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# Presence of an Acid-Labile Alpha-Interferon in Sera from Fetuses and Children with Congenital Rubella

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**In congenital rubella an acid-labile alpha-interferon was present in sera collected from fetuses between weeks 21 and 29 of gestation and from children with active congenital rubella. This interferon was different from the interferon detected in normal amniotic fluid and was not found in sera from uninfected fetuses or from children with postnatally acquired rubella. The fetal interferon is of interest as a complementary marker to confirm the virus contamination of the fetuses during maternal rubella. The role of the prolonged synthesis of this interferon in congenital rubella disease and its immune defects are discussed.**

The possibility of obtaining fetal blood samples in the early stages of pregnancy facilitates the prenatal diagnosis of genetic disorders and congenital infections (5). In the present study, we investigated the capacity of fetuses to produce interferon (IFN) during infections due to toxoplasmosis or rubella. Furthermore, since an alpha-IFN is spontaneously excreted in amniotic fluid (14), it was of interest to explore the presence of such an IFN in fetal sera.

## MATERIALS AND METHODS

**Patient population and clinical specimens.** Fetal blood sampling was carried out between weeks 20 and 29 of gestation from 20 pregnant women with confirmed rubella during pregnancy, 32 pregnant women with toxoplasma infection during pregnancy, and 9 pregnant women to look for genetic disorders. Twenty-three amniotic fluids were collected at the same time. This study population is detailed in Table 1.

Sera were also obtained from 10 children (age, 4 days to 4 1/2 months) with congenital rubella. In all cases, the diagnosis was established by the presence of rubella immunoglobulin M (IgM) antibodies in the serum.

Ten paired sera were collected from patients (age, 5 to 20 years) with acquired and uncomplicated rubella. The first blood specimen was collected on the day of the rash before the appearance of specific antibodies, and the second was taken 7 to 12 days after the rash.

Five sera were obtained from five pregnant women at the time of fetal blood sampling for rubella infection.

The sera and amniotic fluids were stored at −20°C except for fetal sera, which were kept at −70°C.

**Antibody detection.** IgM rubella antibodies of the fetuses were detected by an IgM capture immunoassay (6), using a modified technique (5).

IgM rubella antibodies from congenital and acquired rubella were detected by the same method, by inhibition of hemagglutination assay, or by both after separation by sucrose density gradient centrifugation. IgM toxoplasmosis antibodies were titrated (by Georges Desmonts, Institut de Puériculture, Paris, France) by the assay previously described (7).

**IFN assay.** Sera and amniotic fluid samples were tested without pretreatment. The assays were performed in plastic microplates for tissue culture with Madin-Darby bovine kidney cells, which are very sensitive to alpha-IFN (10).

Dilutions (50 μl) of sera or amniotic fluid were directly mixed with 100 μl of cell suspensions (25,000/0.1 ml) and incubated together for 24 h at 37°C. The cells were then washed and challenged with vesicular stomatitis virus at a multiplicity of infection of 0.1. After an 18-h incubation period, the titer was estimated as the reciprocal of the highest dilution which protected 50% of the cell population. The final titer was expressed in international units (IU) after comparison with the reference sample (NIH GO25 901 527) titrated under the same conditions.

**Identification of the virus inhibitor as human alpha-IFN.** The serum samples were (i) either dialyzed for 24 h at 4°C against a 0.2 M glycine-hydrochloride buffer (pH 2) and then for 3 h against Eagle minimum essential medium or (ii) incubated for 1 h at 20°C in the presence of alpha-IFN antiserum (prepared in sheep with alpha-IFN) (specific activity, $10^7$ IU/mg of protein [kindly provided by Charles Chany, Institut National de la Santé et de la Recherche Médicale Unité 43, Paris, France]), beta-IFN antiserum prepared in rabbits (kindly provided by Jan Vilcek, New York University Medical School, New York, N.Y.), or gamma-IFN antiserum (kindly provided by Howard M. Johnson and G. John Stanton, University of Texas Medical Branch, Galveston).

The three antisera were diluted 1:10 and added at a ratio of 1 volume of serum to 5 volumes of serum from patients (final serum dilution, 1:60). The fibroblast anti-beta-IFNs neutralized 4 IU at a 1:1,200 dilution. The leukocyte anti-alpha-IFNs neutralized 100 IU at a 1:12,000 dilution, and the anti-gamma-IFN neutralized 1,000 IU at a 1:20 dilution.

TABLE 1. Study population of fetal samples

| No. and type of maternal cases | No. of specimens from: | | Pathogen-specific IgM in fetal serum | |
|---|---|---|---|---|
| | Fetal blood | Amniotic fluid | Present | Absent |
| 20 rubella | 23 | 2 | 17 | 6 |
| 32 toxoplasmosis | 32 | 18 | 1 | 31 |
| 9 genetic disorders | 9 | 3 | | |

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TABLE 2. IFN levels in sera from rubella-infected and noninfected fetuses

| Case no. | Time of gestation (weeks) | | Total IgM (mg/100 ml) | Rubella-specific IgM[a] | Alpha-interferon titer (IU) in serum | |
|---|---|---|---|---|---|---|
| | Maternal infection | Fetal blood sampling | | | Without treatment | After pH 2 treatment |
| 1 | 2 | 23 | 8.45 | 3,200 | 16 | <4 |
| 2 | 4 | 25 | 38 | 1,600 | 24 | <4 |
| 3 | 17 | 24 | 7.7 | 200 | 8 | ND[b] |
| 4 | 10 | 23 | 18.75 | 800 | 30 | 4 |
| 5 | 15 | 23 | 60 | 1,600 | 6 | ND |
| 6 | 8 | 29 | 22 | 1,600 | 2–4 | ND |
| 7 | 2 | 22 | 7.6 | 200 | 12 | <4 |
| | | 25 | 12.6 | 1,600 | 6 | ND |
| 8 | 5 | 21 | 5 | 100 | 16 | <4 |
| | | 24 | 10.7 | 800 | 9 | ND |
| 9 | 15 | 21 | 2.2 | <50 | 8 | ND |
| | | 24 | 16 | 800 | 60 | ND |
| 10 | 6 | 20 | 65 | 12,800 | 4 | ND |
| 11 | 14.5 | 23 | 60 | 3,200 | <2 | |
| 12 | 11 | 24 | 50 | 12,800 | 8 | 2 |
| 13 | 2 | 24 | 15 | 800 | 16 | ND |
| 14 | 12 | 22 | 18 | 1,600 | 12 | ND |
| 15 | 14 | 24 | 4 | <50 | <2 | |
| 16 | 17 | 26 | 3.20 | <50 | <2 | |
| 17 | 13 | 23 | 2.35 | <50 | <2 | |
| 18 | 11 | 24 | 2 | <50 | <2 | |
| 19 | 12 | 22 | 3.4 | <50 | <2 | |
| 20 | 15 | 22 | 1.4 | <50 | <2 | |

[a] Reciprocal of the dilution (IgM capture immunoassay).
[b] ND, Not done.

## RESULTS

No IFN activity (less than 2 IU) was detected in the 32 fetal sera collected for toxoplasmosis or in the 9 collected for genetic disorders. On the other hand, in the 23 amniotic fluids collected at the same time, all titers were above 2 IU, with an average of 8 IU (data not shown).

In rubella infection, IFN activity was present in 16 of 17 sera from the infected fetuses and absent in the six fetuses that escaped rubella infection (Table 2).

The IFN titers of fetal sera were analyzed in terms of the age of gestation at the maternal infection and at the fetal blood sampling and in terms of the levels of total and specific IgM antibodies (Table 2).

The IFN titers were not correlated with the interval between maternal infection and fetal blood sampling or with the age of gestation at the maternal infection. For cases 5, 10, 11, and 12, high levels of total and specific IgM globulins were associated with low IFN titers (no titer for case 11). In case 9, IFN was detected just before the increase of rubella-specific IgM.

The acid lability of these fetal IFNs was demonstrated in six of six cases (Table 2). Less than 10% of the IFN activity was resistant to acid pH.

In the sera collected for rubella from five pregnant women, no IFN activity was found in spite of its presence in the fetal blood taken at the same time (data not shown).

In children with congenital rubella, alpha-IFN was present in the sera of 9 of 10 cases at levels ranging from 2 to 48 IU (Table 3). The IFN titers were not correlated with the

TABLE 3. IFN levels in sera from children with congenital rubella

| Case no. | Time of gestation at infection (weeks) | Age at blood sampling (months) | Clinical symptoms | Rubella IgM (% of total IHA[a] activity) | Total serum IHA[a] activity | Alpha-IFN titer (IU) in serum | |
|---|---|---|---|---|---|---|---|
| | | | | | | Without treatment | After pH 2 treatment |
| 1 | 6 | 2.5 | Heart defects | 25 | 80 | 4 | ND[b] |
| | | | Neurological | | | | |
| 2 | 8 | 0.5 | Heart defects | 30 | 640 | 8 | ND |
| 3 | Not specified | 3.5 | Pneumopathy | 50 | 80 | 8 | <2 |
| | | 4.5 | Hepatosplenomegalia | | | 16 | ND |
| 4 | Not specified | 4 days | Hypotrophy | 5 | 160 | <4 | |
| | | 0.5 | | | | <2 | |
| 5 | 12 | 3 | Thrombopenia | 10 | 40 | 48 | 4 |
| | | | Hepatosplenomegalia | | | | |
| | | | Diarrhea | | | | |
| 6 | Not specified | 1.5 | Heart defects | 5 | 160 | 2 | ND |
| | | | Skin rash | | | | |
| 7 | 11 | 3.5 | Heart defects | 25 | 20 | 32 | 2 |
| | | 4 | Hepatosplenomegalia | | | 20 | ND |
| 8 | 8 | 4.5 | Pneumopathy | 30 | 40 | 12 | <4 |
| 9 | 4 | 3.5 | Cataracts | 33 | 80 | 12 | ND |
| | | | Pneumopathy | | | | |
| | | | Skin rash | | | | |
| 10 | 16 | 3.5 | Microcephalia | 25 | 80 | 24 | <4 |
| | | | Skin rash | | | | |

[a] IHA, Inhibition of hemagglutination assay.
[b] ND, Not done.

TABLE 4. Identification of the IFNs from fetuses and children with congenital rubella

| Serum from: | Case no. | Neutralization with serum[a] | | | |
|---|---|---|---|---|---|
| | | Control | Anti-alpha-IFN | Anti-beta-IFN | Anti-gamma-IFN |
| Fetuses | 2 | 24 | <2 | 24 | 24 |
| | 4 | 24 | <2 | 24 | 24 |
| | 7 | 8 | <2 | 8 | 8 |
| | 8 | 12 | <2 | 12 | 12 |
| | 12 | 8 | <2 | 8 | 6 |
| Children | 3 | 8 | <2 | 8 | 8 |
| | 5 | 32 | <2 | 48 | 48 |
| | 7 | 32 | <2 | 32 | 32 |
| | 8 | 8 | <2 | 6 | 8 |
| | 10 | 16 | <2 | 16 | 16 |

[a] Values shown are the IFN titer expressed in international units.

specific IgM response. They decreased more than 90% after pH 2 treatment in five of five sera tested. No relationship could be established between the age of gestation at infection and the IFN titers.

From children with postnatally acquired rubella, IFN was not detected (<4 IU) in sera taken before the skin rash or 7 to 15 days later.

The identification of the IFN activity was performed for sera from five fetuses and five children, all with congenital rubella. They were all completely neutralized with an anti-alpha-IFN serum (Table 4). Anti-gamma-IFN and anti-beta-IFN sera did not modify their titer significantly.

## DISCUSSION

The data presented here show that the amniotic fluid IFN characterized previously (14) is probably not synthesized in the fetus, since in the absence of infection no substances possessing antiviral properties could be detected in fetal blood. In congenital rubella, the IFN detected in fetal serum could be distinguished from amniotic fluid IFN. Indeed, although both were neutralized by alpha-IFN antibodies, the amniotic fluid IFN was stable (14), whereas the fetal serum IFN was labile at acid pH 2. Congenital rubella IFN found in vivo is similar to that induced in vitro with rubella virus, which is also sensitive at low pH (3, 4). This induced IFN is obtained after a 5-day incubation of lymphocytes exclusively from patients already immunized with the rubella virus (4).

Since more than 90% of the virus-infected fetuses synthesized IFN, its presence in a fetal serum in conjunction with rubella IgM further supports the diagnosis of congenital rubella infection. In case 9, IFN appeared before the specific IgM. However, it is possible that high levels of total and specific IgM could be correlated to lower IFN titers. Furthermore, it is of interest that we were unable to detect any type of IFN in maternal sera from infected patients at the time of the fetal blood sampling.

It is surprising that a similar IFN was still detected in the sera from newborns with active congenital rubella, although infection of the fetuses occurred before week 12 of gestation. In other studies (8, 21) with an IFN assay with human fibroblast cells, IFN was not detected in children excreting or not excreting rubella virus. This difference with our results could be explained by the fact that bovine cells are better protected than human cells by some subtypes of alpha-IFN (26).

On the other hand, alpha-IFN was not detected before the synthesis of specific antibodies or during the period of IgM synthesis in benign rubella acquired after birth. The absence of alpha-IFN in the sera and cerebrospinal fluid of patients affected with acute postrubella encephalitis has also been reported (16).

A chronic release of an acid-labile IFN comparable to that in congenital rubella has also been described during systemic lupus (15, 20) and in a chronic active viral disease, immune deficiency syndrome (9). Both of these diseases are associated with immune defects. Immunodeficiency occurs also in congenital rubella patients (21, 24). They excrete virus for several months (1) in spite of the presence of rubella antibodies in the serum. These patients develop high IgM (23) and low IgG and IgA (12) levels. In some cases, a low proportion of T lymphocytes has been transiently observed (27). The question is raised whether this IFN synthesis plays an active role in the defect of immunity of congenital rubella. Indeed, it has been reported that IFN has an inhibitory or stimulatory activity on in vitro antibody synthesis, dependent on the concentration and subtypes of recombinant alpha-IFN employed (13, 19; N. H. Yeh and G. H. Reem, Antiviral Res. 2:26, 1983). This suggests that the variant acid-labile alpha-IFN could contribute to the development of the dysglobulinemia. Moreover, it has been shown that IFN can delay the rejection of allografts (17) and exert in vitro a protective effect on target cells against cytotoxic lymphocytes (2, 28). These properties, as well as the fact that rubella virus yields from chronically infected cells are not affected by IFN (8), could be incriminated in the prolonged excretion of the viruses. Since it has been demonstrated that endogenous IFN increases the severity of disease in mice infected at birth with lymphocytic choriomeningitis virus (11, 22), it is possible that pH 2-labile alpha-IFN also plays a negative role in the evolution of congenital rubella.

The long period of alpha-IFN synthesis is probably due to a defect of its regulation. Immune murine IFN seems to be regulated like antibody production by suppressor T cells (13). In congenital rubella, the viral multiplication begins mainly before lymphocyte maturation, which occurs at 9 to 12 weeks (18, 25), and could interfere with the growth of T-subset lymphocytes involved in the regulation of the acid-labile alpha-IFN synthesis.

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