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## Interferon production by leukocytes in children with otitis media with effusion

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### Abstract

We have previously shown that leukocyte cultures of children suffering from recurrent respiratory tract infections produce less interferon (IFN) than those of healthy children. In the present study this tentative marker of recurrent infections was used to study the pathogenetic background of otitis media with effusion (OME). Altogether 57 consecutive children, aged 2–11 years, who came for tympanostomy and/or adenoidectomy were divided into three subgroups: 25 of them had OME and a history of recurrent acute otitis media (rAOM/OME+), 20 had OME without an infectious background (inf-/OME+), and 12 had a history of recurrent upper respiratory infections (inf+/OME-) without OME. All the children were free of acute illness at the time of sampling. Differences between the groups were seen in IFN yields when leukocyte cultures were stimulated with adeno-, rhino-, corona-, respiratory syncytial or influenza A viruses. Leukocytes from inf-/OME+ children produced more IFN than those of the other two groups. Though no sex differences in the IFN responses were seen among rAOM/OME+ and inf+/OME- children, leukocytes from inf-/OME+ girls produced significantly higher amounts of IFN than those of inf-/OME+ boys, or rAOM/OME+ and inf+/OME- children. These differences between clinically different groups of children support the view that the etiology of OME can be heterogeneous.

**Keywords:** Acute otitis media; Interferon; Otitis media with effusion; Respiratory infection; Secretory otitis media

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## 1. Introduction

Otitis media with effusion (OME, secretory otitis media) is a common childhood disease without a completely clarified etiology. A number of clinical studies suggest an association between OME and upper respiratory tract infections [7,12]. However, all patients do not have a clear history of respiratory tract infections or otitis media [5,11,15], and it has been suggested that OME is not a single clinical entity [5]. On the other hand, it has also been difficult to evaluate the significance of the different infection-related and other pathogenetic factors in OME. While laboratory studies on the pathogenesis or classification of OME have mainly been based on the analyses or characteristics of the middle ear effusion and middle ear mucosa, also more general immunologic factors may contribute to the genesis of OME [21,24]. We have previously shown that leukocytes from children prone to respiratory tract infections have a deficiency in their IFN production capacity when exposed *in vitro* to pathogenic viruses [18]. It is not yet clear, whether the deficiency is a marker of a genetic trend to increased susceptibility to respiratory infections or is secondary to frequent infections. Anyhow, it may serve as a marker for a recent history of recurrent infections. In the present study we investigated whether children suffering from OME with or without a history of recurrent acute otitis media differ with respect to the capacity of their leukocytes to produce virus-induced IFN.

## 2. Materials and methods

### 2.1. Patients

The study population comprised of 57 consecutive children, aged 2–11 years, admitted during January/February 1993 for scheduled operation (tympanostomy and/or adenoidectomy) to the Department of Otolaryngology, Helsinki University Central Hospital, because of OME, or because of recurrent upper respiratory tract infections (acute otitis media included) without OME (inf + /OME –). In this study the OME children were subdivided into a group ( $N = 20$ ) with an asymptomatic incidentally discovered OME but without an apparent infectious background that is otitis or other respiratory tract infections only occasionally or not at all (inf – /OME +) and into a group ( $N = 25$ ) with known recurrent attacks of acute otitis media (rAOM/OME +). Table 1 shows the age and sex distribution of these 57 children. The diagnosis of these children was based on the patients history, pneumatic otoscopy and otomicroscopy. The criteria for diagnosing OME includes the presence of effusion in pneumatic otoscopy, and confirmed in tympanostomy, behind an intact eardrum without sign and symptoms of acute infection. The definition of AOM was that a child had findings suggesting middle ear effusion, and at least one of the following symptoms: otalgia, tugging at or rubbing of the ear, rectal or axillary temperature of at least 38.0°C, irritability, restless sleep, acute gastrointestinal symptoms (vomiting or diarrhea) or other simultaneous respiratory infection. The criteria of rAOM/OME + group were that a child had had at least 4 AOM episodes during the last 6 months. To be included in the inf + /OME –

group a child had to have experienced at least 6 episodes of upper respiratory tract infection during the last 6 months. Such infections included rhinitis, pharyngitis or tonsillitis, occasionally combined with sinusitis or otitis, but without any other complications. The infections were mostly accompanied by a moderate fever. Otherwise all the children were healthy and had grown normally. All the children with known allergy or other diseases were excluded. The patients were enrolled in the study after an informed consent of the parents. In all patients the serum levels of immunoglobulins and complement components were measured by immunoturbidometric methods, and none of the children showed any noticeable immunological aberrations. The children had been healthy for at least one week since the last infection. Blood specimens for leukocyte preparation were drawn using the needle inserted for injecting intravenous anaesthetics, but immediately before that.

### 2.2. Separation of leukocytes

Leukocytes from heparinized blood were separated over a Lymphopaque gradient as described earlier [4]. The mononuclear cells at the interphase of the gradient were collected and washed 3 times with phosphate-buffered saline, adjusted to a concentration of  $2 \times 10^6$ /ml in tissue culture medium (RPMI-1640 supplemented with 10% foetal calf serum) and distributed as 0.1 ml aliquots into 96-well tissue culture plates, 12 wells of each cell batch.

### 2.3. Induction of IFN

Freshly made parallel leukocyte cultures were inoculated with samples of 5 crude virus preparations, 2 wells each. A single preparation of each inducer was used throughout the study and a fresh aliquot was thawed for each experiment. The source and propagation of the inducer viruses was described previously [18]. Influenza, adeno- and rhinoviruses were added into the cultures at a calculated

Table 1  
Age and sex in the different patient groups

	inf - /OME + (N = 20)	rAOM/OME + (N = 25)	inf + /OME - (N = 12)
Age (years)			
Median	7	4	4
Range	2–10	2–11	2–10
Sex			
Male	14	18	6
(<5 years)	(5)	(13)	(1)
Female	6	7	6
(<5 years)	(2)	(4)	(5)

OME = otitis media with effusion; inf - /OME + = otitis media with effusion and a history of otitis or other respiratory infections only occasionally or not at all; rAOM/OME + = recurrent acute otitis media preceding otitis media with effusion; inf + /OME - = recurrent upper respiratory tract infections without otitis media with effusion.

multiplicity of infection of 1 TCID<sub>50</sub>/cell. The titres of the stocks of coronavirus 229E and respiratory syncytial (RS) virus were too low for this and 0.001 and 0.1 TCID<sub>50</sub>/cell, respectively, were used. The plates were sealed with adhesive tape and incubated at 36°C. Cell-free specimens from the culture medium were harvested at day 2 postinfection and stored at –20°C until assayed [16].

#### 2.4. IFN assay

IFN concentrations were measured by a biological micromethod based on the reduction of cytopathic effect caused by vesicular stomatitis virus [13]. A continuous bovine cell line (NBL) was used in the assay. Samples from patient cultures were analysed blindly. A calibrated leukocyte IFN standard (a gift from Dr. Kari Cantell, National Public Health Institute, Helsinki) was included in all assays so that the results could be given in international units (IU). The results given are means of 2 parallel cultures, a maximum of a 2-fold difference was seen between the parallel specimens. A constant donor-specific pattern in the IFN responses has been seen [17]. The subtype of IFN was not specifically assessed in these studies. Virus-induced leukocyte IFN is mostly a mixture of different IFN- $\alpha$ :s and the NBL cell line used in the assays is not sensitive to human IFN- $\gamma$ . Therefore, we believe that the IFN yields recorded represent IFN- $\alpha$ .

#### 2.5. Statistical method

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

### 3. Results

#### 3.1. OME children segregate into two groups

Leukocytes from all 20 inf–/OME+, 25 rAOM/OME+ and 12 inf+/OME– children showed an IFN production response to at least two different inducers. IFN yields from cultures stimulated by adeno-, rhino-, corona-, RS or influenza A viruses were higher for the inf–/OME+ children's leukocytes than for those of the rAOM/OME+ and inf+/OME– children's leukocytes (Fig. 1). Statistically significant differences were seen between inf–/OME+ and rAOM/OME+ children when leukocytes were stimulated by rhino- ( $P < 0.01$ ), corona- and RS viruses ( $P < 0.05$ ). The results with adeno- and influenza A virus showed a similar tendency but not statistically significant differences. A statistically significant difference between inf–/OME+ and inf+/OME– was seen when the leukocytes were stimulated by adeno-, rhino- or coronaviruses ( $P < 0.01$ ), and by RS and influenza A viruses ( $P < 0.05$ ). The difference between rAOM/OME+ and inf+/OME– children was not significant for any inducer.

#### 3.2. Influence of sex on IFN response in inf–/OME+ children

Interferon production was different between the two sexes among the inf–/OME+ children but not among the rAOM/OME+ or inf+/OME– children. IFN yields from cultures stimulated by rhino-, corona- or RS viruses were higher ( $P < 0.05$ ) for the inf–/OME+ girls than for those of the inf–/OME+ boys.

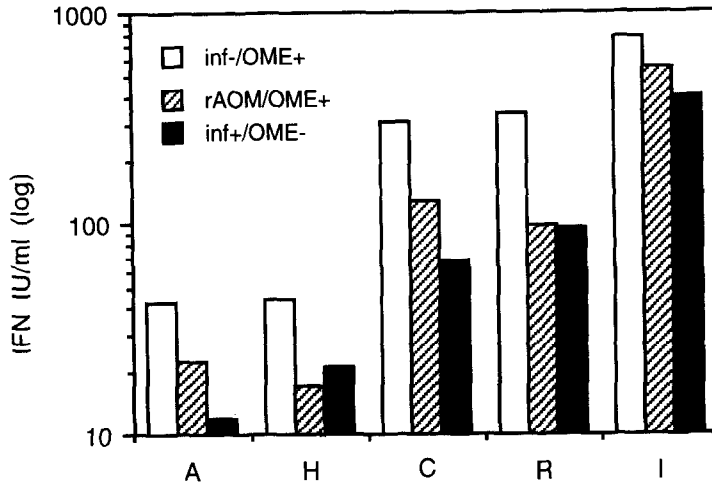


Fig. 1. Geometric means of IFN yields produced by leukocyte cultures from children with inf-/OME+, rAOM/OME+ and inf+/OME-. Significance of the differences: inf-/OME+ vs. rAOM/OME+ with adenovirus  $P < 0.1$ , with rhinovirus  $P < 0.01$ , with coronavirus  $P < 0.05$ , and with RS virus  $P < 0.05$ ; inf-/OME+ vs. inf+/OME- with adenovirus  $P < 0.01$ , with rhinovirus  $P < 0.01$ , with coronavirus  $P < 0.01$ , with RS virus  $P < 0.05$ , and with influenza A virus  $P < 0.05$ . A = adenovirus, H = rhinovirus, C = coronavirus, R = RS virus, I = influenza A virus.

There were also similar, although not statistically significant, differences in the IFN production by adeno- and influenza A viruses (Fig. 2).

Leukocytes from inf-/OME+ girls produced significantly higher amounts of IFN than leukocytes from rAOM/OME+ and inf+/OME- children by all examined viruses ( $P < 0.01$ – $< 0.001$ ). Similar trends were seen for the boys in adeno-, corona- and rhinovirus-stimulated cultures but the differences were not statistically significant (Fig. 3).

There were no statistically significant differences between the IFN levels produced by leukocytes from children 2–5 years of age and from those older than 5 years. Spontaneous IFN production was not detected in the leukocyte cultures of any child.

#### 4. Discussion

Etiologic factors that initiate the pathogenetic process leading to otitis media can be summarised by the term multifactorial. Genetic factors are becoming more important, and are likely to contribute to the anatomy and function of the Eustachian tube and the middle ear cleft, and to immune functions. Though viral infections are commonly associated with recurrent respiratory tract infections (RRTI) and often provoke recurrent acute otitis media [1,20], and may lead to OME, the hypothesis that OME in children is always a manifestation of recurrent or chronic catarrhal infection of the upper respiratory tract has not been proven. Clinically, the course and background of OME varies greatly, and it can be

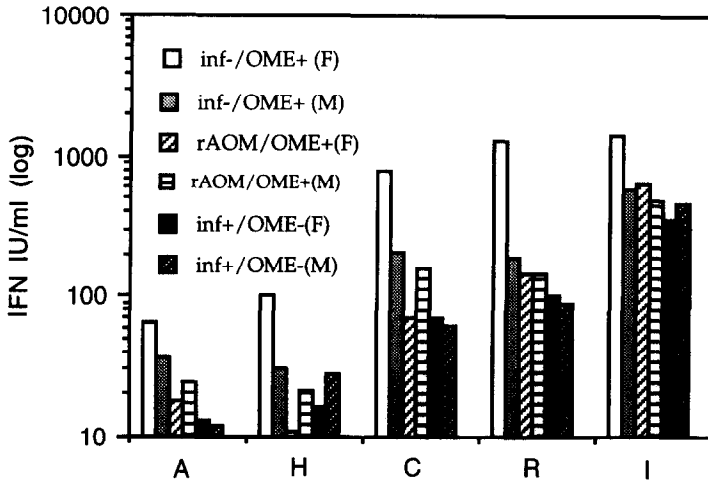


Fig. 2. Sex dependence of IFN production. Significant differences ( $P < 0.05$ ) were seen between inf-/OME+ males and females when stimulated by rhino-, corona-, and RS viruses. The differences between the sexes were not significant among the rAOM/OME+ or inf+/OME- children. F = female, M = male. A = adenovirus, H = rhinovirus, C = coronavirus, R = RS virus, I = influenza A virus.

subdivided into a postinfectious (history of rAOM) and a silent (no history of rAOM) form [11]. However the pathologic events and changes seen in OME often occur as a continuum of events progressing through acute and subacute stages to a chronic phase.

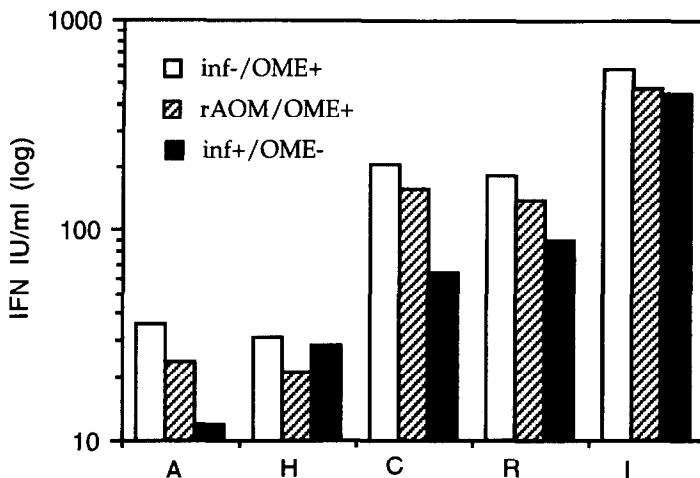


Fig. 3. Geometric means of IFN yields produced by leukocyte cultures from males with inf-/OME+, rAOM/OME+ and inf+/OME-. Statistically significant differences between the different groups of children were not seen. A = adenovirus, H = rhinovirus, C = coronavirus, R = RS virus, I = influenza A virus.

Interferons are considered major factors in the host defence against viral infections in humans. The IFN system is essential for antiviral defence, as has been shown by mice lacking the type I IFN receptor [14]. Our study demonstrated an overall difference in the IFN system between the two types of OME showing that virus-induced IFN production by cultured leukocytes from OME children without an apparent infectious background (inf – /OME +) can differ from that of OME children with a preceding recurrent AOM. Unfortunately healthy children were not included in this study, but we have previously shown that their leukocytes produce more IFN *in vitro* than those of rAOM and RRTI children [18]. Though we cannot yet be certain, if a deficiency in IFN production is a genetic or acquired property in these children, it anyhow seems to be a marker of both rAOM and RRTI. In accordance with this, OME children of the rAOM group showed in this study this marker but those without a history of recurrent infections did not show it as a group. While we did not determine the type of IFN produced, it is highly likely that most of the IFN measured was IFN- $\alpha$  since virus-induced leukocyte IFN is mostly a mixture of different  $\alpha$  IFNs. Significantly increased IFN- $\gamma$  production induced by Concanavalin A has been reported in OME children compared to normal controls [3].

An unexpected observation was that inf – /OME + girls produced higher IFN levels than inf – /OME + boys with any of the five pathogenic viruses tested. This finding is difficult to explain. Boys have a significantly higher risk for developing OME and rAOM than girls [9,19,22]. This does not, however, explain the differences in IFN production between the inf – /OME + children since no systematic difference between the sexes was seen among rAOM/OME + and inf + /OME – children. The independence of IFN production from sex among rAOM/OME + and inf + /OME – children is in accordance with previous reports [3,18]. Boys with inf – /OME + did not significantly differ from boys with rAOM/OME + or inf + /OME – in IFN production although a trend towards a difference was seen for some of the viruses used. However, because of the small numbers of children far-reaching conclusions based on these findings could not be drawn. Maybe the findings only reflect the fact that infections in general are rarer in young girls than in boys and that IFN production by leukocytes may be one factor contributing to this difference.

Because associated diseases, such as allergic rhinitis [2,8] may have a role in the etiopathogenesis of OME, all the children with an allergy or other diseases as well were excluded. Age might also be a confounding variable. The inf – /OME + children were older than rAOM/OME + and inf + /OME – children. A large number of studies have shown an age dependence of rAOM and RRTI [19,23], and also OME with a background of infections seems to concentrate on younger age groups [11] though it is well established that several immunologic phenomena depend on the subject's age [10,21], the age within the ranges met in this study, probably does not affect the IFN production [6,18].

In conclusion, a tentative marker of RRTI and rAOM, an impaired IFN response of virus-infected leukocyte cultures, was also found in the subgroups of OME children with a history of recurrent infections, but not in those without that



kind of background. This difference was statistically significant in girls. These results support the view that OME can develop by different pathogenetic mechanisms.

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