

Suppression of IFN-gamma production in atopic group at the acute phase of RSV infection

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Several studies have suggested that respiratory syncytial virus (RSV) bronchiolitis induced the change of cytokine production profile in childhood. We sought to determine whether the RSV-induced cytokine production was affected by the patient's atopic background. We quantified interferon-gamma (IFN-gamma) and interleukin (IL)-4 in the supernatant of peripheral blood mononuclear cells (PBMCs) cultured for 24 h and in the presence of phytohemagglutinin (PHA), IL-12, or IL-18, from 14 infants who were divided into two groups, those who are non-atopic and an atopic group. In RSV-infected infants with atopic diseases, IFN-gamma production from IL-12- or especially IL-18-stimulated PBMCs was subtotally suppressed in the acute phase, whereas in RSV-infected infants without atopic diseases IFN-gamma production was not suppressed on acute phase. The IFN-gamma suppression observed in the atopic group is not caused by the immaturity of an infant's immune system since reduced IFN-gamma production to RSV is not observed in the infants of non-atopic group. IFN-gamma suppression in regard to RSV infection might be caused by some genetic factor involved in the development of atopic disease such as IL-18 signal cascade.

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Respiratory syncytial virus (RSV) remains the most prevalent infectious cause of lower respiratory tract illness in both infants and children (1–3). Most infections with RSV are symptomatic; however, the range of severity in infants and young children varies greatly. Several studies have suggested that infants in whom RSV bronchiolitis develops will have recurrent wheezing and asthma later in childhood (4). The reasons why this occurs are still unknown; whether RSV is the actual causal factor for asthma or simply targets children who are predisposed to bronchial obstructive disease is still speculation (5). Further complicating the picture is the question of whether there is a link between RSV infection in infancy and the development of wheeze or asthma later in childhood. Results from clinical

studies have been contradictory, with some results supporting a link, and others disproving a link (6, 7).

It has been shown in animal studies that viral infections can increase the risk of allergic sensitization. A viral influence causing a switch from Th2 to Th1 lymphocytes, and thus a lower production of IgE antibodies, has been suggested, but a more complex mechanism may operate in RSV infection, judging from animal studies and the results of the present and other clinical studies (8). It has been hypothesized that an early RSV infection may perpetuate Th2-dominant immune responses (9).

Aberle et al. tried to compare both the levels of IFN-gamma mRNA expression in peripheral blood mononuclear cells (PBMCs) and the distribution of lymphocyte subpopulations in

infants with severe RSV bronchiolitis and those with a milder clinical course of illness (10). Their data indicated that reduced IFN-gamma expression might be an important factor in the pathogenesis of severe RSV disease in infancy.

In this study we tried to determine whether lymphocytes in children with atopy or some genetic background show a different immune response to RSV compared with non-atopic children. Our results showed that the reduced IFN-gamma expression was observed specifically in the atopic group.

Subjects and methods

Subjects

Fourteen children between 1 month and 4 yr of age, were admitted for respiratory tract infection to our hospital during the epidemic period of 2002–2003. All of our cases were admitted in our hospital according to the dyspnea and/or the exhaustion because of the continuance of the high fever. They had infections with a fever and coughing with and without bronchial obstruction. Their nasopharyngeal secretions were positive for the RSV antigen by enzyme-linked immunosorbent assay (ELISA) kit (Directigen RS, Becton, Japan). They were divided into two groups: (i) with no allergic disease or history thereof, and (ii) with allergic disease or a family history and/or a past history of allergic disease. The atopic group in this study consisted of subjects with bronchial asthma and atopic dermatitis or a family history within one generation of allergic diseases. There were no significant differences in age, sex and clinical severity between the non-atopic and atopic group. The diagnosis of bronchial asthma was made according to the criteria of the American Thoracic Society, and that of atopic dermatitis was made according to the criteria of Hanifin. Informed consent was obtained from their parents. All of the subjects were randomly selected among patients in our hospital. This study was approved by Gifu University ethical committee.

Cell preparation and culture

Peripheral blood mononuclear cells were isolated from the heparinized blood of patients by gradient centrifugation in Ficoll-Paque (Pharmacia AB, Uppsala, Sweden). PBMCs were suspended to give a density of 10^6 /ml in the culture medium which consisted of RPMI 1640 supplemented with 10% heat-inactivated fetal calf serum, L-glutamine (2 mmol/l), penicillin (100 U/ml)

and streptomycin (100 µg/ml). PBMCs (10^6 /ml) were cultured in the presence of 10 µg/ml phytohemagglutinin (PHA) (Gibco BRL, Grand Island, NY, USA), interleukin (IL)-12 (5 IU/ml), or IL-18 (400 ng/ml) in a volume of 1 ml in a round-bottom tube (Falcon 2059, Becton Dickinson Labware, Lincoln Park, NJ, USA) at 37°C in a humidified atmosphere containing 5% CO₂. The recombinant IL-18 had been prepared in our laboratory through use of an *Escherichia coli* expression system [according to a method developed by Kato et al. (11)].

Assays for IFN-gamma and IL-4

The test-tube culture supernatants were spun to remove the cells after culturing and were stored, frozen at –80°C until assayed. IFN-gamma concentrations were measured with a human IFN-gamma ELISA kit (Ohtsuka, Tokyo, Japan), and the detection limit was 15.6–1000 pg/ml. IL-4 concentrations of the culture supernatants were measured with a human IL-4 US ELISA kit (Bio Source, Camarillo, CA, USA). When cytokine values were not detectable, for statistical analysis the minimum detectable level was used.

IgE assay

Plasma samples obtained from heparinized blood were kept at –30°C. Plasma IgE concentrations were determined by chemiluminescent enzyme immunoassay. The values were regarded as the serum IgE concentrations. Specific IgE antibodies for house dust and *Dermatofagoides farinae* were determined by fluoroenzyme assay (CAP RAST FEIA, Pharmacia & Upjohn, Sweden).

Statistical analysis

The significance of the difference between the control and atopic group was analyzed by the Wilcoxon–Mann–Whitney test. A statistical significance was assumed for p-values < 0.05.

Results

The patients with RSV infection were divided into non-atopic and atopic group (Table 1). Clinical severity was determined by the period of fever, wheezing and the hospitalization period. No patients had a prior episode to RSV infection. There were no significant differences in age, sex and clinical severity although the hospitalization period of atopic group seemed to be longer than non-atopic group (p = 0.079). In all

Table 1. Clinical features of the patients divided into non-atopic and atopic group

	Sex	Age	FH or PH	Fever (day)	Wheeze	IgE (IU/ml)	RAST	Hospitalization period* (day)
Non-atopic 1	M	1 yr	—	1	—	111.8	HD 0, DF 0	9
Non-atopic 2	M	1 yr	—	4	—	15.7	HD 0, DF 0	13
Non-atopic 3	F	3 month	—	6	+	118.7	HD 0, DF 0	11
Non-atopic 4	F	1 month	—	1	+	0.5	HD 0, DF 0	10
Non-atopic 5	M	3 month	—	2	+	2.1	HD 0, DF 0	6
Non-atopic 6	M	1 yr	—	0	+	19.8	HD 0, DF 0	7
Atopic 1	F	10 month	BA	0	+	57.5	HD 0, DF 0	17
Atopic 2	F	4 yr	BA	1	+	42	HD 0, DF 0	13
Atopic 3	M	1 yr	BA	1	+	200	HD 5, DF 4	6
Atopic 4	M	4 yr	BA	5	+	819.3	HD 3, DF 3	19
Atopic 5	F	4 yr	BA/AD	1	+	6488.4	HD 6, DF 6	9
Atopic 6	M	2 yr	BA	2	+	4.2	HD 0, DF 0	17
Atopic 7	F	1 month	FH	1	+	2.2	HD 0, DF 0	15
Atopic 8	F	1 month	FH	1	+	0.5	HD 0, DF 0	13

Sex: m, male; f, female; FH, family history within one generation of allergic diseases; PH, past history; BA, bronchial asthma; AD, atopic dermatitis; fever, period of fever over 38°C; RAST, CAP RAST scores; HD, house dust; DF, *Dermatophagoides farinae*.

*p-value = 0.079.

patients (n = 14), the IFN-gamma concentration from PHA-stimulated PBMCs between the acute and convalescent phase is shown in Fig. 1a. The concentrations of IFN-gamma in all RSV-infected patients (n = 14) did not show remarkable change between the acute and convalescent phase (Table 2). RSV infected patients were divided into two groups, a non-atopic group (n = 6) and an atopic group (n = 8). In the non-atopic and atopic group, the IFN-gamma concentration from PHA-stimulated PBMCs is shown in Fig. 1b. There was no difference in IFN-gamma production by PHA-stimulated PBMCs in either group. In both groups, the IFN-gamma concentration from IL-12-stimulated PBMCs is shown in Fig. 1c. In the atopic group, the IFN-gamma concentration from IL-12-stimulated PBMCs was significantly lower in the acute phase than that in the convalescent phase ($p = 0.024$). Similarly, in the atopic group the IFN-gamma concentration from IL-18-stimulated PBMCs was significantly lower in the acute phase than that in the convalescent phase ($p = 0.0018$) (Fig. 1d). The change of IFN-gamma production by IL-18-stimulated PBMCs was more significant ($p = 0.0018$) than that by IL-12-stimulated PBMCs ($p = 0.024$). In addition, in the acute phase the IFN-gamma concentrations by IL-12- or IL-18-stimulated PBMCs in the atopic group were significantly lower than those in the non-atopic group ($p = 0.013$ or $p = 0.009$, respectively), while in the convalescent phase the IFN-gamma concentrations by IL-18-stimulated PBMCs in the atopic group were significantly higher than those in the non-atopic group ($p = 0.028$).

The concentrations of IL-4 in all RSV-infected patients (n = 14) between the acute and convalescent phase did not show any remarkable change (Fig. 2). Furthermore, no difference of IL-4 production between the non-atopic and atopic group was detected.

Discussion

In the present study, we have shown that in the atopic group in the acute phase the IFN-gamma concentrations by IL-12- or IL-18-stimulated PBMCs were significantly lower than those in the non-atopic group. Furthermore, the IFN-gamma concentrations by IL-18-stimulated PBMCs in the atopic group were significantly higher than those in the non-atopic group in the convalescent phase. However, no difference of IL-4 production between the non-atopic and atopic group was detected.

We tried to determine whether lymphocytes in children with atopy or a family history of allergic diseases show a different immune response to RSV compared with non-atopic children. Our results suggest that the atopic disease or some genetic background of atopy might be one of the factors which prolongs RSV infection because of a reduced Th1 reaction to RSV, although we could not find any significant differences in the clinical course between the non-atopic group and atopic group.

Our results showed that reduced IFN-gamma expression was observed specifically in the atopic group in the acute phase. As low IFN-gamma expression in RSV is observed specifically in the

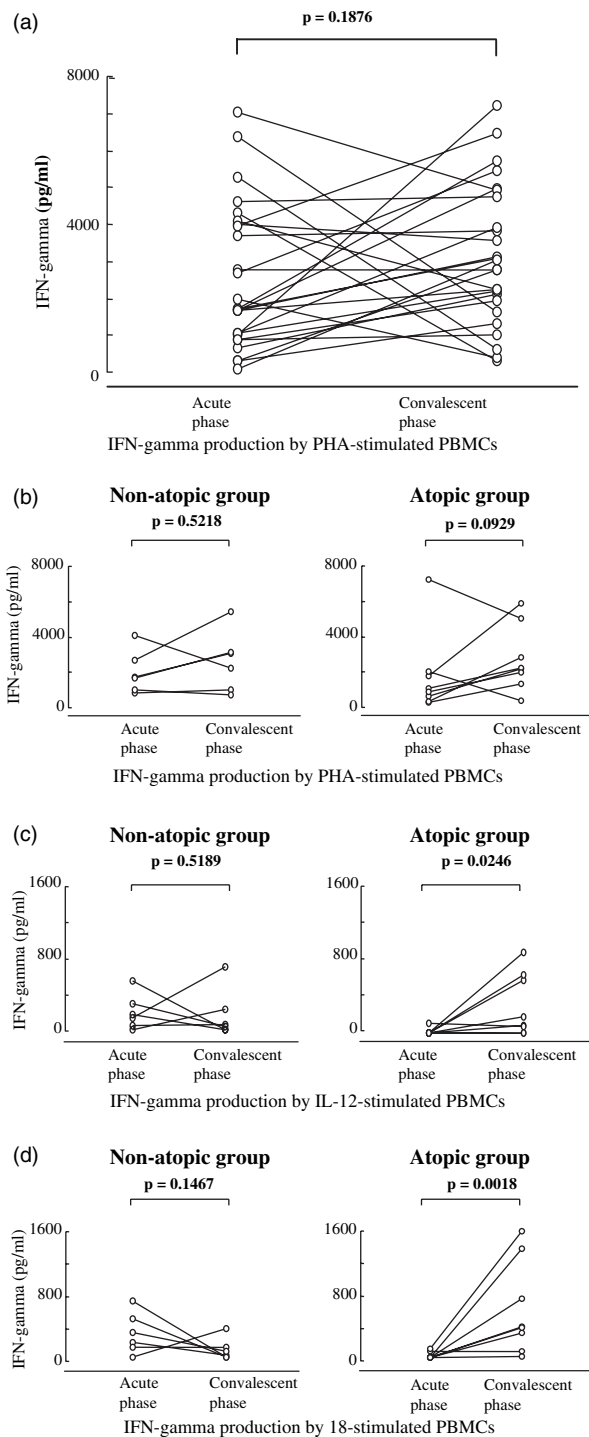


Fig. 1. (a) In all patients ($n = 14$), interferon (IFN)-gamma concentration from phytohemagglutinin (PHA)-stimulated peripheral blood mononuclear cells (PBMCs) between the acute and convalescent phase. (b) In the non-atopic group ($n = 6$) and atopic group ($n = 8$), IFN-gamma concentration from PHA-stimulated PBMCs between the acute and convalescent phase. (c) In the non-atopic and atopic group, IFN-gamma concentration from IL-12-stimulated PBMCs between the acute and convalescent phase. (d) In the non-atopic and atopic group, IFN-gamma concentration from IL-18-stimulated PBMCs between the acute and convalescent phase.

atopic group, it may not be due to an inhibitory effect of RSV, which is capable of suppressing both non-specific and RSV-specific lymphocyte proliferation (12). In addition, there was no significant difference about ages between non-atopic and atopic group (Table 1). Reduced IFN-gamma production was observed only in atopic group. Therefore we assumed that a weak IFN-gamma response to RSV may not result from the immaturity of an infant's immune system.

One factor controlling the level of cytokine expression is genotype. Gentile et al. reported that the IFN-gamma genotype is related to the severity of a lower respiratory illness, the duration of intensive care unit stay and the frequency of otitis media (13). In Japanese children the association of IFN-gamma polymorphism with atopic asthma has been reported. It would be intriguing to investigate IFN-gamma polymorphism in our atopic group.

Several lines of evidence have shown the features of cytokine production in RSV infection. Pala et al. reported that enhanced IL-4 responses in children with a history of RSV bronchiolitis in infancy (14). Association of cytokine responses with disease severity in infants with RSV infection was reported (15, 16). van Bentem et al., showed that RSV-induced bronchiolitis but not upper respiratory tract infection was accompanied by an increased nasal IL-18 response (17). Joshi et al. showed that RSV is associated with lower IFN-gamma production in young babies compared with upper respiratory tract infections (18). These results suggest that RSV infection changes the cytokine production and the RSV-induced cytokine production might be partly affected by the time and tissues of taking the samples.

The change of IFN-gamma production by IL-18-stimulated PBMCs was more significant ($p = 0.0018$) than that by IL-12-stimulated PBMCs ($p = 0.024$). This result suggests that in RSV infection the IL-18 receptor signal cascade might be specifically inhibited. Our previous reports showed that some atopic diseases are caused by impairment of IL-18R α chain 950 del CAG, which downregulates IgE production (19). We have not yet determined what mechanism induces the predominant expression of the IL-18R α chain cDNA in some atopic patients. Viral infection, such as RSV, and environmental factors might be candidate modulators of IL-18R α chain transcript expression (20).

In conclusion, the suppression of IFN-gamma in the acute phase of RSV infection was observed only in the atopic group. These results suggest

Table 2. Interferon (IFN)-gamma and interleukin (IL)-4 production induced by phytohemagglutinin (PHA), IL-12 or IL-18 stimulation

	PHA acute	PHA convalescent	IL-12 acute	IL-12 convalescent	IL-18 acute	IL-18 convalescent	IL-4 acute	IL-4 convalescent
Non-atopic 1	2669.9	5445.4	177.1	15.6	198.1	37.1	70.73	48.07
Non-atopic 2	860.7	1027.1	15.6	244.6	15.6	362.3	39.1	46.3
Non-atopic 3	1001.2	3063.4	144.8	707.2	132	135.8	9.93	41.61
Non-atopic 4	1726.7	3115	547.9	15.6	308	80.4	36.68	10.63
Non-atopic 5	1685.3	2260	301.6	51.3	701.9	15.6	18.68	10.12
Non-atopic 6	4097	722.3	62.8	74.6	476.4	15.6	34.48	41.91
Mean $\pm$ SD	2006 $\pm$ 1209	2605 $\pm$ 1714	208 $\pm$ 193	184 $\pm$ 269	305 $\pm$ 249	107 $\pm$ 132	34 $\pm$ 20	33 $\pm$ 17
Atopic 1	1724.5	5697.3	15.6	523.9	15.6	34.7	34.57	24.38
Atopic 2	653.6	2122.5	15.6	92.7	15.6	372.6	18.83	87.38
Atopic 3	294.1	1315.7	15.6	15.6	15.6	307.2	17.1	60.81
Atopic 4	1990.4	410.2	116.2	81.3	91	92.7	43.27	105.67
Atopic 5	1063.6	2193.8	15.6	15.6	15.6	698.3	78.34	105.7
Atopic 6	317.9	2778.9	15.6	172.1	15.6	364.9	67.97	38.45
Atopic 7	7053.8	4916.7	23	794.7	119	1484.3	32.29	41.54
Atopic 8	869.9	1945.7	15.6	579.2	15.6	1286.2	33.23	10.59
Mean $\pm$ SD	1745 $\pm$ 2229	2672 $\pm$ 1781	29 $\pm$ 35	284 $\pm$ 302	37 $\pm$ 42	580 $\pm$ 538	40 $\pm$ 21	59 $\pm$ 36

Acute, acute phase; convalescent, convalescent phase.

PHA, IL-12, IL-18 = PHA, IL-12 or IL-18-induced IFN-gamma production (pg/ml); IL-4 = PHA-induced IL-4 production (pg/ml).

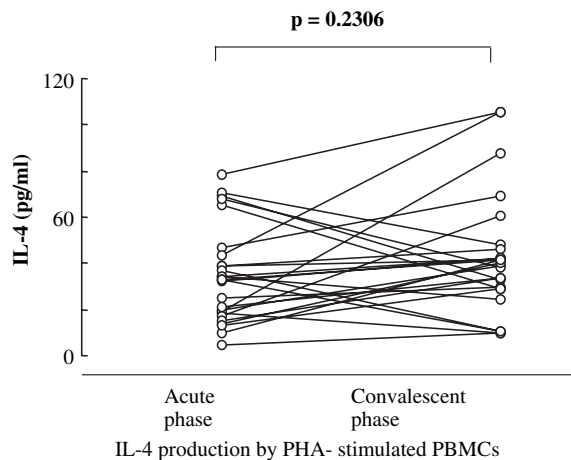


Fig. 2. In all patients (n = 14), IL-4 concentration from phytohemagglutinin-stimulated peripheral blood mononuclear cells between the acute and convalescent phase.

that the significant difference between the non-atopic and atopic group in regard to the immune response to RSV might be caused in part by genetic factors.

References

- FRICK OI, GERMAN DF, MILLS J. Development of allergy children: association with virus infection. *J Allergy Clin Immunol* 1979; 64: 228–241.
- WENNERGREN G, KRISTJANSSON S. Relationship between respiratory syncytial virus bronchiolitis and future obstructive airway diseases. *Eur Respir J* 2001; 18: 1044–58.
- PSARRAS S, PAPADOPOULOS NG, JOHNSTON SL. Pathogenesis of respiratory syncytial virus bronchiolitis-related wheezing. *Paediatr Respir Rev* 2004; 5 (Suppl.): S179–84.
- HENDERSON J, HILLIARD TN, SHERRI A, STALKER D, AL SHAMMARI N, THOMAS HM. Hospitalization for RSV bronchiolitis before 12 months of age and subsequent asthma, atopy and wheeze: a longitudinal birth cohort study. *Pediatr Allergy Immunol* 2005; 16: 386–92.
- HOLT PG, SLY PD, BJORKSTEN B. Atopic versus infectious diseases in childhood: a question of balance? *Pediatr Allergy Immunol* 1997; 8: 53–8.
- BONT L, HEIJNEN CJ, KAVELAARS A, et al. Monocyte IL-10 production during respiratory syncytial virus bronchiolitis is associated with recurrent wheezing in a one-year follow-up study. *Am J Respir Crit Care Med* 2000; 161: 1518–23.
- 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.
- OPENSHAW PJ. Immunity and immunopathology to respiratory syncytial virus: the mouse model. *Am J Respir Crit Care Med* 1995; 152: 59–62.
- ROMAN M, CALHOUN WJ, HINTON KL, et al. Respiratory syncytial virus in infants is associated with predominant Th2-like response. *Am J Respir Crit Care Med* 1997; 156: 190–5.
- ABERLE JH, ABERLE SW, DWORZAK MN, et al. Reduced interferon-gamma expression in peripheral blood mononuclear cells of infants with severe respiratory syncytial virus disease. *Am J Respir Crit Care Med* 1999; 160: 1263–8.
- KATO Z, JEE J, SHIKANO H, et al. The structure and binding mode of interleukin-18. *Nat Struct Biol* 2003; 10: 966–71.
- BONT L, HEIJNEN CJ, KAVELAARS A, et al. Peripheral blood cytokine responses and disease severity in respiratory syncytial virus bronchiolitis. *Eur Respir J* 1999; 14: 144–9.
- GENTILE DA, DOYLE WJ, ZEEVI A, et al. Cytokine gene polymorphisms moderate illness severity in infants with respiratory syncytial virus infection. *Hum Immunol* 2003; 64: 338–44.

14. PALA P, BJARNASON R, SIGURBERGSSON F, METCALFE C, SIGURS N, OPENSHAW PJ. Enhanced IL-4 responses in children with a history of respiratory syncytial virus bronchiolitis in infancy. *Eur Respir J* 2002; 20: 376–82.
15. CHEN ZM, MAO JH, DU LZ, TANG YM. Association of cytokine responses with disease severity in infants with respiratory syncytial virus infection. *Acta Paediatr* 2002; 91: 914–22.
16. DE WAAL L, KOOPMAN LP, VAN BENTEN IJ, et al. Moderate local and systemic respiratory syncytial virus-specific T-cell responses upon mild or subclinical RSV infection. *J Med Virol* 2003; 71: 290–7.
17. VAN BENTEN IJ, VAN DRUNEN CM, KOOPMAN LP, et al. RSV-induced bronchiolitis but not upper respiratory tract infection is accompanied by an increased nasal IL-18 response. *J Med Virol* 2003; 71: 290–7.
18. JOSHI P, SHAW A, KAKAKIOS A, ISAACS D. Interferon-gamma levels in nasopharyngeal secretions with respiratory syncytial virus and other respiratory viral infections. *Clin Exp Immunol* 2002; 131: 143–7.
19. WATANABE M, KANEKO H, SHIKANO H, et al. Predominant expression of 950delCAG of IL-18R α chain cDNA is associated with reduced IFN-gamma production and high serum IgE levels in atopic Japanese children. *J Allergy Clin Immunol* 2001; 109: 669–75.