-type of allergic reactions. Furthermore, the newly induced IgG antibodies may also inhibit IgE-facilitated allergen-presentation to T cells and thus suppress T cell activation and the release of pro-inflammatory cytokines (Wachholz et al., 2003; Rossi et al, 2007; Pipet et al., 2009; Niederberger, 2009).

The results of the present study showed that the allergic rhinitis patients were shown a very good response to immunotherapy. The change in allergic symptoms was significantly lowered in seasonal and perennial allergic rhinitis patients. This study confirms that the allergen immunotherapy is an effective and well tolerated in the treatment of allergic rhinitis.

REFERENCES

- Akdis M, Akdis CA (2007). Mechanisms of allergen-specific immunotherapy. *J. Allergy Clin. Immunol.* 119: 780-91.
- Biewenga J, Stoop A, van der Heijden H, van der Baan S, van Kamp G (1995). Albumin and immunoglobulin levels in nasal secretions of patients with nasal polyps treated with endoscopic sinus surgery and topical corticosteroids. *J. Allergy Clin Immunol.* 96: 334-40.
- Bjorsten B and ISAAC phase III study group (2008). Worldwide time trends for symptoms of rhinitis and conjunctivitis phase III of the international study of asthma and allergies in childhood. *Pediatric*

- Allergy & Immunology* 19: 110-124
- Bousquet J, Cauwenberge V, Khaltaev P, Aria N (2001). Workshop Group: World Health Organization. Allergic rhinitis and its impact on asthma. *J. Allergy Clin. Immunol.* 108: S147-334.
- Bousquet J, Lockey R, Malling HJ (1998). WHO position paper. Allergen IT: Therapeutic vaccines for allergic diseases. *Allergy* 53: 4-42.
- Cook JD (1985). Measurement of iron status. A report of the International Nutritional Anaemia Consultive Group (INACG). New York: Washington DC; Ch.II: pp 4.
- Durham SR, Ying S, Varney VA, Jacobson MR, Sudderick RM, Mackay IS, Kay AB, Hamid QA. 1996. Grass pollen immunotherapy inhibits allergen-induced infiltration of CD4+ T lymphocytes and eosinophils in the nasal mucosa and increases the number of cells expressing messenger RNA for interferon-gamma. *J Allergy Clin Immunol.* 97:1356-65.
- Friedman RB, Young DS (1997). Effects of disease on clinical laboratory tests, 3th ed. AACC press. Washington, DC.
- Hammad H, de Heer HJ, Soullie T, Angeli V, Trottein F, Hoogsteden H C, et al. (2004). Activation of peroxisome proliferator-activated receptor-gamma in dendritic cells inhibits the development of eosinophilic airway inflammation in a mouse model of asthma. *Am. J. Pathol.* 164(1): 263-271.
- Hoffman R, Benz Jr EJ, Shattil SJ, et al. (2005). *Hematology: Basic Principles and Practice.* 4th ed. Philadelphia, Pa: Churchill Livingstone, 768.
- Jacobsen L, Niggemann B, Dreborg S, Ferdousi HA, Halken S, Host A, et al. (2007). Specific immunotherapy has long-term preventive effect of seasonal and perennial asthma: 10-year follow-up on the PAT study. *Allergy* 62(8): 943-948.
- Joint Task Force on Practice Parameters (2007). Allergen immunotherapy: a practice parameter second update. *J. Allergy Clin. Immunol.* 120: S25-85.
- Kay AB (2001). Allergy and allergic diseases (Part I). *N Engl J Med.* 344:30-7.
- Larche M, Akdis AC, Valenta R (2006). Immunological mechanisms of allergen-specific immunotherapy. *Nat. Rev. Immunol.* 6: 761-71.
- Lorenzo GD, Mansueto P, Maria LP, Rizzo M, Castello F, et al. (2009). Evaluation of serum s-IgE/total IgE ratio in predicting clinical response to allergen-specific immunotherapy. *American Academy of Allergy, Asthma & Immunology.* doi:10.1016/j.jaci.2009.02.012
- Magnan A, Mely L, Prato S, et al. (2000). Relationships between natural T cells, atopy, IgE levels, and IL-4 production. *Allergy.* 55:286-90.
- Malone DC, Lawson KA, Smith DH, Arrighi HM, et al., (1997). Cost of illness study of allergic rhinitis in the United States. *J. Allergy Clin. Immunol.* 99: 22-7.
- Merk S, Dykewicz, Stanley F, Nicklas R, et al. (1998). Joint Task Force Algorithm and Annotations for diagnosis and management of rhinitis. *Annals of Allergy Asthma and Immunology* 81:469-77.
- Nathan RA, Meltzer EO, Selner JC, Storms W (1997). Prevalence of allergic rhinitis in the US. *J. Allergy Clin. Immunol.* 99: S808-S 814.
- Neils M, Scadding GK (2000/03). *Allergic Rhinitis.* Health press. p 22-25. ISBN: 9781899541430.

- Niederberger V (2009). Allergen-specific immunotherapy. *Immunology Letters* 122: 131-133
- Norman PS (2004). Immunotherapy. *J Allergy Clin Immunol* 113: 1013-23.
- Passalacqua G, Durham SR (2007). Allergic Rhinitis and its Impact on Asthma update: Allergen immunotherapy. *J Allergy Clin Immunol*. 119:881-91.)
- Persson H, Flicker S, Sadegh MK, Greiff L, Valenta R, Ohlin M (2008). A common idiotype in IgE and its relation to recognition of the grass pollen allergen Phl p 2. *Molecular Immunology* 45: 2715-2720
- Pipet A, Botturi K, Pinot D, Vervloet D, Magnan A (2009). Allergen-specific immunotherapy in allergic rhinitis and asthma. Mechanisms and proof of efficacy *Respiratory Medicine*. 1-13.
- Portnoy JM (2001). Immunotherapy for allergic diseases. *Clin Rev Allergy Immunol*. 21(2-3):241-259..
- Pound JD (1998). *Immunochemical protocols*, 2nd ed. Vol. 80. Humana press, New Jersey, USA.
- Rolland JM, Gardner LM, O'Hehir RE (2009). Allergen-related approaches to immunotherapy. *Pharmacology & Therapeutics* 121: 273-284.
- Rossi RE, Monasterolo G, Coco G, Silvestro L, Operti D (2007). Evaluation of serum IgG4 antibodies specific to grass pollen allergen components in the follow up of allergic patients undergoing subcutaneous and sublingual immunotherapy. *Vaccine* 25: 957-964
- Savant T, Thammapalerd N, Jarronvesma N, Bunnag D (1997). Total serum IgE level in patients with amoebic liver abscess and other parasitic infections. *J. Trop. Med. Pub. Health*. 2:149.
- Sheldon JM, Lovell RG, Mathews KP (1967). *A manual of clinical allergy*. W B Saunders Company, Philadelphia. p506 – 531.
- Skoner DP (2001). Allergic rhinitis: Definition, Epidemiology, Pathophysiology, detection and diagnosis. *J. Allergy Clin. Immunol*. 47:1-44.
- Smith AM, Chapman MD, Taketomi EA, Platts-Mills TA, Sung SS (1998). Recombinant allergens for immunotherapy: a Der p 2 variant with reduced IgE reactivity retains T-cell epitopes. *J. Allergy Clin. Immunol*. 101(3): 423-425.
- Spencer K, Price CP. Influence of reagent quality and reaction conditions on the determination of serum albumin by the Bromocresol Green Dye-binding Method. *Ann Clin Biochem*. 14:105
- Valenta R, Niederberger V (2007). Recombinant allergens for immunotherapy. *J. Allergy Clin. Immunol*. 119: 826-30.
- Wachholz PA, Soni NK, Till SJ, Durham SR (2003). Inhibition of allergen-IgE binding to B cells by IgG antibodies after grass pollen immunotherapy. *J. Allergy Clin. Immunol*. 112(5): 915 - 22.
- Wilson DR, Nouri-Aria KT, Walker SM, Pajno GB, O'Brien F, Jacobson MR, et al. (2001). Grass pollen immunotherapy: symptomatic improvement correlates with reductions in eosinophils and IL-5 mRNA expression in the nasal mucosa during the pollen season. *J. Allergy Clin. Immunol*. 107(6): 971e6.