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Interferon Response of the Fetal Rhesus Monkey After Viral Infection (38113)

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(Introduced by J. L. Sever)

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Both *in vivo* and *in vitro* experiments have suggested that neonatal tissue is significantly less responsive than older tissue in production of interferon after viral infection (1–5). Furthermore, Overall and coworkers (6) demonstrated that the ability of the fetal lamb to produce interferon increases with the development of the fetus. These comparative and developmental studies of interferon production suggest a direct relationship between age and interferon synthesis. The significance of this phenomenon in the explanation of congenital malformations after intrauterine viral infections and the severity of certain infections in young individuals is yet to be determined. However, the existing data appear to support the hypothesis that the inability of the cells of the immature animal to synthesize interferon may play an important role in the pathogenesis of perinatal infections. The present report was designed to investigate the chronology of the interferon response of the developing monkey fetus to a viral interferon inducer.

Materials and Methods. Animals and virus pool. Rhesus monkeys were bred and maintained in our monkey colony at the National Institute of Neurological Diseases. The Chikungunya virus pool was passed 10 times intracerebrally in infant mice and consisted of 10% brain tissue suspension in saline with a titer of $10^{7.5}$ LD_{50} per ml in newborn mice.

Inoculation and collection of specimens from rhesus monkey fetus. For this study the gestation period of the rhesus monkeys was arbitrarily divided into three periods of 55 days. For each period, a group of four pregnant animals was used; two fetuses were inoculated with 0.1 ml of the undiluted Chikungunya virus pool and the other two were injected with the same volume of normal saline solution. For inoculation laparotomy was performed on the pregnant animal and the gravid uterus was exposed for digital palpation. Upon locating the fetus and placenta, the injection was made through the uterine wall into the fetus avoiding the placenta. Using this procedure, the 90- and 135-day-old fetuses (2nd and 3rd periods) were inoculated intramuscularly. The 35-day-old fetuses (1st period) were too small for intramuscular inoculation. Alternatively, they were inoculated either by intraperitoneal or intraathoracic routes which are known to produce as good interferon response as the intramuscular route (7). To reduce trauma to the fetus and to prevent viral contamination of maternal tissues, a 27-gauge, ¼-in. needle was used for the injection.

For virus and interferon assays fetal body fluids or sera were collected 48 hr postinoculation. The 37-day-old fetuses were also taken by cesarean section. However, due to its reduced size the entire fetus was macerated and body fluids collected. Before using these embryo extracts for interferon assays we tested them for the presence of proteolytic enzymes that could possibly denature interferon. This possibility was ruled out after testing standard interferon preparations previously incubated for 2 wk at −20° and −4° with embryo extracts prepared with 35 to 50-day-old rhesus monkey fetuses; with and without exposure to the embryo fluids, the standard interferon samples showed similar titers.

Interferon assay. Interferon titers were determined on samples after treatment at pH 2.0 to destroy residual interfering activity of

Chikungunya virus. A Sindbis virus hemagglutination-reduction assay employing human foreskin fibroblast cells was used as previously described (8).

Virus titrations. Chikungunya virus was titrated in newborn mice; 10-fold dilutions of the virus were inoculated intracerebrally into groups of eight animals, 0.01 ml per animal. The end-point (LD_{50}) was calculated by the Reed and Muench method (9).

Results. Virus titrations with fetal extracts and/or fetal sera showed that the infected fetuses, regardless of gestational age, yielded between $10^{3.5}$ and $10^{4.0}$ LD_{50}/ml. However, similar levels of viral content of the fetuses were not reflected by similar levels of interferon. As seen in Fig. 1, fetuses in the first period of gestation produced negligible amounts of interferon (10 units) whereas fetuses in second and third periods responded to viral infection with production of 650 units/ml and 910 units/ml, respectively. None of the fetuses inoculated with saline produced detectable interferon levels.

Since it has been demonstrated that interferon levels in different tissues usually parallel viral titers in these tissues (10), these results indicate that early in gestation, the monkey fetus is incapable of synthesizing measurable levels of interferon after severe infection with Chikungunya virus. Interferon production occurring as a consequence of virus infection was first measurable by mid-gestation and increased sharply as the fetus approached term.

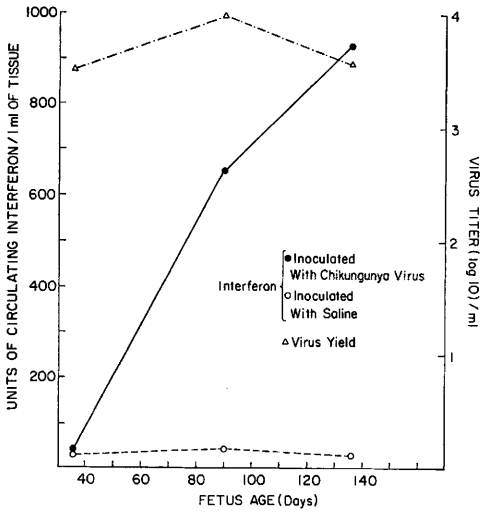

FIG. 1. Each point in the curves represents the arithmetical mean of two determinations. One interferon unit = reciprocal of the dilution which resulted in a $10^{0.5}$ reduction in hemagglutinin yield.

Discussion. The data reported here indicate that the production of interferon by the rhesus monkey fetus is a function of age. This observation is in agreement with the report by Overall and Glasgow for the fetal lamb interferon response after Chikungunya virus infection (6). This correlation between age and ability to synthesize interferon also has been suggested for newborn and adult laboratory animals and man (1–5). In fact, Heineberg *et al.* (3) hypothesized that the difference in outcome of Coxsackie B infection in suckling and older mouse is in large part explained by inability of the cells of the immature animal to elaborate interferon.

Our findings are particularly interesting in view of the increased susceptibility of the young primate fetus to many virus infections which newborn animals have no difficulty in overcoming (11). It is possible that the young fetus' failure to produce interferon is one of the important factors in determining its increased susceptibility to some transplacental infections.

In man fetal death and resorption frequently occur after severe damage due to transplacental infection during the first trimester of pregnancy. Fetal infections during the second and third trimesters of gestation generally are not as lethal but can result in lesions and/or congenital malformations, possibly because at this time the fetus is able to produce IgM and IgG immunoglobulins in small amounts (12). However, Baron *et al.* (13) showed that this relationship holds true in the chick embryo which does not produce immunoglobulins until after hatching, suggesting that interferon alone may be an essential factor in recovery from many viral infections. Thus, interferon and probably cellular immune response also appear to be important in the fully formed fetus as mechanisms for controlling infections. Very little is known about the involvement of these systems in the pathogenesis of transplacental infections in man. However, since the interferon system is immature during the first third of the gestation period in at least one primate and this time of embryonic development coincides with peak susceptibility to virus in-

fection, similar studies should be conducted in man.

Summary. Determination of interferon levels in the fetal rhesus monkey 2 days after Chikungunya virus infection showed that during the first third of gestation the fetus was incapable of producing measurable amounts of interferon. During the second and third periods substantial amounts of circulating interferon (600/900 units/ml) were detected. Viremia was present in all fetuses inoculated with Chikungunya ranging from $10^{3.5}$ to $10^{4.0}$ mouse LD_{50}/ml. Control fetuses inoculated with normal saline did not produce circulating interferon. The possible importance of the interferon system in controlling transplacental viral infections in primates is suggested.

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