

Influence of viral infection on the development of nasal hypersensitivity

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Summary

Background The underlying relationship between viral infections and allergic diseases of the upper respiratory tract has not been well clarified.

Methods In order to clarify the relationship between viral infection and nasal hypersensitivity, mice were sensitized with ovalbumin (OVA) and then infected intranasally with respiratory syncytial virus (RSV), after which their nasal sensitivity to histamine or antigen was examined.

Results Non-sensitized mice showed transient mild nasal hypersensitivity following nasal administration of histamine after intranasal RSV inoculation. In mice sensitized with OVA, RSV infection significantly exaggerated their nasal hypersensitivity to histamine and OVA. Treatment of these mice with a neurokinin (NK)-1/NK-2 receptor antagonist, but not with anti-IL-5 antibodies, reduced their hypersensitivity. The infiltration of nasal mucosa with eosinophils was temporarily associated with accelerated rate of RSV elimination in these animals.

Conclusion RSV infection induced transient nasal hypersensitivity. Several mechanisms, including impairment of nasal epithelial cells are thought to mediate this effect. In allergen-sensitized mice, RSV inoculation strongly enhanced nasal hypersensitivity.

Keywords histamine, nasal hypersensitivity, RSV

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Introduction

Recent epidemiological evidence has suggested that acute respiratory viral infections exacerbate the symptoms of pre-existing reactive airway diseases and is the most important trigger of acute asthmatic attacks [1–4]. Viruses, rather than bacteria, cause most acute respiratory tract infections, and asthma attacks in children are often preceded by viral infection [5–7].

The nasal cavity is often the first target of invading viruses, because it is the point of entry into the respiratory tract. The common cold is the most widespread viral infectious condition and is usually caused by viruses such as rhinoviruses, parainfluenza viruses, influenza viruses, adenoviruses and respiratory syncytial virus (RSV) [8, 9]. However, the relationship between viral infections and allergic diseases in the upper respiratory tract has not been well defined. The results from studies that have examined the influence of atopy on the development of the symptom after viral infections are controversial [10–13]. Bardin et al. [11] observed more severe cold symptoms in atopic subjects than in non-atopic subjects after experimental rhinovirus infection. However, in another study, augmented nasal allergic inflammation induced by

antigen provocation before viral inoculation did not result in a worsening of cold symptoms [12]. The effects of the common cold on nasal hypersensitivity or allergic rhinitis have not been clearly established.

Nasal responses to viral infection are thought to differ depending on the viral species. Although rhinoviruses causes little damage to epithelial cells in the respiratory tract, RSV induces marked cytopathic effects [13]. RSV is an RNA virus infection which usually results in common cold symptoms, although progression to lower respiratory tract symptoms, the most common being bronchiolitis, frequently occurs in infants. RSV causes about 60% of the bronchitis cases in children [14, 15]. In prospective studies, as many as 75–90% of infants with a clinical diagnosis of bronchiolitis subsequently developed recurrent episodes of wheezing suggestive of childhood asthma and experienced airway histamine or methacholine hypersensitivity which persisted for several years [16–22].

In the present study, we have shown that RSV infection contributes to the exacerbation of nasal hypersensitivity in an allergic rhinitis mouse model.

Materials and Methods

Animals

Eight-week-old male C57BL/6 mice (Nippon Clea, Shizuoka, Japan) that were raised on ovalbumin (OVA)-free chow were

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used in this study. Hartley strain guinea-pigs (Nippon Clea) were also used to measure passive cutaneous anaphylaxis (PCA). The use of these laboratory animals was approved by the local Animal Ethics Committee (Yamanashi Medical University) and the experiments were conducted in conformity with the guidelines of the committee.

Experimental infection with respiratory syncytial virus

The long strain of RSV (prototype RSV group A strain) was grown in HEP-2 cells in minimal essential medium (MEM) supplemented with 2% fetal calf serum (FCS), 2 mM L-glutamine and antibiotics. RSV was partially purified by polyethylene glycol precipitation, followed by centrifugation in a 35–65% discontinuous sucrose gradient, as described elsewhere [23]. RSV (1×10^6 plaque-forming units (PFU)) in a volume of 20 μ L was administered intranasally to mice. Uninfected Hep-2 cells were processed similarly and used as controls.

Virus assay

Lungs and nasal tissues were collected and homogenized in MEM containing 2% FCS and were stored at -70°C until they were assayed. RSV was assayed by the plaque method using HEP-2 cells in 24-well microplates. The overlay for the plaque assay consisted of MEM supplemented with 2% FCS, antibiotics and 1% methylcellulose. Plates were incubated for 7 days at 37°C . After the methylcellulose was removed, the plaques were fixed with 10% formaldehyde and stained with 0.1% crystal violet.

Evaluation of sensitivity to histamine in nasal mucosa

One microlitre of various concentrations of histamine, diluted in phosphate-buffered saline (PBS), was administered into each nostril of the experimental mice. The number of nasal rubbing attacks that occurred during the ensuing 10 min was then counted.

Experimental protocol for sensitization with ovalbumin

Mice were immunized with 10 μ g OVA (grade V, Sigma Chemical Co., St Louis, MO, USA) intraperitoneally with alum once a week for 4 weeks. Heat-killed bordetella pertussis (1×10^8 bacterial units) was used as an adjuvant in the first immunization. Five days after the last immunization, the mice were either inoculated with RSV or sham-infected with sonicated non-RSV-infected HEP-2 cells. Two micrograms OVA in 2 μ L PBS was administered intranasally for 5 consecutive days after the inoculation. Sensitized mice were divided into the following experimental groups and treated as follows. Group 1 consisted of 30 mice treated with a neutralizing IL-5 antibody or a neurokinin (NK)-1/NK-2 antagonist. A rat neutralizing monoclonal antibody (mAb) directed against mouse IL-5 (PharMingen, San Diego, CA, USA) and a control isotype mouse IgG1 mAb (PharMingen) were used. Antibodies were injected intraperitoneally twice a week at a dose of 0.1 mg for 1 week before RSV inoculation, and were administered intranasally for 5 consecutive days after inoculation. Group 2 consisted of 10 OVA-sensitized mice who received 0.04 μ g of the NK-1/NK-2 antagonist [24]

FK224 (Fujisawa Co Ltd, Osaka, Japan) intranasally for 5 consecutive days after RSV inoculation. On the day following the last nasal administration of OVA, the nasal rubbing attacks were counted for 10 min. The sensitivity of the mice to histamine was examined 24 h later in a similar manner.

Treatment of ovalbumin-sensitized mice with a neutralizing anti-interferon- γ monoclonal antibody or with interferon- γ

OVA-sensitized mice received 0.1 mg of anti-IFN- γ neutralizing mAb (PharMingen) or control mAb intraperitoneally twice a week and then intranasally for 5 consecutive days before nasal provocation with OVA. Other OVA-sensitized mice were administered 1 μ g of IFN- γ (PharMingen) intranasally for 5 consecutive days before provocation with OVA.

Detection of ovalbumin-specific immunoglobulin E antibody

OVA-specific IgE antibodies were detected by PCA [25]. Briefly, 100 μ L of undiluted and twofold diluted serum samples were injected intradermally into the dorsal skin of shaved guinea-pigs. Three days later, the animals were challenged intravenously with 1 mg OVA together with 1% Evans blue. A blue lesion of a diameter greater than 5 mm, as determined 30 min after the challenge, was considered to be positive. PCA titres were expressed as the reciprocal of the highest dilution giving a positive reaction.

Histological examination

On the 4th day after RSV inoculation the mice were killed by CO_2 overdose. The heads of the mice were detached along the line between the upper and lower jaws, and they were then fixed in formalin and decalcified. The section of the nasal cavity anterior to the eyeball was examined and processed for paraffin sectioning. Tissue sections were stained with PAS and the number of infiltrating eosinophils in the whole nasal septum mucosa of each section was determined.

Fluorescence-activated cell sorting analysis

Nasal mucosal tissue from the above mice was cut into small pieces, which were then teased gently through a nylon mesh using frost glass slides. The disrupted mucosa was then suspended in RPMI-1640 containing 10% FCS, penicillin (100 units/mL) and streptomycin (100 μ g/mL). After washing twice with medium, $\text{CD}3^+$ T cells were purified in 0.2 mL of RPMI-1640 using magnetic beads (Dynal, Great Neck, NY, USA). Following purification, the medium was supplemented with 10% FCS. 10^6 nasal $\text{CD}3^+$ T cells collected from seven RSV-infected OVA-sensitized mice or from non-infected OVA-sensitized mice were stained with fluorescein-conjugated anti- $\text{CD}4$ antibody (PharMingen) and fixed overnight with 4% paraformaldehyde (Sigma Chemical Co). The fixed cells were permeabilized by incubation in PBS with 1% bovine serum albumin and 2% saponin (Sigma Chemical Co) for 10 min. A phycoerythrin-conjugated anti-IFN- γ antibody (PharMingen) or an anti-IL-5 antibody (PharMingen), diluted to 20 μ g/mL in PBS, was then added. After a 30 min incubation, the cells were washed with PBS and were

analyzed using a FACScan (Becton Dickinson, Fullerton, CA, USA).

Statistical analysis

Comparisons between groups were evaluated using Student's *t* test and Wilcoxon's test.

Results

Viral replication and nasal histamine sensitivity

After nasal inoculation with 10^6 PFU of RSV, mild replication of RSV in the respiratory tract was observed with peak levels occurring in the lung on day 4 and the levels then declined until day 7 as shown previously [26]. RSV was recovered from the nasal mucosa for 12 days after inoculation.

Non-specific stimulation of the nasal mucosa of mice also resulted in nasal rubbings. The number of nasal rubbing attacks observed in 20 normal mice following nasal installation of 2 μ L PBS was 9.4 ± 2.9 (mean $\pm$ SD). Thus, the lowest histamine concentration administered intranasally in a volume of 2 μ L that was needed to induce more than 20 nasal rubbing attacks was defined as the threshold level of nasal histamine hypersensitivity. After RSV inoculation, the threshold decreased and reached its lowest on day 4. It returned to normal by day 14 (Fig. 1(a)).

Influence of respiratory syncytial virus infection on ovalbumin-sensitized mice

The threshold of nasal hypersensitivity to histamine decreased in OVA-sensitized mice and RSV infection in OVA-sensitized mice induced a dramatic enhancement of nasal sensitivity to

histamine (Fig. 1(b)). The threshold of nasal hypersensitivity to histamine observed in RSV-infected mice increased gradually after the last nasal administration of OVA and 14 days later, it was the same as that of non-infected mice (data not shown). Fluorescence-activated cell sorting analysis of nasal mucosal T lymphocytes in the RSV-infected OVA-sensitized mice not only revealed an increased expression of IFN- γ , but also of IL-5 (Table 1). Anti-IL-5 treatment of RSV-infected OVA-sensitized mice using neutralizing antibodies reduced the histamine sensitivity in some degree ($P < 0.05$) and the treatment with an NK-1/NK-2 antagonist resulted in a marked reduction ($P < 0.001$) of the sensitivity (Fig. 1(b)).

After OVA nasal provocation the frequency of nasal rubbing attacks dramatically increased in RSV-infected OVA-sensitized mice, compared with non-infected sensitized mice (Fig. 2). However, anti-IL-5 treatment of RSV-infected OVA-sensitized mice did not significantly improve nasal symptoms after OVA administration. On the other hand, an NK-1/NK-2 antagonist resulted in a significant improvement (Fig. 2).

The number of eosinophils in the nasal mucosa was markedly increased in RSV-infected OVA-sensitized mice compared with those in non-infected OVA-sensitized mice (Fig. 3). The PCA titre, on the other hand, was not significantly different between the two groups (mean $\pm$ SD; 21.1 ± 21.0 in infected sensitized mice, 16.6 ± 11.4 in non-infected sensitized mice). Anti-IL-5 treatment of RSV-infected OVA-sensitized mice significantly reduced the number of infiltrated eosinophils, however, the treatment with an NK-1/NK-2 antagonist had no effect on eosinophil infiltration.

The nasal administration of IFN- γ to OVA-sensitized mice increased the number of nasal eosinophils, but had no effect on nasal symptoms (Fig. 4). Treatment with anti-IFN- γ neutralizing antibodies did not affect nasal symptoms or eosinophil infiltration (Fig. 4).

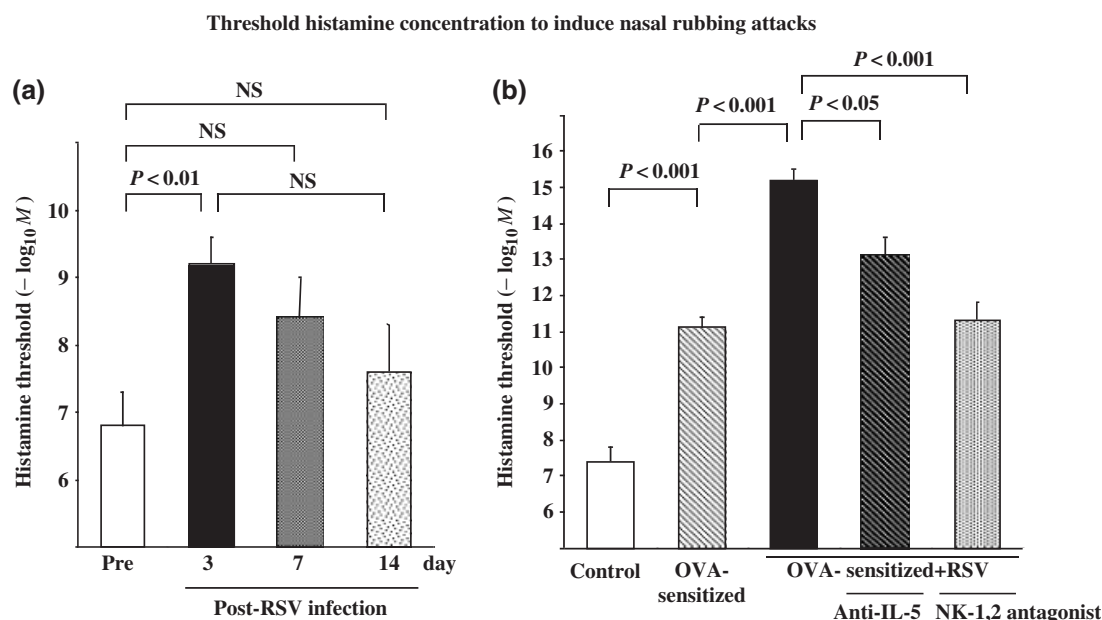


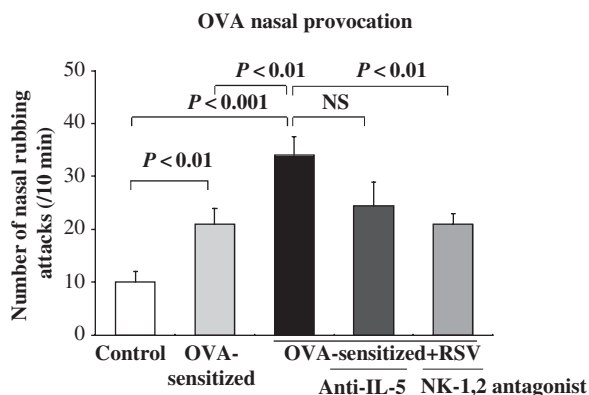
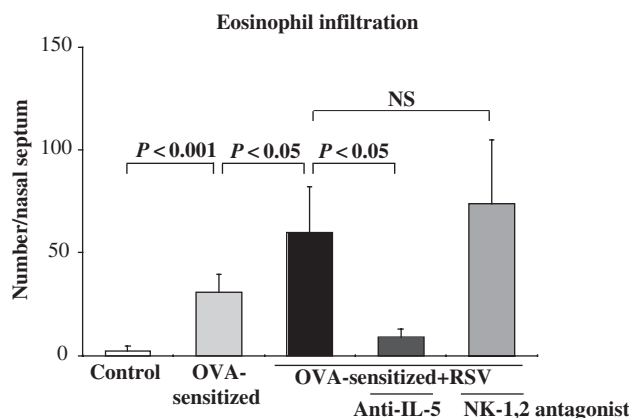
Fig. 1. Threshold histamine concentration needed to induce nasal rubbing attacks in respiratory syncytial virus (RSV)-infected non-sensitized mice (a) and in ovalbumin (OVA)-sensitized mice (b). After RSV inoculation, the threshold decreased transiently and reached its lowest on day 4. Although the threshold decreased in OVA-sensitized mice, RSV infection in OVA-sensitized mice induced a dramatic reduction of the threshold. The treatment with neurokinin (NK)-1/NK-2 receptor antagonist but not with anti-IL-5 neutralizing antibodies improved the reduction. Non-OVA-sensitized mice were used as controls.

Table 1. IL-5 and IFN- γ expression of nasal mucosal T lymphocytes from OVA-sensitized mice*

	RSV-infected mice (%)	Sham-infected mice (%)
IL-5	11.9	6.2
IFN- γ	17.4	11.4

OVA, ovalbumin; RSV, respiratory syncytial virus.

*Mean of two groups and each group consisted of T lymphocytes collected from nasal mucosa of seven mice.

**Fig. 2.** The number of nasal rubbing attacks in ovalbumin (OVA)-sensitized mice following OVA provocation. Respiratory syncytial virus (RSV) infection in OVA-sensitized mice induced a dramatic enhancement of number of attacks. The anti-IL-5 treatment reduced the enhancement in some degree and the topical administration of the neurokinin (NK)-1/NK-2 receptor antagonist did more.**Fig. 3.** The number of eosinophils in the nasal mucosa. Respiratory syncytial virus (RSV) infection markedly increased the eosinophil infiltration in ovalbumin (OVA)-sensitized mice. The treatment with anti-IL-5 antibodies reduced the number significantly but not with neurokinin (NK)-1/NK-2 receptor antagonists.

RSV replication on day 4 was significantly reduced in OVA-sensitized mice. However, the use of anti-IL-5 did not exhibit any influence on viral replication and no reduction in viral shedding was observed in anti-IL-5-treated OVA-sensitized mice (Fig. 5).

Discussion

The above studies were designed to examine the mechanism of nasal hypersensitivity observed during viral infections. A

murine RSV infection model was used in which the quantitative analysis of nasal rubbing attacks was evaluated as a measure of nasal hypersensitivity. Sneezes in mice are not clearly distinguishable as in humans and are difficult to quantify precisely. The evaluation of nasal obstruction is also difficult, because mice cannot survive by breathing orally. BALB/c mice are known to be sensitive to allergic reactions [27], particularly in the lower respiratory tract, although their nasal reactivity to histamine and other antigens is quite low (data not shown). While C57BL/6 mice are known to mount a Th1 dominant immune response [28], IgE production is inducible in these animals if the correct adjuvant, such as alum, is used, and nasal hypersensitivity can be observed after the topical administration of histamine or antigens. In light of the above and because RSV replication in the nose of BALB/c mice is tolerated well by these animals, we chose to use C57BL/6 mice in our study.

The observations summarized in this report suggest that experimentally induced infection with RSV results in significant enhancement of nasal sensitivity to OVA and histamine in previously sensitized animals. OVA-sensitized animals also exhibited increased expression of IL-5 and IFN- γ and pronounced accumulation of eosinophils in the nasal mucosa after RSV infection.

The mechanisms underlying the development of hypersensitivity states after viral infections such as RSV have not been clinically defined. It is possible that viral infection-associated mucosal damage; recruitment of mast cells, eosinophils and other cellular mediators of hypersensitivity; and activation of cholinergic, adrenergic or non-adrenergic non-cholinergic neurogenic mechanisms may play an important role in the development of mucosal hypersensitivity states [29–31].

In the present studies, pre-treatment with anti-IL-5 resulted in significant decrease in the accumulation of eosinophils. However, such treatment did not influence the degree of viral induced hypersensitivity. In fact, anti-IL-5 treatment was associated with decreased viral elimination in the nasal cavity, and as a result eosinophils may be associated with accelerated RSV elimination. It has been shown that eosinophil cationic protein and eosinophil-derived neurotoxin may act as rebonuclease-dependent antiviral agents [32]. In the present studies, it is interesting to note that use of IFN- γ was associated with increasing eosinophil counts but did not influence nasal hypersensitivity reactions. Thus, although eosinophils may play an important role in viral induced allergic inflammation [33, 34], eosinophils did not seem to contribute to nasal hypersensitivity to OVA in the current experimental setting. IFN- γ is a classical Th1 cytokine that has been shown to reduce allergic reactions when administered during sensitization [35]. However, treatment of OVA-sensitized animals with anti-IFN- γ neutralizing antibody did not decrease nasal sensitivity to OVA during RSV infection.

The observation of particular interest in the current studies is the significant reduction of nasal hypersensitivity detected after the use of NK-1/NK-2 antagonists, although such treatment did not influence eosinophil counts. Recently, it has been shown that infection with RSV frequently is associated with activation of NK receptor sites [36–38]. Tachykinin family of neuropeptides such as substance P have been shown to exhibit strong binding affinity for NK receptors especially NK-1. Such receptor-neuropeptide interactions are associated

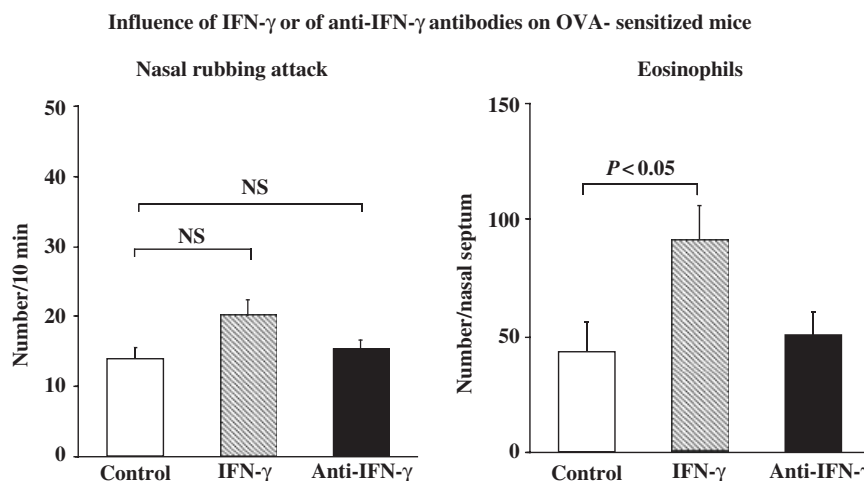


Fig. 4. Influence of IFN- γ and anti-IFN- γ antibodies on ovalbumin (OVA)-sensitized mice. The nasal administration of IFN- γ increased the number of eosinophils, but did not affect the nasal symptoms. Anti-IFN- γ treatment had no effect on either nasal symptoms or eosinophil numbers.

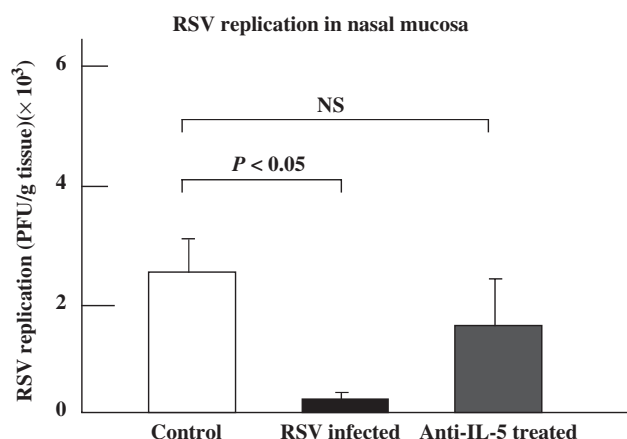


Fig. 5. Respiratory syncytial virus (RSV) replication in the nasal mucosa on day 4 after RSV inoculation. Replication was reduced in ovalbumin (OVA)-sensitized mice, but this reduction was abolished in anti-IL-5-treated OVA-sensitized mice. Non-OVA-sensitized mice were used as controls.

with a wide variety of biologic inflammatory effects, including changes in vascular permeability, mucous secretion, leucocyte chemotaxis and bronchoconstriction [39–41]. It is thus suggested that RSV-associated increase in allergic nasal hypersensitivity to OVA and possibly to other allergens may in part be related to activation of neuropeptide receptors during acute viral infection of the nasal mucosa.

It is possible that increased eosinophil recruitment is mediated by chemokines induced by IFN- γ . Recently induction of eotaxin 3 and IP-10 by IFN- γ in mucosal cell cultures has been demonstrated after experimental RSV infection in *in vivo* settings [42–44]. Based on these reports and the present studies, it is proposed that a possible relationship exists between IFN- γ and induction, recruitment and/or activation of eosinophils in allergic sensitization in the nasal mucosa during viral infections.

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References

- 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; 324:1–5.
- Message SD, Johnston SL. Viruses in asthma. *Br Med Bull* 2002; 61:29–43.
- Gern JE. Viral and bacterial infections in the development and progression of asthma. *J Allergy Clin Immunol* 2000; 105: s497–502.
- Johnston SL, Plattermore PK, Sanderson G et al. The relationship between upper respiratory infections and hospital admissions for asthma: a time-trend analysis. *Am J Respir Crit Care Med* 1996; 154:654–60.
- Tuffaha A, Gern JE, Lemanske Jr RF. The role of respiratory viruses in acute and chronic asthma. *Clin Chest Med* 2000; 21:289–300.
- Schwarze J, Gelfand EW. The role of viruses in development or exacerbation of atopic asthma. *Clin Chest Med* 2000; 21:279–87.
- Johnston SL, Plattermore PK, Sanderson G et al. Community study of role of viral infections in exacerbations of asthma in 9–11 year old children. *BMJ* 1995; 310:1225–9.
- Makela MJ, Puhakka T, Ruuskanen O et al. Viruses and bacteria in the etiology of the common cold. *J Clin Microbiol* 1998; 36:539–42.
- Irwin RS, Madison JM. The diagnosis and treatment of cough. *N Engl J Med* 2000; 343:1715–21.
- Doyle WJ, Skoner DP, Fireman P et al. Rhinovirus 39 infection in allergic and non-allergic subjects. *J Allergy Clin Immunol* 1992; 89:968–78.
- Bardin PG, Fraenkel DJ, Sanderson G et al. Amplified rhinovirus colds in atopic subjects. *Clin Exp Allergy* 1994; 24:457–64.
- Avila PC, Abisheganaden JA, Wong H et al. Effects of allergic inflammation of the nasal mucosa on the severity of rhinovirus 16 cold. *J Allergy Clin Immunol* 2000; 105:923–32.
- Folkerts G, Busse WW, Nikamp FP, Sorkness R, Gern JE. Virus-induced airway hyperresponsiveness and asthma. *Am J Respir Crit Care Med* 1998; 157:1708–720.

- 14 Van Schaik SM, Welliver RC, Kimpen JK. Novel pathways in the pathogenesis of respiratory syncytial virus disease. *Pediatr Pulmonol* 2000; 30:131–8.
- 15 Shay DK, Holman RC, Newman RD, Liu LL, Stout JW, Anderson LJ. Bronchiolitis-associated hospitalizations among US children, 1980–1999. *JAMA* 1999; 282:1440–6.
- 16 Sigurs N, Bjarnason R, Sigurbergsson F, Kjellman B. Respiratory syncytial virus bronchiolitis in infancy is an important risk factor for asthma and allergy at age 7. *Am J Respir Crit Care Med* 2000; 161:1501–7.
- 17 Sigurs N, Bjarnason R, Sigurbergsson F, Kjellman B, Bjorksten B. Asthma and immunoglobulin E antibodies after respiratory syncytial virus bronchiolitis: a prospective cohort study with matched controls. *Pediatrics* 1995; 95:500–5.
- 18 Hall CB, Hall WJ, Gala CL, McGill FB, Leddy JP. Long-term prospective study in children after respiratory syncytial virus infection. *J Pediatr* 1984; 105:358–64.
- 19 Eisen AH, Bacal HL. The relationship of acute bronchiolitis to bronchial asthma—a 4 to 14-year follow up. *Pediatrics* 1963; 31: 859–61.
- 20 Sims DG, Downham MAPS, Gardner PS, Webb JKG, Weighman D. Study of 8-year-old children with a history of respiratory syncytial virus bronchiolitis in infancy. *BMJ* 1978; 1:11–4.
- 21 Welliver RC, Duffy L. The relationship of RSV-specific immunoglobulin E antibody responses in infancy, recurrent wheezing, and pulmonary function at age 7–8 years. *Pediatr Pulmonol* 1993; 15:19–27.
- 22 Pullan CR, Hey EM. Wheezing, asthma, and pulmonary dysfunction 10 years after infection with respiratory syncytial virus in infancy. *Br Med J* 1982; 284:1665–9.
- 23 Ueba O. Respiratory syncytial virus 1 concentration and purification of the infectious virus. *Acta Med Okayama* 1978; 32:265–72.
- 24 Morimoto H, Murai M, Maeda Y et al. FK224, a novel cyclopeptide substance P antagonist with NK1 and NK2 receptor selectivity. *J Pharmacol Exp Ther* 1992; 262:398–402.
- 25 Mota I, Wong D. Homologous and heterologous passive cutaneous anaphylactic activity of mouse antiserum during the course of immunization. *Life Sci* 1969; 8:813–20.
- 26 Matsuoka T, Okamoto Y, Matsuzaki Z et al. Characteristics of immunity induced by viral antigen or conferred by antibody via different administration routes. *Clin Exp Immunol* 2002; 130: 386–92.
- 27 Sato M, Iwakabe K, Ohta A, Sekimoto M, Kimura S, Nishimura T. Self-priming cell culture system for monitoring genetically controlled spontaneous cytokine-producing ability in mice. *Immunol Lett* 1999; 70:173–8.
- 28 Shankar AH, Titus RG. T cell and non-T cell compartments can independently determine resistance to *Leishmania major*. *J Exp Med* 1995; 181:845–55.
- 29 Fryer AD, Jacoby DB. Parainfluenza virus infection damages inhibitory M₂ muscarinic receptors on pulmonary parasympathetic nerves in the guinea-pig. *Br J Pharmacol* 1991; 102:267–71.
- 30 Jacoby DB, Fryer AD. Interaction of viral infections with muscarinic receptors. *Clin Exp Allergy* 1999; 29: 59–64.
- 31 Adamko DJ, Yost BL, Gleich GJ, Fryer AD, Jacoby DB. Ovalbumin sensitization changes the inflammatory responses to subsequent parainfluenza infection: eosinophils mediate airway hyperresponsiveness, M₂ muscarinic receptor dysfunction, and antiviral effects. *J Exp Med* 1999; 190:1465–77.
- 32 Domachowske JB, Dyer KD, Adams AG, Leto TL, Rosenberg HF. Eosinophil cationic protein 1 R Nase 3 is another RNase A-family ribonuclease with direct antiviral activity. *Nucleic Acids Res* 1998; 26:5327–32.
- 33 Kay AB. Modulation of eosinophil function in vitro. *Clin Exp Allergy* 1990; 20:31–4.
- 34 Terada N, Konno A, Tada H, Shirotori K, Ishikawa K, Togawa K. The effect of recombinant human interleukin-5 on eosinophil accumulation and degranulation in human nasal mucosa. *J Allergy Clin Immunol* 1992; 90:160–8.
- 35 Young HA, Hardy KJ. Role of interferon-gamma in immune cell regulation. *J Leukocyte Biol* 1995; 58:373–81.
- 36 Piedimonte G, Rodriguez MM, King KA, McLean S, Jiang X. Respiratory syncytial virus upregulates expression of the substance P receptor in rat lung. *Am J Physiol* 1999; 277:L831–40.
- 37 King KA, Hu C, Rodriguez MM, Jiang X, Piedimonte G. Exaggerated neurogenic inflammation and substance P receptor upregulation in RSV-infected weanling rats. *Am J Respir Cell Mol Biol* 2001; 24:101–7.
- 38 Tripp RA, Barskey A, Goss L, Anderson LJ. Substance P receptor expression on lymphocytes is associated with the immune response to respiratory syncytial virus infection. *J Neuroimmunol* 2002; 129:141–53.
- 39 Konno A, Numata T, Terada N, Hanazawa T, Nagata H, Motosugi H. Role of substance P in the vascular response of nasal mucosa in nasal allergy. *Ann Otol Rhinol Laryngol* 1996; 105:648–53.
- 40 Gungor A, Baroody FM, Naclerio RM, White SR, Corey JP. Decreased neuropeptide release may play a role in the pathogenesis of nasal polyps. *Otolaryngol Head Neck Surg* 1999; 121: 585–90.
- 41 Okamoto Y, Shirotori K, Kudo K et al. Cytokine expression after the topical administration of substance P to human nasal mucosa. *J Immunol* 1993; 151:4391–8.
- 42 Yamamoto S, Kobayashi I, Tsuji K et al. Upregulation of IL-4R by IFN- γ : enhanced IL-4 induced eotaxin-3 production in airway epithelium. *Am J Respir Cell Mol Biol* 2004.
- 43 Azuma MA, Szczepanik M, Tsuji RF et al. Early delayed-type hypersensitivity eosinophil infiltrates depend on T helper 2 cytokines and interferon- γ via CXCR3 chemokines. *Immunology* 2004; 111:306–17.
- 44 Li H, Chunsong H, Guobin C et al. Highly up-regulated CXCR2 expression on eosinophils in mice infected with *Schistosoma japonicum*. *Immunology* 2004; 111:107–17.