Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

ELSEVIER

Journal of Clinical Virology 14 (1999) 199–205

Journal of Clinical Virology

www.elsevier.com/locate/jcv

Lowered yields of virus-induced interferon production in leukocyte cultures and risk of recurrent respiratory infections in children

Anne Pitkäranta [a,*], Johanna Nokso-Koivisto [a,b], Virva Jäntti [b], Aino Takala [c], Terhi Kilpi [c], Tapani Hovi [b]

[a] *Department of Otorhinolaryngology, University of Helsinki, PL 220, 00029 HYKS, Helsinki, Finland*
[b] *Department of Virology, National Public Health Institute, Mannerheimintie 160, FIN-00300 Helsinki, Finland*
[c] *Department of Vaccines, National Public Health Institute, Mannerheimintie 160, FIN-00300 Helsinki, Finland*

Received 26 June 1999; accepted 25 August 1999

Abstract

Objective: To study the correlation between the yield of virus-induced interferon (IFN) production in leukocyte cultures and the risk of recurrent respiratory infections. *Methods*: A sample of 71 consecutive children enrolled in the Finnish Otitis Media Cohort Study were selected. Children suffering from frequently recurring respiratory infections (FRRIs) were defined as the highest quintile of the entire cohort of 329 children, as regards the number of upper respiratory infections (URIs) and/or episodes of acute otitis media (AOM) during the follow-up period from 2 to 24 months. *Results*: In the sample of 71 children, there were 18 children with FRRI (≥ 9 URI and/or ≥ 4 AOM). Leukocyte cultures, prepared from blood drawn from these 18 children at 6 months of age, produced lower yields of IFN than those of the remaining 53 children, when stimulated with adenovirus ($P < 0.001$), coronavirus ($P < 0.001$) or rhinovirus ($P = 0.002$). The difference in IFN yields was even greater ($P < 0.001$ with all three viruses) if the comparison was made between children with FRRI and those with no or maximally one URI during the follow-up period. When the IFN production capacity induced by rhinovirus was measured at the age of 24 months, a statistically significant difference between the children with FRRI and the others was also seen ($P = 0.002$). Influenza A virus-induced IFN production capacity did not differ between the groups at either age ($P = 0.209$). *Conclusions*: Lowered IFN responses in children suffering from recurrent URIs and/or AOM may, in a subgroup of the children, be due to a genetic property of the child. However, because of the great interindividual variations, we cannot use the IFN production capacity as such for prediction of forthcoming respiratory infections and/or otitis media.

Keywords: Child; Interferon; Otitis media; Respiratory infection; Virus

* Corresponding author. Tel.: +358-9-47173697; fax: +358-9-47175010.
E-mail address: anne.pitkaranta@helsinki.fi (A. Pitkäranta)

1386-6532/99/$ - see front matter
PII: S1386-6532(99)00056-6

1. Introduction

Recurrent upper respiratory tract infections (URIs) and acute otitis media (AOM) are common problems in children, and some children seem to suffer from apparently continuous or frequently recurring URIs and AOMs. Despite intensive study of risk factors of recurrent respiratory infections, relatively little is known about the factors that could, with some certainty, predict the proneness to respiratory infections. Certain socio-environmental factors, like attendance at day-care centers, are known risk factors for recurring respiratory infections and otitis media, but no unifying biological cause has been discovered for the situation except in very rare cases of primary immunodeficiency (Lyall et al., 1991).

Interferons (IFN) are considered to have a key role in animal host defense against virus infections (Muller et al., 1994). Type I IFNs are synthesized in most nucleated cells as a response to virus infection and comprise several subtypes of IFN-α, a closely related IFN-ω and the more distantly related IFN-β. Production of IFN-γ (type II IFN) is limited to cells of bone marrow origin. It is produced by activated T-lymphocytes and NK cells, but, according to recent reports, also by cells of the myeloid line. Previous studies have shown that induction of IFN-α expression is under genetic control in mice (Muller et al., 1994), whereas regulation of IFN-α production in humans has not been studied extensively. Leukocyte cultures derived from different donors are known to vary remarkably with respect to the yields of IFN induced by standard amounts of viruses (Katschinski et al., 1994). Reduced yields of virus-induced IFN by leukocyte cultures were previously associated with recurrent respiratory infections in children (Isaacs et al., 1981; Bondestam et al., 1984; Vanecek and Lehovcova, 1985; Pitkäranta et al., 1993, 1996). However, in these case-control studies the baseline IFN-responsiveness of the leukocytes of these children was not available and the question remained uncertain whether the decreased IFN response reflected an underlying predisposition or was secondary to the recurrent respiratory infections.

We have now investigated prospectively a cohort of 71 children to determine if the lowered IFN-producing capacity is present before the onset of the occurrence of frequent respiratory infections and to evaluate the possible predictive value of IFN responsiveness in relation to respiratory infection and otitis media development.

2. Material and methods

2.1. Children and study set-up

This study was carried out in the context of the Finnish Otitis Media (FinOM) Cohort Study. A total of 71 children born in the Hervanta area, Tampere, were enrolled consecutively in the study at their second routine visit to the Hervanta Well Baby Clinic at 2 months of age. The entire cohort comprised 329 children. The enrolment of this subcohort took place from 16 May 1995 through 31 August 1995, and the clinical follow-up continued until 31 July 1997. Written informed consent was obtained from the parents during the enrolment visit. The study protocol and consent form were approved in advance by the Ethical Committees of the Finnish National Public Health Institute, Tampere University Hospital and the Departments of Social and Health Care of Tampere city.

The children were followed from their 2nd to their 24th month of age at a special study clinic in which one or two physicians and two or three study nurses worked full time to carry out the clinical follow-up. The children were examined at regularly scheduled monthly visits up to the age of 6 months, thereafter every 3 months up to the age of 18 months, and finally at 24 months of age. A blood sample for preparation of mononuclear cells was scheduled to be obtained from this subcohort at the age of 6 and 24 months.

In addition to the regular visits, the study children had access to the study physician at any day of the week in case of respiratory infection. The parents were asked to bring their child to the study clinic whenever the child needed medical care for acute respiratory infection, especially if they suspected AOM. On these sickness visits,

history was taken in a structured way and physical examination was performed.

The definition of an infection-prone child or a child with frequently recurring respiratory illnesses is difficult and, consequently, the prevalence figures of infection-prone children vary in the literature according to the definitions used and population under study (Westbom and Kornfält, 1987; Tupasi et al., 1990). Our aim was to identify the most infection-prone quintile of the entire cohort of 329 children. The number of URIs and AOM episodes experienced by each child was counted. After reviewing these data, we concluded that the highest quintile had experienced at least nine URIs and/or at least four AOM episodes during the follow-up. These criteria were used to define frequently recurring respiratory infections (FRRIs) in this study.

AOM was defined as a visually abnormal tympanic membrane (with regard to color, position and/or mobility) suggesting middle ear effusion with at least one of the following symptoms or signs of acute infection: fever, earache, irritability, diarrhea, vomiting, acute otorrhea not caused by otitis externa, or other symptoms of respiratory infection (Karma et al., 1987). Moreover, in this analysis only such events were taken into account as AOM events, in which the presence of middle ear effusion was confirmed by collection of at least one middle ear fluid sample. In the FinOM Cohort Study, myringotomy was a routine procedure for AOM with marked symptoms. Consequently, one or two middle ear fluid samples were obtained from 88% of the clinically diagnosed cases of AOM.

URI was diagnosed if the patient had symptoms and/or signs of acute infection and if a nasopharyngeal aspirate (NPA) sample was obtained. AOM and/or URI episode was considered to start when the respective diagnosis was made by the study physician. A new episode could start when at least 30 days had elapsed since the beginning of the previous episode.

2.2. Interferon induction and assay

Preparation of peripheral blood mononuclear leukocytes from heparinized blood has been described earlier (Pitkäranta et al., 1993, 1996). Mononuclear leukocytes were stored frozen at -70°C in RPMI-1640 supplemented with 10% fetal calf serum containing 20% DMSO until assayed for IFN responsiveness. To study virus-induced IFN production, leukocytes were thawed rapidly, sedimented to remove DMSO containing medium and resuspended in growth medium at 2×10^6 cells/ml. Leukocyte cultures were then inoculated with standard amounts of adenovirus type 7a, human coronavirus 229E, human rhinovirus (a clinical isolate provided by T. Ziegler), and influenza A Beijing/353/89/H3N2 (a gift from R. Pyhälä) as reported earlier (Pitkäranta et al., 1993). A single preparation of each virus was used throughout the study stored at -70°C as small aliquots. Culture medium for IFN assay was harvested on day 2 and clarified by centrifugation. The supernatants were stored at -70°C until assayed. For logistic reasons, we could not use freshly isolated leukocytes in the induction assays. The feasibility of using frozen cells was tested in advance with cells from several volunteers. The absolute yields of IFN were usually two-fold lower from frozen-thawed cell preparations than those in the corresponding fresh cultures, but the donor-specific pattern of IFN yields induced by different viruses was retained (data not shown).

IFN concentration was measured by a micromethod based on the reduction of cytopathic effect caused by a standard amount of vesicular stomatitis virus in monolayers of a continuous bovine cell line (NBL), which is not sensitive to human IFN-γ. Hence, we assume that most of the measured IFN activity was IFN-α. A standardized leukocyte IFN preparation (a gift from K. Cantell) was included in all assays so that results could be expressed in IU/ml.

2.3. Statistical methods

Differences between groups in IFN production were tested with a Mann–Whitney U-test.

3. Results

3.1. Children and respiratory tract infections

The demographic and clinical data of the 71 children studied are shown in Table 1. Among the 71 children, 18 (25%) children had ≥ 9 URI and/or ≥ 4 AOM during their first 2 years and thus belonged to the FRRI group. A total of eight (11%) children did not have any URI or AOM at all, and seven (9%) children had only one URI during their first 2 years of life. There were altogether 35 boys and 36 girls in the sample. There was no trend for either sex being overrepresented among the children with FRRI.

The first URI occurred at a mean age of 4.4 months (range 1.5–13 months) in the FRRI group and at 8.8 months (range 2.5–21.5 months) in the remaining children (excluding the eight children who had no respiratory infections during the follow-up). Altogether, these 71 children experienced 289 URI and 127 AOM episodes during their first 2 years of life: 50% of all URI and 64% of all AOM episodes occurred in the 18 children with FRRI.

3.2. In vitro IFN responsiveness

Leukocytes of 71 children were examined for IFN production after stimulation with adeno-, corona-, rhino- and influenza A viruses at 6 months of age. IFN yields from cultures stimulated by common respiratory viruses were lower for the 18 children with FRRI than for the remaining 53 children (Table 2). Significant differences existed between these two groups of children with adenovirus, coronavirus, rhinovirus, but not with influenza A virus (Table 2). When IFN production by virus-stimulated leukocytes from 18 FRRI children was compared with the 15 children who had none or only one URI, the differences were even more striking (Table 3). This was due to an enhanced IFN responsiveness in the subgroup of children with no or only one URI episodes.

At the age of 2 years, leukocytes were available from 18 children with FRRI and from 33 other children. Again, the IFN yields induced by rhinovirus were statistically significantly lower among the FRRI children than among the children with less frequent illness. No significant difference was seen when IFN was induced by influenza A virus (Table 2). The leukocyte amounts recovered from the frozen specimens did not allow testing for other viruses.

When the comparison of IFN production capacity at the age of 6 months was carried out only for those children whose first URI and/or AOM started after 6 months of age, there was a tendency of the leukocytes from the seven FRRI children to produce less IFN than those from the 31 non-FRRI children when stimulated with

Table 1
Demographic and clinical data of 71 children followed from 2 to 24 months of age divided according to the number of respiratory infections and acute otitis media

	≥ 9 Respiratory infections and/or ≥ 4 acute otitis media ($n = 18$)	≤ 8 Respiratory infections and ≤ 3 acute otitis media ($n = 53$)
Gender		
Male	9	26
Female	9	27
Number of acute otitis media episodes (mean)	3.9	1.2
The age of the child at the first URI/otitis episode		
Mean (months)	4.4	8.8[a]
Range (months)	1.5–13	2.5–21.5

[a] Not including eight children who did not have any respiratory infections.

Table 2
Virus induced interferon produced by leukocyte cultures from 18 children with frequently recurring respiratory infections (FRRI) and from 53 other children

Inducer	Median (min–max) IFN yield (IU/ml)		P^b
	Children with FRRI ($n=18$)	Non-FRRI children ($n=53$)	
Rhinovirus			
At 6 months	10 (3–160)	40 (3–320)	0.002
At 2 years[a]	5 (5–160)	40 (5–320)	0.002
Adenovirus at 6 months	5 (3–40)	20 (5–640)	0.001
Coronavirus at 6 months	10 (5–40)	40 (10–160)	0.001
Influenza A virus			
At 6 months	320 (160–2560)	640 (160–2560)	0.209
At 2 years[a]	320 (80–1280)	320 (160–1280)	0.209

[a] At the age of 2 years there were leukocytes available from 18 children with ≥9 URI and/or ≥4 AOM and from 33 children with ≤8 URI and/or ≤3 AOM.
[b] Statistical significance of the difference assessed by the Mann–Whitney *U*-test.

Table 3
Virus-induced interferon produced by leukocyte cultures from 18 children with ≥9 URI and/or ≥4 AOM compared with 15 children with ≤1 URI at the age of 6 months

Inducer	Median (min–max) IFN yield (IU/ml)		P^a
	Children with ≥9 URI and/or ≥4 AOM ($n=18$)	Children with ≤1 URI ($n=15$)	
Rhinovirus	10 (3–160)	160 (5–320)	<0.001
Adenovirus	5 (3–40)	80 (5–640)	<0.001
Coronavirus	10 (5–40)	80 (10–160)	<0.001
Influenza A virus	320 (160–2560)	320 (80–640)	0.784

[a] Statistical significance of the difference assessed by the Mann-Whitney *U*-test.

Table 4
Virus induced interferon produced by leukocyte cultures at the age of 6 months from seven FRRI[a] children compared with 31 non-FRRI children whose first URI and/or AOM started after 6 months of age

Inducer	Median (min–max) IFN yield (IU/ml)		P^b
	FRRI children ($n=7$)	Non-FRRI children ($n=31$)	
Rhinovirus	10 (3–160)	40 (5–320)	0.263
Adenovirus	5 (5–10)	20 (3–320)	0.054
Coronavirus	10 (5–40)	40 (10–160)	0.006
Influenza A virus	320 (160–2560)	640 (80–2560)	0.845

[a] FRRI, frequently recurring respiratory infection.
[b] Statistical significance of the difference assessed by the Mann–Whitney *U*-test.

adeno-, corona- and rhinovirus. Statistically significant difference was seen with cultures stimulated with coronavirus (Table 4).

The production of IFN was not influenced by the gender of the children. Spontaneous IFN production was not detected in leukocyte cultures of children.

4. Discussion

Our previous studies (Pitkäranta et al., 1993, 1996) indicated that impaired virus-induced production of IFN by cultured leukocytes ex vivo showed a correlation with a record of frequently recurring respiratory illnesses. The studies did not determine whether this deficiency reflected a genetic trend to increased susceptibility to respiratory infections or was a secondary consequence of the frequent illnesses. The present study was designed to investigate the correlation in a prospective set-up. As a group, leukocytes from the 18 children with frequent illnesses (≥ 9 URI and/or ≥ 4 AOM) produced decreased IFN amounts when compared to the IFN production by leukocytes from 53 children with less frequent illnesses (≤ 8 URI and/or ≤ 3 AOM). A similar pattern existed at 6 months and 2 years of age with respect to IFN responsiveness to rhinovirus. The findings suggest that the impaired IFN production in some FRRI children might indeed be a genetic property. We also made a comparison of IFN yields between the children with FRRI and those with no or maximally one URI during the follow-up period. The differences in IFN responsiveness were even greater than in the primary comparison, a finding which further supports the view that the inherent intensity of the virus-induced IFN response might play a role in the susceptibility to URI and/or AOM. Of note, there are many examples of genetically determined aberrations in the production of other cytokines that result in increased severity of other infections (McGuire et al., 1994; Stuber et al., 1996; Westendorp et al., 1997), including such a common disease as periodontitis (Kornman et al., 1997).

It is remarkable that this difference was seen when the leukocytes were stimulated with adeno-, corona- or rhinoviruses but not with influenza A virus. This virus-specific difference was also seen in our earlier studies (Pitkäranta et al., 1991, 1993) and similar observations have been reported by others (Neustock et al., 1993). It is noteworthy, that the overall IFN yields obtained with the influenza A virus in our study are much higher than those seen with the other viruses revealing the difference between the groups of children. Influenza viruses are very effective IFN inducers which may mask differences in production capacity. In addition to the virus inducer-specific differences, there were also great interindividual variations in IFN yields. This makes it difficult to use the IFN producing capacity as such as a prognostic marker for recurrent respiratory infections at the individual level.

In conclusion, we have shown that reduced IFN-responsiveness in the leukocytes of children suffering from recurrent respiratory infections and/or otitis media may, in a subgroup of children, be due to a genetic predisposition of the child. Unfortunately IFN production capacity cannot presently be used to predict the risk of future respiratory illnesses because of great interindividual and virus-specific variations. Nevertheless, our results encourage the search for ways to identify children who might have genetic predisposition to recurrent respiratory infections and otitis media.

Acknowledgements

We wish to thank all the children and their parents who participated in the study. We thank personnel in the special study clinic in Tampere: Ritva Syrjänen, MD, study doctor, and the study nurses Mervi Martola, RN, Marja-Leena Hotti, RN, Päivi Tervonen, RN, study nurse. Kirsi Ijäs and Riitta Heino are acknowledged for their expert technical assistance.

References

Bondestam M, Alm GV, Foucard T. Interferon production in children with undue susceptibility to infections. Acta Paediatr Scand 1984;73:197–202.

Isaacs D, Clarke JR, Tyrrel DA, Webster ADB, Valman HB. Deficient production of leukocyte interferon (interferon-α) in vitro and in vivo in children with recurrent respiratory tract infections. Lancet 1981;31:950–2.

Karma P, Palva T, Kouvalainen K, Kärjä J, Mäkelä PH, Prinssi VP, et al. Finnish approach to the treatment of acute otitis media. Report of the Finnish concensus conference. Ann Otol Rhinol Laryngol 1987;96(suppl.):1–19.

Katschinski DM, Neustock P, Kluter H, Kirchner H. Influence of various factors on interferon-α production in cul-

ture of human leukocytes. J Interferon Res 1994;14:105–10.

Kornman KS, Crane A, Wang H-Y, et al. The interleukin-1 genotype as a severity factor in adult periodontal disease. J Clin Periodontol 1997;24:72–7.

Lyall EGH, Eden OB, Dixon R, Sutherland R, Thomson A. Assessment of a clinical scoring system for detection of immunodeficiency in children with recurrent infections. Pediatr Infect Dis J 1991;10:673–6.

McGuire W, Hill AVS, Allsop CEM, Greenwood BM, Kwiatkowski D. Variation in the TNF-a promoter region associated with susceptibility to cerebral malaria. Nature 1994;371:508–11.

Muller U, Steinhoff U, Reis LFL, Hemmi S, Pavlovic J, Zinkernagel RM, et al. Functional role of type I and type II interferons in antiviral defense. Science 1994;264:1918–21.

Neustock P, Kruse A, Bock S, St. Pierre B, Kirchner H. Deficient interferon-alpha response of newborn in comparison to adults. Lymphokine Cytokine Res 1993;12:109–14.

Pitkäranta A, Linnavuori K, Hovi T. Virus-induced interferon production in human leukocytes: a low responder to one virus can be a high responder to another virus. J Interferon Res 1991;11:17–23.

Pitkäranta A, Karma P, Hovi T. Deficiency in interferon production by leukocytes from children with recurrent respiratory infections. Clin Diagn Virol 1993;1:101–8.

Pitkäranta A, Karma P, Hovi T. Virus-induced interferon production in leukocyte cultures from children with recurrent respiratory infections. A follow-up study. Clin Diagn Virol 1996;6:11–6.

Stuber F, Petersen M, Bokelmann F, Schade U. A genomic polymorphism within the tumor necrosis factor locus influences plasma tumor necrosis factor-a concentrations and outcome of patients with severe sepsis. Crit Care Med 1996;24:381–4.

Tupasi TE, de Leon LE, Lupisan S, Torres CU, Leonor ZA, Sunico MES, et al. Community-based studies of acute respiratory tract infections in young children. Rev Infect Dis 1990;12(suppl. 8):S940–9.

Vanecek K, Lehovcova A. Interferon production in children with respiratory diseases. Acta Paediatr Scand 1985;74:118–21.

Westbom L, Kornfält R. Chronic illness among children in a total population. An epidemiological study in a Swedish primary health care district. Scand J Soc Med 1987;15:87–97.

Westendorp RGJ, Langermans JAM, Huizinga TWJ. Genetic influence on cytokine production and fatal meningococcal disease. Lancet 1997;349:170–3.