

Influenza Virus- and Cockroach Allergen-Specific IgE in Virus Induced Exacerbation of Asthma

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Abstract

IgE mediated hypersensitivity may be an important component of the complex mechanism of virus induced exacerbation of asthma. The present study investigated virus- and an environmental allergen (cockroach)-specific IgE in asthmatics suffering with acute exacerbation of symptoms associated with influenza virus infection. Of the 196 cockroach (*Periplaneta americana*) hypersensitive asthmatics (eliciting positive skin tests to cockroach extract), 40 patients who suffered acute exacerbations of symptoms of asthma associated with upper respiratory tract infections, were studied. Throat gargles were collected from these 40 patients in acute phase of infection for influenza virus isolation. Blood samples were collected from these patients during acute and convalescent phases of infection for serological diagnosis of influenza infection by haemagglutination inhibition and also for estimating influenza virus- and cockroach allergen-specific IgE levels by radioallergosorbent test. Influenza virus infection was detected in 20/40 of patients. Only 12 of these 20 patients completed the study protocol. Influenza virus specific IgE was detected in seven of them, 6 showing higher virus-specific IgE levels in convalescent phase. As a control, sera from pre-infection stage of two patients were also tested and no virus specific IgE levels were observed in their pre-infection as well as acute phase sera. But the sera collected during convalescent phase from both of these patients showed the presence of virus specific IgE. Five of the 7 patients showed cockroach allergen-specific IgE, which increased from acute to convalescent phase in 4 of them. Our results suggest that influenza virus infection of upper respiratory tract causes exacerbation of symptoms in asthmatics by producing virus-specific IgE and/or boosting environmental allergen-specific IgE.

Keywords: Bronchial of asthma, Influenza virus infection, Acute and convalescent phases, Virus-specific IgE, Cockroach-specific IgE.

INTRODUCTION

Epidemiological studies have shown that viral

respiratory infections (VRI) are the most common cause of acute episodes of wheezing and exacerbation of symptoms in asthmatics¹⁻³. Current treatment modalities for asthmatics during exacerbation of symptoms provide poor response⁴. Viruses like rhinovirus, respiratory syncytial virus (RSV), influenza virus, parainfluenza virus, etc. may induce exacerbation of asthma by infecting bronchial epithelium and inducing local inflammation, interaction

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of virus and allergen induced inflammation, generation of a systemic immune response and/or neurogenic inflammation⁴⁻⁷. Hence, effect of viral infection on airway function is multifactorial and involves a complex interplay of nervous system, inflammation, epithelium and IgE related hypersensitivity⁷. However, actual mechanism of virus induced exacerbation of asthma has not yet been completely understood.

One common feature of asthma is production of an enhanced serum IgE response to some environmental allergen(s). We feel that during VRI exacerbation of symptoms in asthmatics could be due to the production of virus-specific IgE and/or potentiation of some environmental allergen(s)-specific IgE. Therefore, the present study was conducted to investigate the virus- and an environmental allergen (cockroach)-specific IgE levels in patients suffering with exacerbation of asthma during influenza virus infection.

MATERIALS AND METHODS

1. Selection of Patients

Patients with bronchial asthma⁸ (11-63 years) attending the outpatient department of VP Chest Institute, University of Delhi, Delhi were studied.

Intradermal tests were performed on these patients with cockroach (*Periplaneta americana*) whole body extract. Histamine (100 µg/ml in phosphate buffered saline; PBS) and PBS were used as positive and negative controls, respectively⁹. Patients (n=196) showing positive skin test response to cockroach extract were studied. Forty of these patients, who suffered acute exacerbation of asthma⁸ associated with upper respiratory tract infections, were examined during and after the episode of infection. Respiratory infection in these patients was diagnosed on the basis of clinical history and their physical examination.

Ethical and technical aspects of the study protocol were approved by Board of Research Studies, University of Delhi and written informed consent was obtained from each patient.

2. Evaluation of influenza virus infection

Throat gargles of patients were collected in virus transport medium (Hanks balanced salt solution)

during acute phase of infection. The specimens were kept at 4°C (2 hours), centrifuged (3000 rpm, 10 minutes) and supernatants were inoculated into the amniotic cavity of hen's embryonated eggs¹⁰. The inoculated eggs were incubated sequentially at 35°C and 4°C for 72 and 12 hours, respectively. The allantoic and amniotic fluids were collected separately and tested for the presence of influenza virus by haemagglutination (HA) test¹¹. The results of HA tests were confirmed by repeating egg inoculation. Specimens were considered positive on the basis of presence of HA after 3 consecutive passages.

3. Serological diagnosis of influenza virus infection

Blood samples were collected from patients during acute (1-3 days of onset) as well as convalescent phase (10-14 days after onset) of the infection, sera separated and stored at -20°C. Patients' sera were sequentially treated with trypsin solution (0.8%) and potassium periodate (0.01 M) to remove nonspecific inhibitors. Haemagglutination inhibition (HI) tests¹¹ were performed with these sera for serologic diagnosis of influenza virus infection.

4. Estimation of IgE levels

Virus- and cockroach allergen-specific IgE levels were determined in the sera of patients by radioallergosorbent test (RAST).

4.1. Virus specific IgE

4.1.1. Preparation of virus material

Influenza virus was purified from harvested amniotic fluid. Briefly, amniotic fluid of eggs showing presence of influenza virus was pooled for virus purification¹². The pooled amniotic fluid was centrifuged and the supernatant was placed in an ice bath. A cold suspension of 10% fowl erythrocytes was added to it and kept for 2 hours with gentle intermittent shakings. This suspension was centrifuged and the pellet of aggregated erythrocytes was suspended in PBS and incubated at 37°C in a water bath for one hour with gentle agitation. The suspension was centrifuged and the supernatant containing virus particles was collected and extensively dialyzed (Spectrapor 3, Spectrum Medical Industries, Inc., Los

Angeles, USA; molecular weight cut off = 3.5 kd) against 0.05 M ammonium bicarbonate, lyophilized and stored at -20°C.

4.1.2. Preparation of solid phase virus coupled discs

Cellulose paper discs (6 mm diameter; Whatman No. 50) were activated by soaking them in cyanogen bromide solution in dimethyl formamide (2.5%) for 45 minutes (4°C; pH 10-11)¹³. The activated discs were sequentially washed with ice-cold 0.1 M sodium bicarbonate, distilled water and cold acetone, and dried at room temperature. The discs were then incubated with virus material in borate buffered saline (BBS; pH 8.0) for 72 hours at 4°C. The uncoupled sites on the discs were blocked by soaking them in 1M ethanolamine (4°C; 24 hours). Thereafter, the discs were washed with BBS and RAST wash (0.02 M potassium dihydrogen phosphate, 0.08 M dipotassium hydrogen phosphate, 1% Tween 20 and 0.1% sodium azide; pH 7.4), and stored in RAST diluent (0.2% BSA in RAST wash; pH 7.4) at -20°C¹³.

4.1.3. Performance of RAST

Virus specific IgE in the acute as well as convalescent phase sera of patients was evaluated by RAST¹⁴ using influenza virus coupled discs. Briefly, one disc was incubated (overnight at room temperature) with 100 ml of patient's serum (1:1 v/v in RAST diluent) in polystyrene tubes (12 x 75 mm). The discs were then washed, followed by overnight incubation with 100 ml of radiolabelled (I¹²⁵) anti-human IgE (200 ng/ml; Dako, Denmark). Thereafter, the supernatant was carefully aspirated, discs were washed and radioactive counts bound were determined in a Gamma Counter. As a negative control, similar tests were also performed with a pool of sera of non-allergic healthy volunteers (n = 20).

RAST ratio was calculated on the basis of radioactive counts bound to the disc as under:

$$\text{RAST Ratio} = \% \text{ CPM with patient's serum} / \% \text{ CPM with normal human sera pool}$$

CPM = Counts per minute.

A RAST was considered positive when the RAST ratio was 2 or more.

As a control, sera from pre-infection stage of two patients were also tested for the presence of virus specific IgE levels.

4.2. Cockroach allergen specific IgE

Solid phase cockroach allergen coupled discs¹³ were prepared. Allergen specific IgE in the sera of patients was estimated by RAST¹⁴ using these allergen coupled paper discs.

RESULTS

Of the 40 cockroach sensitive asthmatics, who developed exacerbation of symptoms associated with upper respiratory tract infection, influenza virus infection was detected in 20 patients. The results of HI tests with patients' sera showed the presence of antibodies to various serotypes of influenza virus (H1N1/H3N2 and B/Lee). The sera showing four-fold or more increase in the titer of virus specific antibodies in HI test during convalescent phase were considered positive. Serotypes and strains of influenza virus identified in these patients are presented in Table 1.

Of the 20 patients, 8 discontinued participation in the study at different time intervals and only 12 completed the study protocol. Virus specific IgE antibodies were detected in the sera of 7 out of these 12 asthmatics either in acute or convalescent phase of virus infection. Of these 7 patients, 2 showed the presence of virus specific IgE in acute as well as convalescent phases (NK, D), 4 in convalescent phase (PK, PS, PB and SN) and 1 in acute phase (K) only (Table 2).

Of the 7 asthmatics, who showed virus specific IgE, 6 elicited higher RAST ratios in convalescent phase as compared to acute phase (Table 2, Figure 1). Pre-viral infection phase sera of two of these patients (PK, PS) were also available. Both of them did not show any influenza virus specific IgE antibodies in their sera collected in pre-infection as well as acute phase of infection. However, virus specific IgE was detected in these patients only during convalescent phase (Table 2).

Cockroach allergen specific IgE was estimated in the sera of these 7 patients in both acute as well as convalescent phases. Cockroach allergen specific IgE were detected in acute phase of viral infection in 5 of

Table 1. Results of haemagglutination inhibition tests with the sera of asthmatics showing presence of antibodies against different serotypes of influenza virus

Patients	Virus serotypes and their strains		
	Influenza A		Influenza B
	H1N1	H3N2	Lee
P	+	-	-
BL	+	-	-
DK	+	-	-
PB	+	-	-
NK	+	-	-
PS	+	-	-
M	-	+	-
D	-	+	-
SN	-	+	-
JY	-	+	-
CR	-	+	-
RS	-	+	-
SRC	-	-	+
RK	-	-	+
AV	-	-	+
R	-	-	+
PC	-	-	+
PK	-	+	+
VJ	+	+	+
K	+	+	+

Note: +, Present; -, Absent.

these 7 patients (Table 2). In 4 of these 5 patients (PK, SN, NK and D) allergen specific IgE levels increased in convalescent phase, and remained almost the same in the fifth (PS) patient (Figure 2).

Cockroach allergen specific IgE was also estimated in the sera of 3 out of the 5 patients (RS, RK and DK) who did not show any virus specific IgE. Interestingly, all the 3 patients revealed no change in RAST ratios in cockroach RAST.

DISCUSSION

VRI generate local as well as systemic immune responses to clear the viral pathogen. These responses along with neural signals are altered in asthmatics, provoking severe bronchial hyperresponsiveness and exacerbation of asthma⁴. An understanding of the mechanisms, by which these virus mediated changes occur, may help the clinicians in deciding the course of treatment in patients suffering with virus associated exacerbation of asthma^{4,6}.

Influenza virus has been reported to be one of the most important respiratory pathogen in Indian asthmatics¹⁵. The first evidence of association of influenza type A infection and asthma was given by Minor and associates¹⁶. Subsequently, other workers also reported the association of influenza infection and worsening of asthma^{15,17}. In the present study,

Table 2. Virus- and cockroach allergen-specific IgE in the paired sera of 12 asthmatics suffering with exacerbations of symptoms associated with influenza virus infection

Patient	Virus specific IgE (RAST Ratio)			Cockroach specific IgE (RAST Ratio)	
	Pre-infection	Acute	Convalescent	Acute	Convalescent
PK	1.7	1.7	3.1	7.5	12.7
PS	1.5	1.8	3.7	2.2	1.8
SN	-	1.8	3.4	2.0	3.7
NK	-	3.3	3.9	2.9	4.2
D	-	3.1	3.5	2.1	2.4
K	-	2.2	1.8	1.0	0.9
PB	-	1.4	2.0	1.8	1.2
RS	-	1.5	1.5	0.7	0.9
RK	-	1.5	1.8	0.9	1.0
DK	-	0.7	1.5	1.0	0.9
R	-	1.4	1.3	ND	ND
VJ	-	1.7	1.4	ND	ND

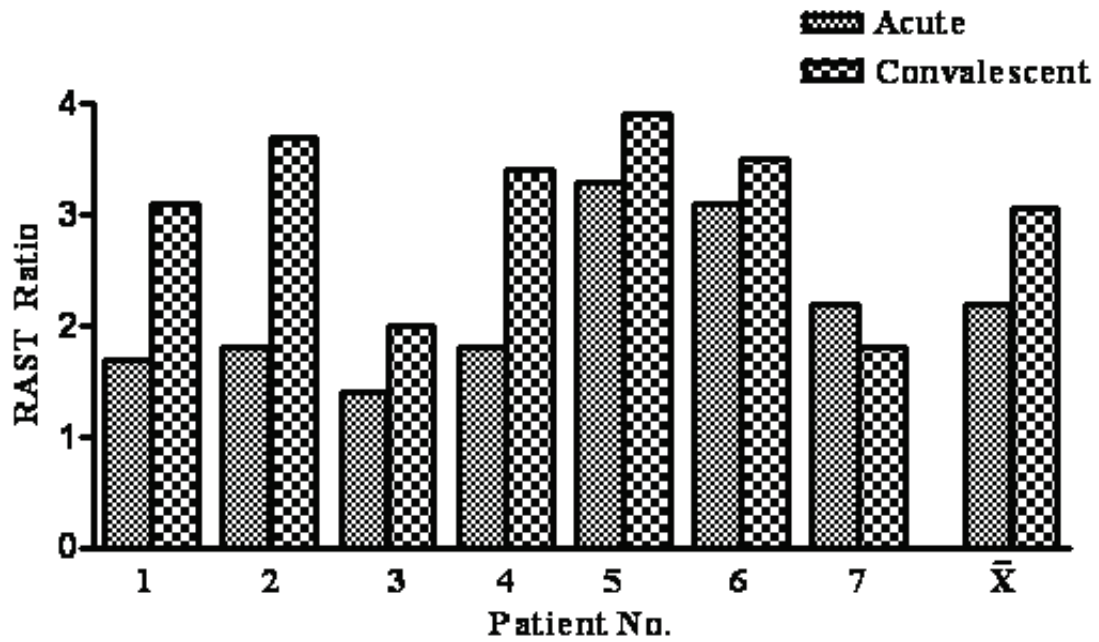


Fig 1. Influenza virus-specific IgE in the sera of seven asthmatics during acute and convalescent phase of viral infection associated with exacerbation of symptoms. X represents mean values for seven patients.

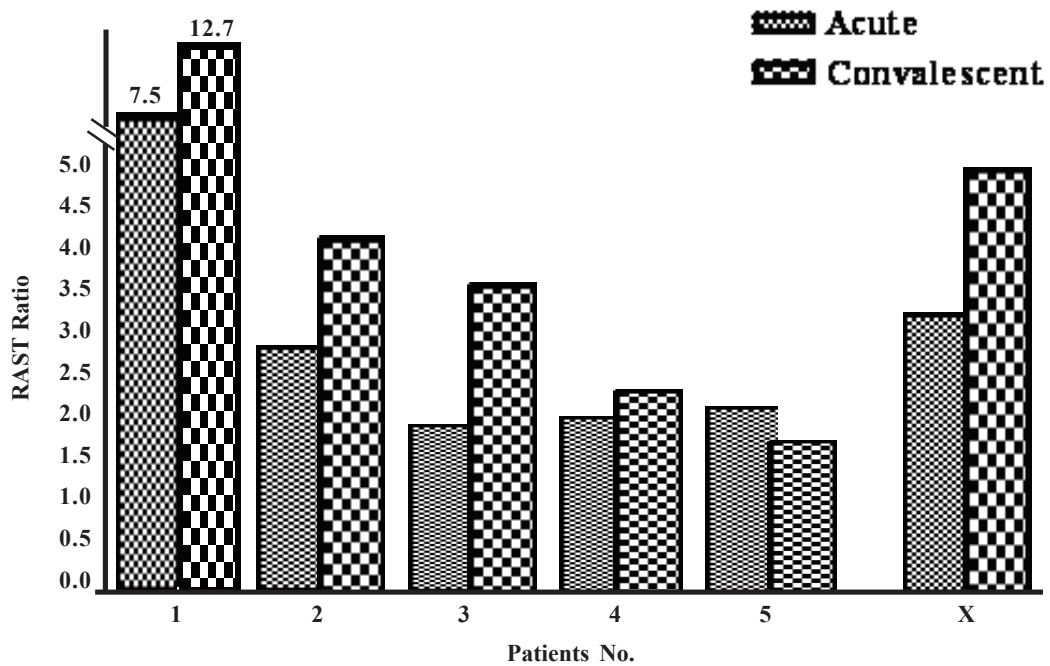


Fig 2. Cockroach allergen-specific IgE in the sera of five asthmatics who showed the presence of virus specific IgE. X represents mean values for five patients.

serological evidence of influenza virus infection during exacerbation of asthma symptoms was found in 20/40 (50%) of cases. Some workers have reported a lower rate of influenza virus infection in adult asthmatics^{18, 19}. The higher rate of viral infection observed in our study may be due to higher virulence of influenza virus in this region²⁰.

VRIs are known to activate T helper cells in atopic individuals and produce IgE during acute phase of infection²¹. An increase in total IgE levels of patients has been reported after infections with cytomegalovirus, Epstein Barr virus, rhinovirus and measles virus²¹⁻²³. Welliver and coworkers have reported RSV and parainfluenza virus specific IgE in nasal secretions of children with croup and/or wheezing²⁴. However, the presence of influenza virus specific serum IgE has not been studied so far. We observed influenza virus specific IgE levels in the sera of 7/12 patients suffering with exacerbation of asthma associated with influenza virus infection. These levels were higher in 6 out of 7 patients in convalescent phase of infection (Figure 1). Interestingly, limited number of patients (n=2), where sera from pre-infection stage were also available, no virus specific IgE levels were observed in pre-infection as well as in acute phase sera. But sera of convalescent phase from both of these patients showed the presence of virus specific IgE. It is quite possible that higher levels of virus-specific IgE appear in serum only after localized production in the respiratory tract²⁵. Thus, the presence of IgE in the sera of these patients also suggests its presence in respiratory tract epithelium, which may be responsible for exacerbation of symptoms during virus infection in asthmatics.

An important parameter of allergic asthma is production of specific IgE against inhaled environmental allergens. In adult asthmatics, acute exacerbation of asthma was reported to be strongly associated with the combination of allergic sensitization, exposure to high levels of an allergen and concurrent viral infection²⁶. In the present study, environmental allergen (cockroach) specific IgE was also observed in patients' sera (n=5) during influenza virus infection. A concomitant increase in cockroach as well as virus-specific IgE levels from acute to convalescent phase was observed in 4 of these 5 patients (Figure 2). It is quite possible that influenza

virus infection may also have a boosting effect on allergen specific IgE response in asthmatics. In this context, it may be pointed out that in murine model, the timing of exposure of virus and allergen has been reported to be crucial in determining whether viral infection will enhance the development of inhalant allergen sensitization. It may also be noted that in rats, IgE response to ovalbumin could be triggered only during acute stage of influenza viral infection^{27,28}. Our results suggest that influenza virus infection of upper respiratory tract may cause exacerbation of symptoms in some asthmatics by production of virus specific IgE and/or boosting allergen specific IgE. To the best of our knowledge, this is the first report of the influenza virus specific IgE production in asthmatics suffering with exacerbation of symptoms associated with influenza virus infection.

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REFERENCES

1. Micillo E, Bianco A, Dauria D, Mazzarella G, Abbate GF. Respiratory infections and asthma. *Allergy* 2000; **55**(61): 42-45.
2. Tan WC. Viruses in asthma exacerbations. *Curr Opin Pulm Med* 2005; **11**(1): 21-26.
3. MacDowell AL, Bacharier LB. Infection triggers of asthma. *Immunol Allergy Clin North Am* 2005; **25**(1): 45-66.
4. Papadopoulos NG, Xepapadaki P, Mallia P, Brusselle G, Watelet JB, Xatzipsalti M, et al. Mechanisms of virus-induced asthma exacerbations: state-of-the-art. A GA²LEN and InterAirways document. *Allergy* 2007; **62**: 457-470.
5. Mallia P, Contoli M, Caramori G, Pandit A, Johnston SL, Papi A. Exacerbations of asthma and chronic obstructive pulmonary disease (COPD): Focus on virus induced exacerbations. *Current pharmaceutical design* 2007; **13**(1): 73-97.
6. Corne JM, Holgate ST. Mechanisms of virus induced exacerbations of asthma. *Thorax* 1997; **52**: 380-389.
7. Papadopoulos NG, Velissariou IM. The role of respiratory viruses in the pathogenesis of pediatric asthma. *Pediatr Ann* 2006; **35**(9): 637-642.
8. Global Initiative for Asthma. Global strategy for Asthma Management and Prevention. NHLBI/WHO Workshop report. 2002. NIH Publication 02-3659. Update 2004. Available on line at www.ginasthma.com.

9. Agarwal MK, Jhamb S. Immunodiagnosis of Type I respiratory allergic disorders. *Cardiothoracic J* 1995; **1**(6): 36-50.
10. WHO technical report series. *Expert committee on respiratory virus disease*. 1959; **170**: 4-10.
11. Casals J. Immunological techniques for animal viruses. In: Maramorosch K, Koprowski H, eds. *Methods in Virology*. New York: Academic Press, 1967: 113-198.
12. Sheffield FW, Smith W, Belyavin G. Purification of influenza virus by red cell adsorption-elution. *Brit J Exp Path* 1954; **35**: 214-222.
13. Adolphson CR, Gleich GJ, Yunginger JW. Standardization of allergens. In: Rose NR et al, eds. *Manual of Clinical Laboratory Immunology*. Washington DC: American Society for Microbiology, 1986: 652-659.
14. Hamilton RG, Adkinson NF. Immunological tests for diagnosis and management of human allergic disease: Total and allergen-specific IgE and allergen-specific IgG. In: Rose NR et al, editors. *Manual of Clinical Laboratory Immunology*. Washington DC: American Society for Microbiology, 1997: 881-892.
15. Prasad AK. Influenza type A, parainfluenza and adenovirus infections in patients with asthma. First joint conference of Indian College of Allergy and Applied Immunology and Indian Immunology Society, Srinagar, 1979, Abstract no. 106.
16. Minor TE, Dick EC, Baker JW, Ouellette JJ, Cohen M, Reed CE. Rhinovirus and Influenza Type A infections as precipitants of asthma. *Am Rev Respir Dis* 1976; **113**: 149-153.
17. Lin C, Kuo W, Lin W, Lin C. Immunomodulation of influenza virus infection in precipitating asthma attack. *Chest* 1988; **93**: 1234-1238.
18. Huhti E, Mokka T, Nikoskelainen J, Halonen P. Association of viral and mycoplasma infections with exacerbations of asthma. *Ann Allergy* 1974; **33**: 145-149.
19. Hudgel DW, Langston L Jr, Selner JC, McIntosh K. Viral and bacterial infections in adults with chronic asthma. *Am Rev Respir Dis* 1979; **120**: 393-398.
20. Park JE, Park K. Textbook of preventive and social medicine. Jabalpur: Banarsidas Bhanot Publishers, 1991: 123-125.
21. Perelmutter L, Potvin L, Phipps P. Immunoglobulin E response during viral infections. *J Allergy Clin Immunol* 1979; **64**: 127-130.
22. Bahna SL, Horwitz CA, Fiala M, Heiner DL. IgE response in heterophil-positive infectious mononucleosis. *J Allergy Clin Immunol* 1978; **62**: 167-173.
23. Griffin DE, Cooper SJ, Hirsch RL, et al. Changes in plasma IgE levels during complicated and uncomplicated measles virus infections. *J Allergy Clin Immunol*. 1985; **76**(2 Pt 1): 206-213.
24. Welliver R, Wong D, Middleton E, Sun M, McCarthy N, Ogra P. Role of parainfluenza virus-specific IgE in pathogenesis of croup and wheezing subsequent to infection. *J Pediatr* 1982; **101**: 889-896.
25. Lawrence EC, Blaese RM, Martin RR, Stevens PM. Immunoglobulin secreting cells in normal human bronchial lavage fluids. *J Clin Invest* 1978; **62**: 167-173.
26. Green RM, Custovic A, Sanderson G, Hunter J, Johnston SL, Woodcock A. Synergism between allergens and viruses and risk of hospital admission with asthma: case-control study. *BMJ* 2002; **324**: 763.
27. Lebec H, Sarlo K, Burleson GR. Effect of influenza virus infection on ovalbumin-specific IgE responses to inhaled antigen in the rat. *J Toxicol Environ Health*. 1996; **49**: 619-630.
28. Yamamoto N, Suzuki S, Suzuki Y, et al. Immune Response Induced by Airway Sensitization after Influenza A Virus Infection Depends on Timing of Antigen Exposure in Mice. *J Virol*. 2001; **75**(1): 499-505.