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Virus-induced interferon production in leukocyte cultures from children with recurrent respiratory infections. A follow-up study

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Abstract

Background: Lowered yields of virus-induced interferon (IFN) by leukocyte cultures were previously suggested to be associated with recurrent respiratory infections in children (Pitkäranta et al. (1993) *Clin. Diagn. Virol.* 1, 101–108)

Objectives: To investigate if the observed lowered IFN producing capacity was secondary to the underlying disease and, consequently, would be normalized after recovery of the child from the chain of infections.

Study design: Forty-eight 3–12-year-old children suffering from recurrent upper respiratory tract infections (acute otitis media included) were followed-up for 2 years. Their clinical condition and virus-induced interferon production in cultures of peripheral blood leukocytes were examined at the beginning and end of this period.

Results: In 24 children the health improved strikingly during the follow-up, in 12 children a mild improvement took place, while 12 children remained constantly ill. IFN yields in cultures stimulated with corona- and respiratory syncytial viruses improved along with the clinical situation of the children. Parallel cultures induced with adeno-, influenza A or rhinoviruses did not show a similar correlation.

Conclusion: These results suggest that the relationship between interferon production by leukocyte cultures and recurrent infections is complex and may be virus-specific.

Keywords: Interferon; Child; Respiratory infection

1. Introduction

Although primary genetic defects of interferon (IFN) production have not been described in man, various patients with different viral infec-

tions have been identified to have disturbances of IFN production (Isaacs et al., 1981; Bondestam et al., 1984; Rossol et al., 1989; Kakumu et al., 1990). This phenomenon is usually considered to be secondary to the underlying disease. However, in the above studies it was not known how the patients had previously (or afterwards) handled viral infections. Hence, the lowered IFN production might also represent a primary disorder.

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Leukocyte cultures from healthy individuals produce varying quantities of IFN when stimulated by standard amounts of a given inducer virus and the yields obtained with different viruses in successive blood specimens show a fairly regular donor-specific pattern (Pitkäranta et al., 1991). We reported previously that leukocyte cultures of children suffering from recurrent respiratory tract infections produce less IFN than those of healthy children (Pitkäranta et al., 1993). This also raised the question whether the phenomenon was secondary to the underlying disease, or whether it was a genetic trait of the children possibly predisposing them to recurrent respiratory infections.

We have now reinvestigated the IFN production in leukocyte cultures of these infection-prone children after a two year interval, and report here that IFN responses have changed in a virus-specific manner.

2. Materials and methods

2.1. Patients

Fifty children were admitted in May–August 1992 for scheduled operations (adenoidectomy and/or tympanostomy) to the Department of Otolaryngology, Helsinki University Central Hospital, because of a history of frequently recurring upper respiratory tract infections and/or frequent middle ear infections. Inclusion criteria comprised at least six episodes of upper respiratory tract infection and/or at least four attacks of acute otitis media within the last 6 months. In May–August 1994 the patients were invited for a second voluntary visit by sending an information letter to the parents. Informed consent was obtained from all the parents. Two children could not come. Characteristics of the 48 children, who came for this examination are presented in Table 1. Like 2 years previously, history was taken and routine ear, nose and throat examination was performed and a heparinized blood sample was drawn. During this visit two patients had an ongoing mild respiratory tract infection, while two were suffering from a chronic respiratory condition.

2.2. IFN induction

Preparation of mononuclear leukocytes from heparinized blood has been described earlier (Bøyum, 1968; Pitkäranta et al., 1988). Freshly made parallel leukocyte cultures were inoculated with standard amounts of five crude virus preparations. Propagation of adenovirus 7a, rhinovirus strain 4270 (a local isolate), coronavirus 229E, and respiratory syncytial (RS) A3 virus took place as earlier reported (Pitkäranta et al., 1993). Influenza A H3N2 virus (a gift from Dr. R. Pyhälä) was grown in embryonated eggs. A single preparation of each inducer virus was used throughout the study and a fresh aliquot was thawed for each experiment. The leukocyte culture conditions were strictly standardized with the inducer dosage being standardized according to the infectivity as described before (Pitkäranta et al., 1993). Virus TCID₅₀ titres were rechecked by end-point dilution in tube cultures since the time between these two experiments was two years. Culture medium for IFN assay was harvested on day 2 (Pitkäranta et al., 1988), cleared from cells by centrifugation, and stored at 20°C until assayed.

Table 1
Distribution of characteristics of children according to clinical healing

	Group 1 (n = 24)	Group 2 (n = 12)	Group 3 (n = 12)
Age			
Median (years)	3.6	3	3
Range (years)	2.7–10.9	2.7–12	2.7–6.6
Sex			
Male	14	9	5
Female	10	3	7
Median duration Of infection chain (Years)	2	2.3	>2.6
Age at onset Of infection chain			
Median (years)	0.7	0.6	0.4
Range (years)	0.2–5	0.2–3.3	0.2–1.3

Group 1, strikingly improved.

Group 2, mildly improved.

Group 3, constantly ill.

2.3. IFN assay

IFN concentrations were measured by a micromethod based on the reduction of cytopathic effect caused by vesicular stomatitis virus in monolayers of NBL cells (Linnavuori, 1988). A standardized human leukocyte IFN preparation (a gift from Dr. K. Cantell, National Public Health Institute, Helsinki) was included in each assay so that results could be expressed in IU/ml. Results given are means of two parallel cultures; a maximum of 2-fold difference was seen between the parallel cultures. The subtype of IFN was not specifically assessed in these studies. Virus-induced leukocyte IFN is mostly a mixture of different alpha IFNs and the bovine cell line (NBL) used in the assays is not sensitive to human gamma IFN.

2.4. Statistical methods

Differences between groups were measured with the Mann-Whitney *U*-test.

3. Results

Children segregated into three groups according to their clinical condition after the follow-up period (Table 1): in 24 children health had strikingly improved, i.e. they had no significant respiratory tract infections at all during the last 6 months (Group 1), in 12 children the health had mildly improved (respiratory tract infections less than 6 episodes and/or acute otitis media less than 4 episodes during the last 6 months, (Group 2), while 12 children had suffered 6 or more upper respiratory tract infections and/or 4 or more episodes of acute otitis media during the last 6 months (Group 3). The children of Group 3 were significantly younger than those in the improved Group 1 ($P < 0.02$), (Table 1). The age of the children in the improved Group 1 and in the moderately improved Group 2 did not significantly differ. Children in Group 3, in spite of being younger, had a significantly longer duration of the infection chain than children in Group 1 already by 1994, although their infection chain

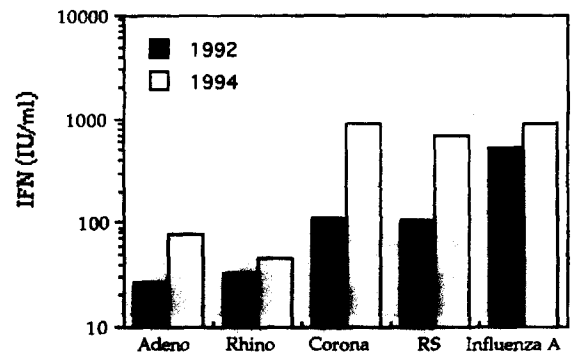


Fig. 1. Geometric means of IFN yields produced by leukocyte cultures from 24 children suffering from recurrent upper respiratory tract infections and for 2 years later when the chain of infections had come to an end (see text for definition). Significance of the differences between the IFN titres in 1992 vs. 1994: adenovirus, $P < 0.05$; rhinovirus, not significant; coronavirus, $P < 0.001$; RS virus, $P < 0.001$; influenza A virus, $P < 0.05$.

had not yet come to an end ($P < 0.05$), (Table 1). Duration of the infection chain in Groups 1 and 2 did not differ from each other. Median age at the onset of the infections differed also between Groups 1 and 3 ($P < 0.05$), (Table 1). Six out of the 12 children in Group 3 had their onset before the age of 6 months while in Group 2 and 3 only 2/12 and 3/24 children did so.

In Group 1 children with improved health, geometric means of the IFN titres were now higher than 2 years previously, and a statistically significant difference was seen in cultures stimulated with corona- and RS-viruses (Fig. 1). In Group 2 a similar tendency was seen (Fig. 2), although the finding was not statistically significant. In the third group, where the children were still suffering from recurrent respiratory tract infections, the mean IFN titres had not changed during the two years (Fig. 3).

Relatively little variation in individual IFN yields between the two specimens was seen among the children in Group 3. Leukocyte cultures from all the children showed a maximum difference of 1–2 dilution steps in the IFN assay between 1992 and 1994, when stimulated with four out of the five different viruses. Somewhat more variation was seen in cultures stimulated with rhinovirus.

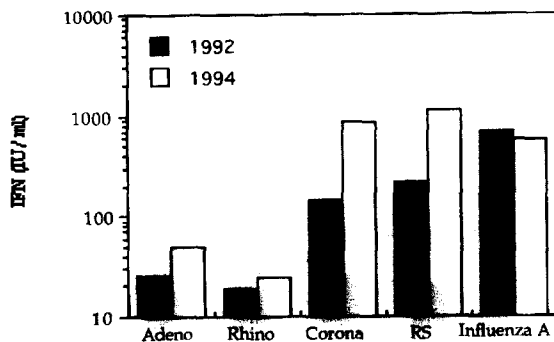


Fig. 2. Geometric means of IFN yields produced by leukocyte cultures from 12 children whose clinical state during the last two years had mildly improved (see text for definition). Significance of the differences between the IFN titres in 1992 vs. 1994: adenovirus, not significant; rhinovirus, not significant; coronavirus, $P < 0.001$; RS virus, $P < 0.001$; influenza A virus, not significant.

In Group 1, the IFN levels had increased or remained similar to those obtained in 1992 except one child who showed a decline. In Group 2, the IFN responses showed quite a similar tendency as in the group 1. Of the 10 children whose leukocytes in 1992 failed to produce any detectable IFN when stimulated with either adeno-, rhino- or coronavirus, none showed now a total lack of IFN production, and some showed a striking increase in the IFN yields produced. Only two of them still suffered from recurrent infections.

Spontaneous IFN production was not detected

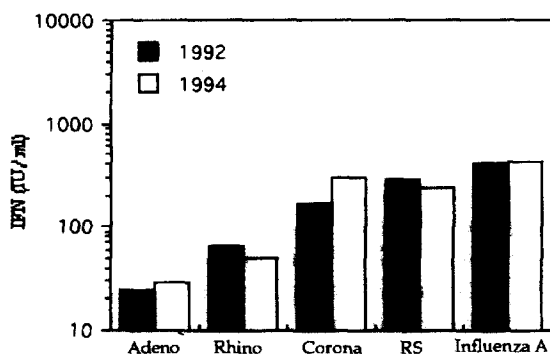


Fig. 3. Geometric means of IFN yields produced by leukocyte cultures from 12 children suffering from continuous chain of respiratory tract infections (see text for definition). Statistically significant differences between the IFN titres in 1992 and 1994 were not seen for any virus.

in any leukocyte cultures. The production of IFN was influenced by neither the age nor the sex of the children.

4. Discussion

The present investigation was designed to study the correlation of production of IFN in leukocyte cultures and continuation of infections in children known to be prone to respiratory tract infections. The yields of IFN obtained in leukocyte cultures are known to vary according to both the inducer used and the cell donor. The inducer can be standardized but the inherent time-dependent variation of blood leukocyte composition and of physiological status of the donor might compromise attempts to determine an IFN yield typical of an individual. Previously, we reported that by using a set of different inducer viruses, it is possible to demonstrate that the yields of IFN obtained under standard conditions remain relative constant for successive blood specimens of healthy individuals, and that especially the ratio of IFN induced by different viruses shows a reproducible typical pattern (Pitkäranta et al., 1991). In a subsequent study, we showed that leukocytes from children with recurrent respiratory tract infections produce significantly less IFN than cells from normal children, when stimulated with standard amounts of common respiratory pathogenic viruses (Pitkäranta et al., 1993). It has been earlier suggested, based on circumstantial evidence, that the lowered IFN yields in this group of patients may be secondary to the underlying disease, as some children had been shown to present improved responses after the cycle of recurrent respiratory infections was over (Chadda et al., 1983). Our current follow-up study tested this hypothesis with a larger group of patients and with five different natural respiratory pathogens as IFN inducers. To avoid possible seasonal variation (Pugliese et al., 1985) retesting was performed during the same summer months, May–August, similar to the initial study two years earlier. Unfortunately, it was not possible to collect follow-up specimens from the healthy control children of our previous study (Pitkäranta et al., 1993).

While responses of leukocytes stimulated with corona- or RS-viruses improved in accordance with the improved health, those due to rhino- and influenza viruses did not. This virus-dependent divergence of correlations is, as such, not surprising, as leukocyte cultures of individual donors were also previously shown to differ strikingly in their relative IFN responses to a given virus (Pitkäranta et al., 1991, 1993). It appears that if we had tested the responses only with corona- or RS-virus, we could have easily concluded that the observed lowered IFN responses of leukocyte cultures from children with recurrent respiratory infections are only secondary to the underlying disease. However, the lack of statistically significant improvement of the responses obtained with the other inducers makes the interpretation more difficult. While the lowered response to corona- and RS-virus stimulation appears to be associated with the continuing infectious situation, for the time being, it cannot be suggested as a diagnostic marker for this condition as the yields of IFN from leukocytes of healthy children vary widely (Pitkäranta et al., 1993). One possible explanation for lower IFN-responses in children suffering from recurrent infections may be due to putative continuous stimulation of the IFN system which may lead to hyporeactivity. However acute respiratory infection has not been found to impair IFN production by leukocytes in vitro (Bondestam et al., 1984; Vanecek and Lehovcova, 1985; Pitkäranta et al., 1993).

The age of the child is an important factor in predicting the risk of respiratory tract infections. The age of the continuously sick children was lower than that of those children whose infections had come to an end. The incidence of the illness has been shown to be higher in children under the age of three years than among older children (Wald et al., 1988; Pönkä et al., 1991). The number of respiratory tract infections in early childhood can partly be due to immunological immaturity (Prellner et al., 1992). Although contradictory findings of age-dependence in IFN production have been reported (Abb et al., 1984; Kishida et al., 1986) the ages within the range they showed in our study children do not affect IFN production (Cantell et al., 1968). Thus the

age factor cannot explain the differences in our IFN yields. Furthermore, when IFN yields of leukocytes from the youngest and oldest thirds out of the entire follow-up group were compared, we could not find any differences (data not shown). This corroborates previously published findings (Bondestam et al., 1984; Pitkäranta et al., 1993).

The children still suffering from the recurrent disease were younger than those who showed improved health. Yet they already had a longer history of disease and, accordingly, had had an earlier onset of the chain of infections known to be a characteristic of some selected children suffering from recurrent respiratory tract infections (Klein, 1994). It is obvious that children suffering from recurrent respiratory tract infections are not a homogeneous group of patients but rather may comprise distinct pathogenetic subgroups. Our current results do not exclude the possibility that a subset of patients has a genetic trait resulting in lowered IFN production connected to proneness to respiratory infections. This possibility should be investigated in a prospective cohort assessing the predictive value of IFN yields produced by leukocytes in relation to later respiratory infections.

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