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Note

Lack of Induction by Rhinoviruses of Systemic Type I Interferon Production or Enhanced MxA Protein Expression During the Common Cold

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Abstract To study whether MxA protein expression is systemically upregulated during rhinovirus infection, blood specimens were collected from 40 patients with common cold and MxA expression in mononuclear cells analyzed by flow cytometry. None of the patients with a confirmed rhinovirus infection ($n=15$) or with an infection of unknown etiology ($n=20$) had elevated expression of the MxA protein (median fluorescence intensity, 549 and 582, respectively) when compared to healthy controls ($n=11$, median 590). Patients with influenza infections had significantly elevated values ($n=5$, median 750), and interferon could be detected only in serum samples from influenza patients. In conclusion, expression of MxA in blood lymphocytes and an apparently systemic type I interferon response is not induced during rhinovirus infection or during most other cases of common cold in young adult patients.

Introduction

Interferons (IFNs) form the first line of defence against viral infections. Type I interferons (IFN-α/β) are often produced by many types of cells, and IFN-α can be detected in serum. Measurement of IFN-α in serum has been used as a marker to differentiate viral and bacterial infections [1–3]. The sensitivity of these assays for indicating viral infection ranges from 50 to 79%. Therefore, other assays based on the expression of IFN-inducible genes have been developed.

One of these IFN-α/β-inducible genes, MxA, encodes for a cytoplasmic GTPase. This protein has antiviral activity against many viruses [4, 5] and is expressed in lymphocytes and in some tissues under strict regulation by type I IFNs [6]. Previously, we showed via flow cytometry that nearly all febrile children with a documented viral infection have elevated MxA levels [7].

Recent studies suggest that rhinovirus is the single most important respiratory virus because of its role in the common cold and in the pathogenesis of otitis media, sinusitis and exacerbations of asthma [8]. Little is known about the significance and function of the IFN system in rhinovirus infections. In the present study, the induction of a systemic IFN response as reflected by the expression of MxA protein in blood lymphocytes of patients with rhinovirus infection was examined.

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Materials and Methods

A total of 51 subjects were recruited for the study. Blood was drawn from 40 patients with common cold within 48 h of the onset of symptoms. The median age of the patients was 23.9 years (range 19.4–37.1 years). Diagnosis of viral infection was performed as described previously [9]. Briefly, antigen detection or culture from nasopharyngeal aspirate and serological tests were used for detection of adenovirus, respiratory syncytial virus, influenza viruses A and B and parainfluenza virus types 1, 2 and 3. Rhinoviruses were detected both by culture and by polymerase chain reaction (PCR). Based on the viral diagnosis, the patients were divided into three groups. There were 15 rhinovirus-positive subjects (8 culture positive, 7 of whom were also PCR positive;

and 7 PCR positive, culture negative), yet in 20 patients, the causative viral etiology could not be identified. One patient included in the rhinovirus group also had a rise in antibodies against the Coronavirus 229 E. The influenza group consisted of three patients with influenza virus A infection and two with influenza virus B infection ($n=5$). The control group consisted of 11 healthy adults.

Serum type I IFN levels were also analyzed by two different assays as described below. Since we were not able to detect elevated MxA levels in the rhinovirus-positive patients in the first phase of the study, we wanted to study whether these patients would have type I interferon in serum. For this purpose, two assays were used as described below. There was no serum left of the influenza virus-positive patients analyzed in the MxA assay, and, therefore, the serum specimens from influenza virus-positive patients whose lymphocytes were used for MxA expression analysis were not available. Therefore, in our analysis, we used 15 paired serum samples from patients with a marked rise in serum antibodies against influenza A. These frozen serum samples were obtained from a collection of serum samples stored at the Department of Virology, University of Turku (J.I.).

MxA protein was analyzed from peripheral blood mononuclear cells by indirect immunofluorescence and flow cytometry as described previously [7]. The lymphocyte population was identified by the typical pattern of side scatter (intracellular structure) and forward scatter (size) parameters in flow cytometry. The MxA level was indicated by the median channel of logarithmic fluorescence counts from the cytometer. The nonparametric Mann-Whitney U test was used for comparisons between the groups.

Serum IFN-α levels were determined using a commercial assay kit (Human IFN alpha ELISA; Endogen, USA) according to the manufacturer's instructions. The sensitivity of the assay was theoretically 3 pg/ml, which corresponds to approximately 0.5–1 IU/ml of IFN-α. Only two of the influenza virus patients were positive by this assay, suggesting inadequate sensitivity. Therefore, serum specimens were also analyzed by a biological IFN assay. The presence of biologically active IFN-α/β in sera was measured in HEp-2 cells by a vesicular stomatitis virus plaque reduction assay as described previously [10]. The results are expressed as international units (IU/ml), using an international control IFN-α prepared as a laboratory standard.

The ethical committee of the Turku University Hospital approved the study. Informed consent was obtained from all patients.

Results and Discussion

The median intensity values of MxA-specific fluorescence in peripheral blood mononuclear cells of different patient groups are shown in Figure 1. Based on earlier data from healthy children and patients with bacterial infections, the cut-off value was set at 650, which is two standard deviations above the mean of healthy children [7]. In the present study, the median fluorescence intensity value for the 11 healthy adults was 590 (Table 1). None of the patients with rhinovirus infection ($n=15$) had values above 650, whereas all influenza virus-positive patients ($n=5$) had values above this limit, with an upper range of 816. The median value in the rhinovirus-negative group ($n=20$) was 582, which does not differ significantly from the median value in the rhinovirus-positive group ($P=0.15$) but is significantly lower than the value in the influenza group ($P=0.0005$).

To study whether the capacity of lymphocytes to produce MxA protein was blocked by some repressors or other inhibitory factor, we also cultured the cells for 18 h in the presence of exogenous IFN-α (100 IU/ml). In all groups the induced values were significantly higher than the baseline values (Table 1). IFN-α/β levels were measured in serum, since MxA expression is regulated by type I IFNs. None of the patients with rhinovirus infection had detectable IFN-α/β in serum as measured by either enzyme immunoassay or by a biological assay. A similar observation was made in the rhinovirus-negative patients and in the control group. IFN-α/β was detectable in the serum of seven of the 15

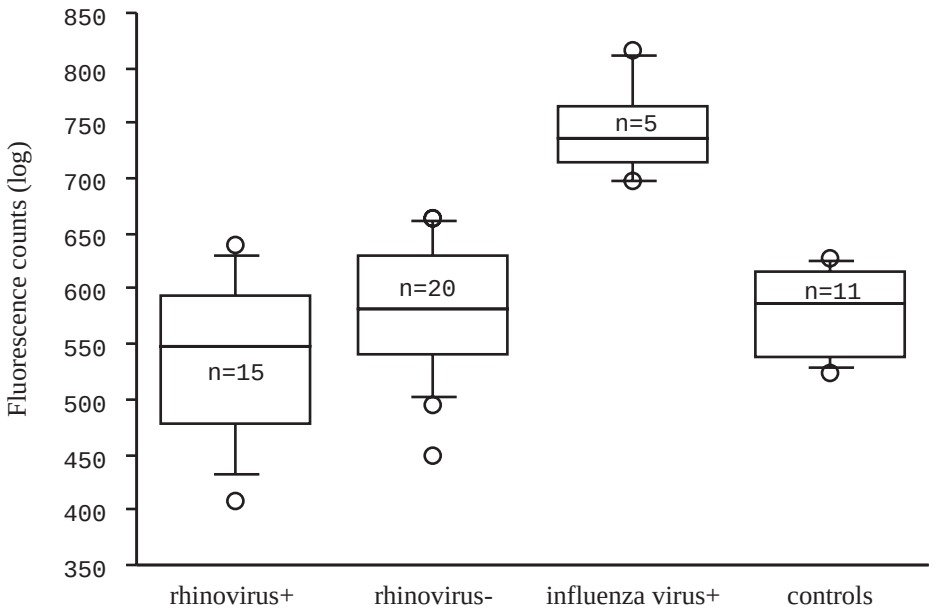

Figure 1 The intensity of MxA-specific fluorescence in peripheral blood mononuclear cells of different patient groups. The horizontal line in the box shows the median value of the fluorescence intensity in the specific group. The outlines of the boxes are the 25th and 75th percentiles, whereas the bars outside the boxes show the 10 and 90th% percentiles. The open circles indicate the values outside this range. The numbers inside the boxes indicate the number of samples

Table 1 The level of MxA protein in blood lymphocytes (as median of fluorescence intensity) before and after induction with exogenous IFN-α in patients with the common cold

Group	No.	Unstimulated	Stimulated	Percent increase	Intraquartile range
Rhinovirus positive	15	549	686	29.2	16.0–43.0
Rhinovirus negative	20	582	714	18.3	11.1–35.7
Influenza	5	750	nd	–	–
Control	11	590	673[a]	14.1	6.3–29.5

[a] Stimulation was done in 10 patients
nd, not done; –, not calculated

patients with influenza virus infection, albeit at low levels [detection limit <3 IU/ml, highest value 30 IU/ml (range 10–30 IU/ml)]. In six of the seven patients, IFN was detected in the acute-phase serum but not in the convalescent serum, and in one patient, IFN was detected only in the convalescent serum sample.

Low MxA values in patients with common cold were not expected, particularly in light of our earlier findings in children with viral infections in whom MxA was induced in practically all patients with influenza, adenovirus, respiratory syncytial virus and rotavirus infection [7]. Moreover, rhinovirus has been shown to induce IFN in vitro [11], and IFN has been found in the nasal secretions of volunteers infected with rhinovirus [12]. Stimulation with exogenous IFN-α, on the other hand, resulted in significant induction, suggesting that there is no functional defect associated with the rhinovirus infection per se. It is known that viruses and, probably, virus strains differ in their ability to induce IFN production, and there is also significant individual variation [11]. IFN synthesis is often short-lived, but MxA is a stable protein with a half-life of several days [6]. Since all patients were examined within 48 h after the onset of symptoms, it is unlikely that potential MxA upregulation would have been missed due to delayed analysis [7, 13].

The MxA gene is under the strict control of type I IFNs, and thus expression of the MxA protein indirectly reflects the presence of IFN in the host. Serum IFN-α/β levels were therefore measured as well. The findings were in accordance with the study of Parry and Parry [14], who found interferon in the sera of nine of their 14 patients with influenza but in none of the 11 patients with rhinovirus infection.

Our data suggests that type I IFN production in serum is not comparable to that seen in most other respiratory viral infections in vivo, since the rhinovirus-positive patients had only low or no expression of the MxA protein and no IFN-α/β was detectable in serum samples. There is still uncertainty about the presence of rhinovirus in different parts of the airways, but according to recent studies with experimental infections in humans, rhinoviruses seem to infect epithelial cells both in the upper and lower airways [15]. It is possible that, in most cases, the rhinovirus infection is limited mainly to the respiratory epithelium without major systemic influence.

In conclusion, this study suggests that the systemic type I IFN response and subsequent upregulation of IFN-inducible MxA protein expression is poor in rhinovirus infection. MxA protein expression may, therefore, not be a suitable marker of viral infection in the common cold. It is still unknown whether the lack of a systemic IFN response plays a significant pathophysiological role during rhinovirus infection. Although we did not find IFN or clearly induced MxA protein expression in the peripheral blood of any subject with rhinovirus infection, we cannot rule out the possibly significant role of IFN in local epithelial response against the virus.

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