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Circulating Interferon in Sudden Infant Death Syndrome (40057)

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Although the etiology of Sudden Infant Death Syndrome (SIDS) is unclear, its epidemiology strongly suggests that viral infections may play a significant role (1). Attempts to demonstrate the etiologic importance of viruses have been directed towards direct viral isolation from SIDS cases with recovery rates varying up to 37.5% (2, 3). This variation has partly been attributed to the different methods used for specimen processing and isolation.

Since circulating interferon has been detected in infections produced by live virus vaccines (4), in patients with naturally occurring viral infections (5) with some studies showing a correlation between circulating interferon and viremia (6), another suggested approach of establishing the occurrence of viral infections in SIDS is indirectly through the detection of circulating interferon (7).

This report describes our attempts to search for evidence of viral infections in SIDS by detecting the presence of circulating interferon.

Materials and methods. Study Population. Specimens were obtained from 56 infants who were autopsied at the Office of the Chief Medical Examiner, Baltimore, MD, during the 13-month period between January 1967 and February 1968. Forty-four infants were classified as SIDS by fulfilling the definition proposed at the Second International Conference on Sudden Death Syndrome of Infancy in that their deaths were unexpected by history and unexplained by the postmortem examination (8). This group included 20 females and 24 males from 6 weeks to 12 months of age with 37 cases being under 6 months. Specimens collected included tissue from lung, trachea, heart, brainstem, cortex, and serum from heart blood samples. These were frozen at −70° until used. In several instances the blood samples were unclotted.

In the control group of 12 infants with explainable deaths, there were six males and six females between 4 weeks and 14 months. The causes of death were attributed to trauma, burns, asphyxia, drowning, meningitis with adrenal hemorrhage, central nervous system malformation and hydrocephaly, trisomy 13–15, congenital heart disease, and pneumonia.

A second control group was included consisting of 10 adults between 24 and 74 years of age whose deaths were either caused by arteriosclerotic cardiovascular disease (ASCVD) or by gun shot or knife wounds (homicide). Despite the difference in age, inclusion of this group was thought to be necessary because of the insufficient numbers of traumatic and natural deaths in the infant control group.

Interferon Assay. Serial twofold dilutions of sera were made in Eagle's minimal medium (MEM) supplemented with 3% fetal calf serum (FCS). One ml of the dilutions was added in triplicate to confluent monolayer cultures of human diploid fibroblast (Wi-38) cells prepared in 16 × 125 mm screw-capped tubes. After 18 hours of incubation at 37°, the diluted sera were aspirated, and the cells were challenged with 100 $TCID_{50}$ of Sindbis virus. The cultures were maintained at 37° and read in 72 hr when the virus controls showed 100% (4+) cytopathic effect (CPE). The result was expressed in units/ml as the reciprocal of the dilution which reduced CPE by 50% (2+), as compared to the virus control.

Virus Isolation. Specimens of lung, trachea, heart, cortex, and brainstem were made into 10% suspensions by dilution and grinding in an appropriate volume of MEM. Attempts at virus isolation were limited to only those cases in which circulating interferon was detected. Aliquots of tissue suspension were inoculated into duplicate screw cap tubes

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containing monolayer cultures of human amnion, human embryonic kidney, and Wi-38 cells. The cultures were maintained at 37° and observed daily for CPE. After 14 days, the cells were scraped from the walls of the negative cultures, and the cell suspension used as inocula for new cultures that were in turn observed for 2 weeks. Hemadsorption was tested for by using a 1% suspension of guinea pig erythrocytes.

Results. A viral inhibitor was detected in the serum of 3 cases of SIDS and of 1 control (Table I). This inhibitor had properties characteristic of interferon. It was species specific, nondialyzable, trypsin sensitive, stable at pH_2, was not sedimented by ultracentrifugation at 110,000*g* for 3 hr, and did not directly inactivate the challenge virus.

Characteristics of the infants in whom interferon was detected are listed in Table II. The interferon titers in units/ml in the three cases of SIDS were 32–64, in comparison to a titer of eight in the one control case. Virus cultures were negative in all four cases. The pathological findings in the three SIDS cases were negative, as expected, but in the control case the findings included pneumonia, subdural hematoma, and bilateral otitis media with middle ear cultures growing Klebsiella–Aerobacter bacteria.

Discussion. Our results show that circulating interferon is not frequently detected in SIDS. Interferon was found in only three of 44 cases of SIDS in comparison to one of 22 control cases with the frequency and titers not being significantly different. The finding of interferon in the infant with pneumonia and otitis media with middle ear cultures positive for Klebsiella–Aerobacter bacteria is not surprising since circulating interferon has been detected in patients with gram negative infections (9).

Our data could be interpreted in several ways. The infrequent finding of circulating interferon could be due to too early sampling before there was an interferon response, to an insensitive assay system, or to inactivation of the interferon by proteolytic enzymes released at death. Arguments against this include the observation that interferon usually appears in serum early in the course of infection at the time that viremia occurs (6), the use of a similar type of interferon assay as reported here in detecting circulating interferon in children with acute respiratory infections (5), and studies showing that there is an accurate correlation in antemortem and postmortem measurements of serum proteins such as globulins and albumin (10).

The results could best be interpreted to suggest that virus infections in SIDS are not of a disseminated type since circulating interferon was not frequently detected. This is consistent with the findings in most studies involving viral isolation in SIDS where recovery of viruses has been limited to the respiratory and gastrointestinal tracts (3). Our studies are similar to those previously reported in which attempts were made to detect viremia in SIDS by measuring circulating interferon (7). They also support the hypothesis that if viruses do play a role in the final

TABLE I. CIRCULATING INTERFERON IN SIDS.

Group	Number	Number with interferon
SIDS	44	3
*Control	12	1
**Control	10	0

* Infants with known causes of death.
** Adult death due to homicide or arteriosclerotic cardiovascular disease (ASCVD).

TABLE II. INFANTS WITH CIRCULATING INTERFERON.

Case	Interferon Titer	Age	Sex	Month of Death	Virology Cultures	Pathology
SIDS	64	6 months	Male	April	—	—
SIDS	64	5 months	Male	December	—	—
SIDS	32	6 weeks	Female	January	—	—
Control	8	2 months	Female	February	—	Pneumonia, subdural hematoma, bilateral otitis media with middle ear culturing Klebsiella—Aerobacter bacteria

* Expression in units/ml as the reciprocal of the dilution of sera which reduced CPE by 50% (2+) as compared to the virus control.

common pathway of SIDS, they may possibly function in a local manner (8), the mechanism of which remains to be determined.

Summary. Circulating interferon, which may be indirect evidence of viral infections, was searched for in the Sudden Infant Death Syndrome (SIDS). A viral inhibitor with the properties of inteferon was detected in the serum of three out of 44 cases of SIDS, and in one out of 22 control cases. These results show that circulating interferon is not frequently detected in SIDS, and suggest that if viruses do play a role in the final common pathway of SIDS, they may possibly function in a local manner, rather than in a disseminated type of infection.

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