The Effect of Interferon Induction in Parturient Sows and Newborn Piglets on Resistance to Transmissible Gastroenteritis

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ABSTRACT

High titers of interferon were found in the serum and milk of three sows treated two days after farrowing with polyinosinic:polycytidylic acid complexed with poly-L-lysine and carboxymethylcellulose, but circulating interferon was not found in the piglets suckled by these sows. When two treated sows and their suckling piglets were exposed to infection with transmissible gastroenteritis virus eight hours after treatment, the sows showed no signs of disease, although they developed circulating interferon in response to the virus infection. The piglets suckled by the treated sows developed signs of transmissible gastroenteritis which were identical to those seen in a control litter of piglets suckled by an untreated sow. Piglets treated at two days of age with the polyinosinic:polycytidylic acid complex showed a delay in onset of clinical signs when exposed to infection with transmissible gastroenteritis virus, compared with untreated control piglets. When two sows were treated with the polyinosinic:polycytidylic acid complex before farrowing, neither circulating interferon nor activated natural killer cells were found in the piglets after birth.

RÉSUMÉ

Les auteurs ont détecté un titre élevé d'interféron dans le sérum et le lait de trois truies auxquelles ils avaient administré, deux jours après la parturition, de l'acide polyinosinique:polycytidylique, en même temps que de la poly-L-lysine et du carboxyméthylcellulose; ils n'en décelèrent toutefois pas dans le sérum des porcelets qu'allaitaient ces truies. L'infection de deux truies, huit heures après l'administration des substances précitées, et des porcelets qu'elles allaitaient, avec le virus de la gastro-entérite transmissible, se solda par l'absence de signes cliniques, chez les truies, mais par la production d'interféron circulant; quant à leurs porcelets, ils développèrent des signes cliniques de gastro-entérite transmissible, identiques à ceux que manifestèrent les porcelets d'une portée qu'allaitait une truie témoin. Les porcelets qui reçurent les substances précitées, à l'âge de deux jours, affichèrent un délai dans l'apparition des signes cliniques, après une infection avec le virus de la gastro-entérite transmissible, comparativement à des porcelets témoins. L'administration des substances précitées à deux truies, avant la parturition, se solda par l'absence d'interféron circulant et de lymphocytes meurtriers naturels activés, chez leurs porcelets.

INTRODUCTION

Previous studies in this laboratory (1) demonstrated that natural killer (NK) activity against target cells infected with transmissible gastroenteritis (TGE) virus was lacking in newborn piglets, and depressed in parturient sows. It was suggested that this might contribute to the high susceptibility of newborn piglets and parturient sows to TGE. Interferon (IFN) induction in newborn piglets with polyinosinic:polycytidylic acid (poly IC) complexed with poly-L-lysine and carboxymethylcellulose (poly ICLC) produced transient NK activation and some evidence of an increase in the resistance of the piglets to challenge with TGE virus (2).

The objectives of the experiments described in the present paper were to determine whether IFN could be induced in parturient sows, whether IFN could be transferred to the newborn piglets, in the milk or across the placenta, and whether the suckling piglets, as well as the treated sows, would be more resistant to challenge with TGE virus. Three sows were treated with poly ICLC shortly after parturition, and their sera and milk, together with sera from their suckling piglets, were assayed for IFN. Two litters of piglets from treated sows were exposed to infection with virulent TGE virus, together with an untreated control sow and her litter, and as positive controls some of the suckling piglets were treated with poly ICLC shortly after birth. Two further sows were treated with poly ICLC on the day before the expected farrowing date. Their sera, and sera from their piglets at birth, were tested for IFN, and in addition peripheral blood lymphocytes (PBL) from their newborn piglets were tested for NK activity as an indirect indication of placental transfer of IFN.

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MATERIALS AND METHODS

PREPARATION AND ADMINISTRATION OF POLY ICLC

The poly ICLC was prepared as described (2). Cross-bred Yorkshire sows from a specific pathogen-free herd which lacked virus neutralizing antibodies against TGE virus were housed in isolation and given 0.06 mg/kg of poly ICLC by intravenous injection either within two days after farrowing, or on the day before the anticipated farrowing date. Newborn piglets were given 0.5 mg/kg of poly ICLC intravenously at two days of age.

ASSAY AND CHARACTERIZATION OF INTERFERON

The serum samples were tested for antiviral activity by a plaque inhibition assay in Madin-Darby bovine kidney cells challenged with vesicular stomatitis virus as previously described (3). Milk samples were diluted 1:20 in Eagle's minimum essential medium (Gibco Laboratories) and filtered through a 450 nm Millipore membrane filter before they were tested. The antiviral activity of selected samples was characterized as IFN according to standard criteria (4), and found to be abolished by trypsin treatment, partially susceptible to heat, and resistant to pH 2.0 and ultracentrifugation, consistent with the properties of type I (α/β) IFN.

ASSAY OF NATURAL KILLER ACTIVITY OF LYMPHOCYTES

Immediately after birth, the piglets from the two sows treated with poly ICLC before farrowing were anesthetized with halothane (Somnothane: Hoechst Canada Inc.) and 30 mL of blood were collected by cardiac puncture, after which each piglet was killed. Peripheral blood lymphocytes were isolated and purified by centrifugation over Ficoll-Hypaque, with removal of monocytes by plastic adherence (1). Natural killer activity of the PBL was determined by the single cell cytotoxicity assay (2), utilizing PK-15 cells as targets.

CHALLENGE WITH TRANSMISSIBLE GASTROENTERITIS VIRUS

Two days after farrowing, when poly ICLC was administered to the treated sows, two piglets were removed from each litter and dosed orally with 1.5 mL of a 20% suspension of small intestinal mucosa and contents from a gnotobiotic piglet which had been infected 48 h previously with the Purdue strain of TGE virus. The piglets were fed with 50% evaporated cows' milk (Carnation Milk) while they were away from the sows, to which they were returned 8 h later. The purpose of these piglets was to serve as a source of virus for contact transmission to the remainder of each litter. They showed clinical signs of TGE on the day after infection. The sows and piglets were examined clinically twice daily for the duration of the experiment, which was terminated five days after farrowing, when the sows and piglets were necropsied.

RESULTS

INTERFERON INDUCTION IN POSTPARTURIENT SOWS

The three sows which were treated with poly ICLC two days after farrowing were mildly depressed 4 h after treatment. The titers of IFN in the serum and milk of these sows peaked at 8 h postinduction and declined to relatively low levels by 24 h postinduction (Fig. 1). The titers in the serum were higher than in the milk except at 24 h postinduction. Serum samples from the suckled piglets were monitored for IFN over the same 24 h period, and were invariably negative. Interferon was not found in the serum or milk of an untreated sow, nor in her piglets, during the same neonatal period.

RESPONSE TO CHALLENGE WITH TRANSMISSIBLE GASTROENTERITIS VIRUS

In this experiment, two sows were treated with poly ICLC two days after farrowing, and 8 h later two piglets infected with TGE virus as described above were returned to each litter to provide a source of infection for the sows and their piglets. An untreated control sow and her litter were similarly challenged with TGE virus. The IFN titers in samples of serum and

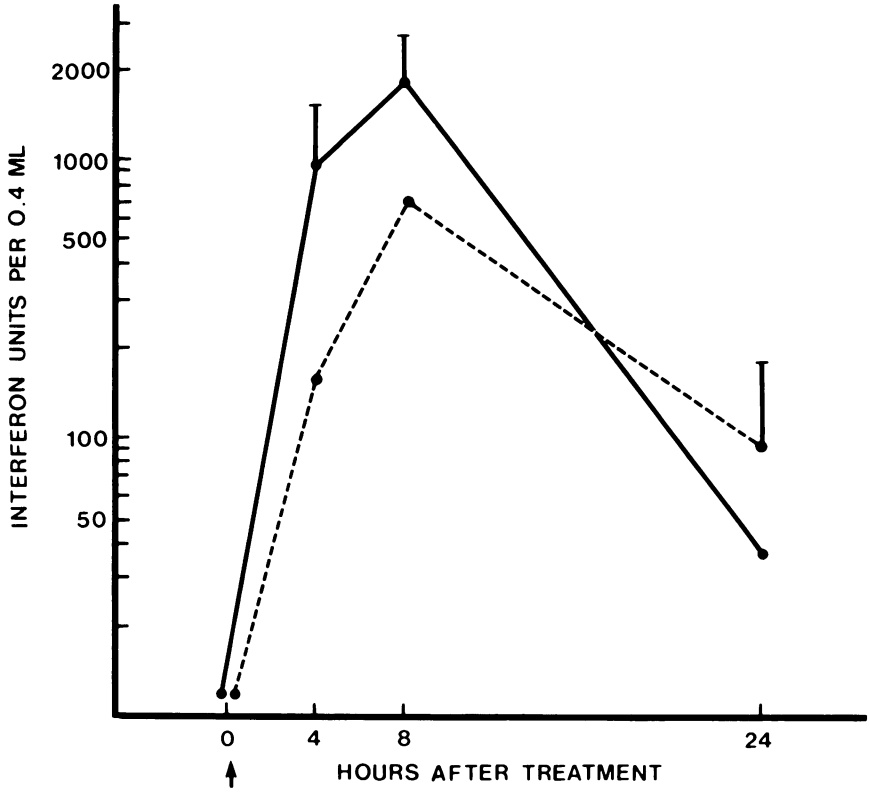

Fig. 1. Mean interferon titers in the serum (●———●) and milk (●- - - - - - - - -●) of three sows treated with poly ICLC (arrow). Standard deviations are shown as vertical bars.

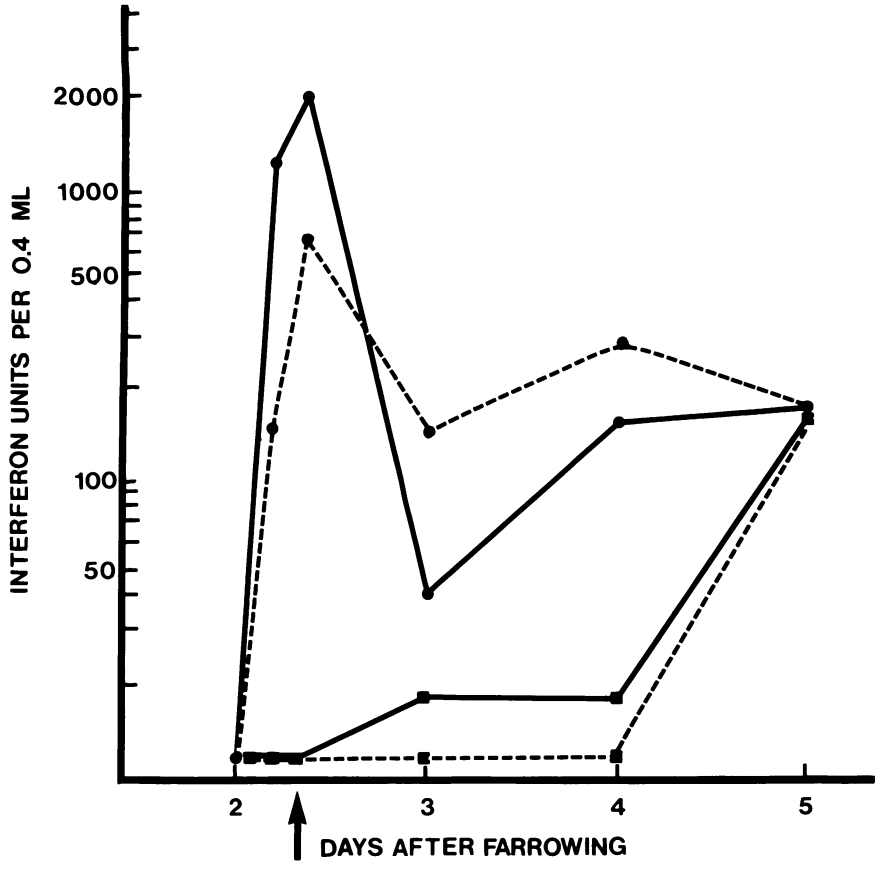

Fig. 2. Interferon titers in the serum (———) and milk (- - - - - - - -) of sows treated with poly ICLC (•) two days after farrowing, and of an untreated sow (■), exposed to TGE virus 8 h later (arrow). The values for the treated sows are means from two animals.

milk from these sows are shown in Fig. 2. The treated sows showed an IFN response to treatment with poly ICLC similar to that described above, peaking at 8 h postinduction and falling to relatively low levels by 24 h postinduction. The titers then rose again in the treated sows in response to exposure to TGE virus, while the untreated sow showed a similar but somewhat slower response to exposure to TGE virus. Both of the treated sows remained clinically normal throughout the experiment, while the untreated sow developed vomiting and diarrhea two days after exposure to TGE virus.

Eleven of the piglets born to the treated sows and four of those born to the untreated control sow were left untreated, and their IFN responses are shown in Fig. 3. The IFN responses to exposure to TGE virus were similar in the piglets suckled by the treated sows and those suckled by the untreated sow except at five days after farrowing when the IFN titers in the former were higher than those in the latter. Vomiting and diarrhea began in all these untreated piglets within 24 h of exposure to the orally infected piglets in each litter. There were no differences in the time of onset of clinical signs of TGE, or in the subsequent course of the disease and postmortem findings at five days of age.

Three piglets born to one of the treated sows and three piglets born to the untreated sow were themselves treated with poly ICLC two days after farrowing. Four hours after inoculation with poly ICLC the treated piglets were dull and listless, but they had recovered by 8 h postinoculation. The IFN responses in these piglets are shown in Fig. 4. They showed an initial response to the injection of poly ICLC at 8 h postinduction, and a second response to exposure to TGE virus. The only diffrence between the responses of the piglets suckled by the treated sow and those suckled by the untreated sow was at five days after farrowing, when the IFN titers in the former were higher than in the latter. All of these treated piglets showed a delay in onset of clinical signs of TGE of approximately 8 h, compared with their untreated litter mates. The subsequent course of the disease, and the postmortem findings, which were typical of TGE, were indistinguishable between the treated and untreated piglets.

INTERFERON INDUCTION IN PREPARTURIENT SOWS

Two sows were treated with poly ICLC on the day before the anticipated farrowing date. Each sow had a serum IFN titer of 2560 units/0.4 mL 6 h after treatment. The first sow farrowed three days after treatment. No antiviral activity was found in her piglets' serum immediately after birth, and no significant NK cell activity was demonstrated in the piglets' PBL. The second sow farrowed on the day after induction with poly ICLC, when the piglets were also negative for IFN and NK cell activity.

DISCUSSION

The sows showed a good response to IFN induction with poly ICLC. The serum IFN titers in the sows were higher than those observed in the poly ICLC-treated piglets, although the dose of poly ICLC for the sows was relatively lower than that used in the neonatal piglets. High levels of IFN were also found in the milk of the treated sows, and the kinetics of the milk IFN responses were similar to those in serum, as was demonstrated in mice (5). In spite of the high IFN titers in the sows' milk, circulating IFN was not found in the piglets which were suckled by the treated sows, in contrast to the findings in mice (5). Failure of the neonatal piglets to absorb detectable amounts of IFN may indicate digestion of the IFN, or simply that insufficient IFN was present in the milk to be detected after absorption.

When newborn piglets were challenged by exposure to TGE virus, the clinical responses of the piglets suckled by sows which had been treated with poly ICLC were indistin-

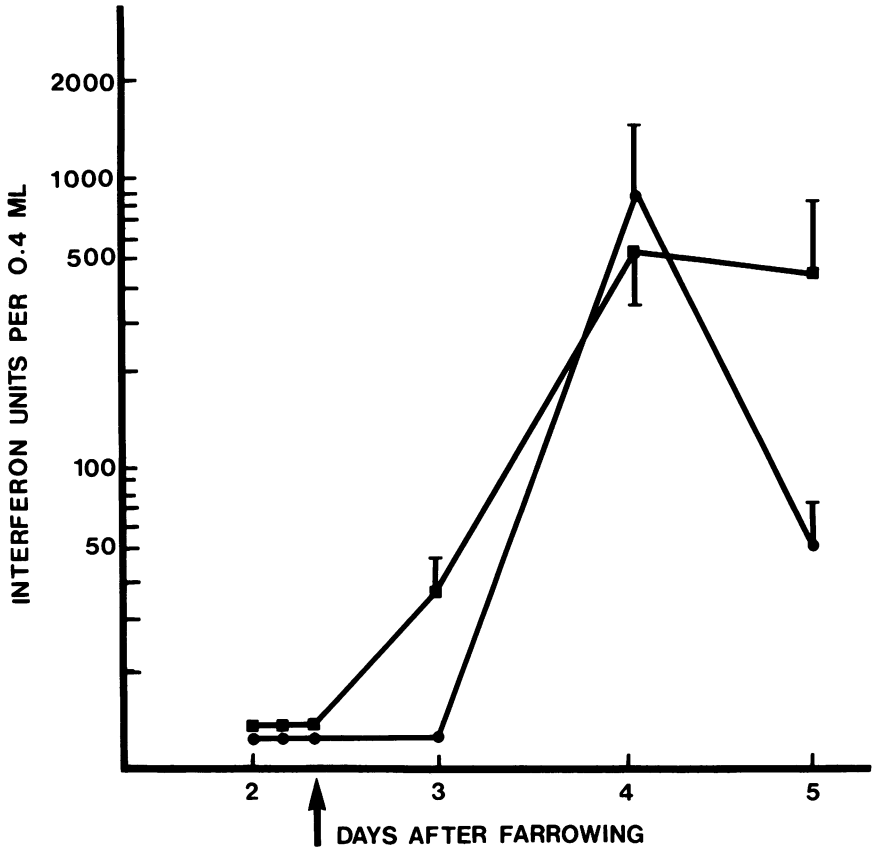

Fig. 3. Mean interferon titers in serum from 11 piglets suckled by sows treated with poly ICLC two days after farrowing (■——■) and from four piglets suckled by an untreated sow (●——●), all exposed to TGE virus (arrow). Standard deviations are shown by vertical bars.

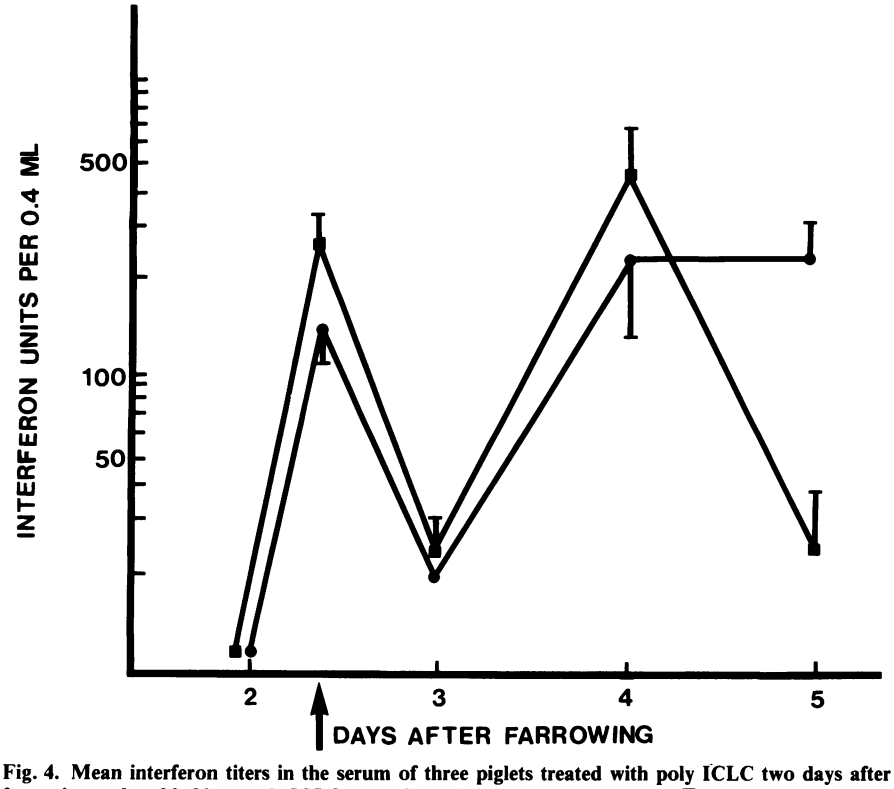

Fig. 4. Mean interferon titers in the serum of three piglets treated with poly ICLC two days after farrowing and suckled by a poly ICLC-treated sow (●), or an untreated sow (■) and exposed to TGE virus 8 h later (arrow). Standard deviations are shown by vertical bars.

guishable from those of the piglets which were suckled by an untreated sow. Therefore the IFN ingested in the milk failed to protect the piglets against TGE virus infection, in contrast to the situation in infant mice which resisted oral challenge with vesicular stomatitis virus when their mothers were treated with an IFN inducer (5). It should be noted, however, that bovine IFN-α given orally to newborn piglets failed to protect them against challenge exposure to TGE virus (6), although the IFN inhibited TGE virus replication *in vitro*. While the failure of the two treated sows to develop TGE when exposed to the virus, in contrast to the untreated sow, suggests a protective effect of poly ICLC in the sow, additional numbers would be required to verify this. Although the treated sows showed no clinical signs of TGE, they did show a serum IFN response after exposure to the virus.

We obtained no evidence that IFN, induced in the sow by treatment with poly ICLC, crossed the placenta. Although Korsantiya and Smorodintsev (7) claimed to have demonstrated transplacental transmission of endogenous IFN in pregnant mice inoculated with influenza or Newcastle disease viruses, others (5) pointed out that these data could be interpreted as transfer of IFN in the milk rather than across the placenta. More recent studies (8) showed that exogenous IFN given to pregnant mice failed to cross the placenta, and in sheep no evidence of placental transfer of IFN was found (9).

Finally, the administration of poly ICLC directly to newborn piglets caused a delay in the onset of clinical signs when the piglets were challenged with TGE virus, although the subsequent course of the disease, and the IFN response to challenge, did not differ from the controls. We interpret this as evidence of a transient increase in resistance to TGE, confirming earlier findings in this laboratory (2).

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