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INTERFERON RESPONSE IN COLOSTRUM-DEPRIVED NEWBORN CALVES INFECTED WITH BOVINE ROTAVIRUS: ITS POSSIBLE ROLE IN THE CONTROL OF THE PATHOGENICITY

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Résumé

INDUCTION D'INTERFÉRON AU COURS DE L'INFECTION DU VEAU NOUVEAU-NÉ PAR LE ROTAVIRUS : RÔLE POSSIBLE DANS LE CONTRÔLE DE LA PATHOGÉNICITÉ. — Des veaux nouveau-nés privés de colostrum sont inoculés dès la naissance ou à l'âge de trois jours au moyen de rotavirus produit en culture de cellules. La cinétique d'apparition d'interféron dans la circulation en réponse à différentes doses de virus est mise en corrélation avec l'excrétion virale. Les fortes doses de virus conduisent à l'induction précoce d'interféron ; aucun symptôme clinique n'est observé dans ces conditions. Aux faibles doses par contre, la production d'interféron est beaucoup plus tardive ; elle suit l'apparition d'une diarrhée intense mais de courte durée. Plusieurs vagues d'induction sont observées dans tous les cas. Nos observations plaident en faveur du rôle de l'interféron endogène dans la résistance du veau à l'infection virale.

Rotavirus is very often associated with neonatal calf diarrhœa (Pastoret and Schœnaers, 1977). It was shown recently to induce the production of interferon, both in the small intestine and in the serum of experimentally infected animals (La Bonnardière *et al.*, 1981).

However, a functional role of interferon in the control of pathogenicity and in the recovery from rotaviral infection could not be ascribed from the available data.

Colostrum-deprived newborn calves infected with high doses of cell culture rotavirus did not show any clinical symptom (Schwers *et al.*, 1982), while calves inoculated with lower doses of the same virus isolate presented diarrhœa (Schwers *et al.*, 1983a). The virus produced in cell culture conserves therefore its pathogenicity for newborn calf.

A possible explanation for the paradoxical dose effect observed in these experiments is a control of the viral pathogenicity by endogenous interferon. It was therefore essential to analyze interferon production after experimental rotavirus infection.

The clinical and virological studies have been presented in a separate paper (Schwers *et al.*, 1983b). We report here the kinetics data concerning the interferon induction, in parallel with viral excretion, in eleven colostrum-deprived calves experimentally infected with different doses of bovine rotavirus.

Materials and Methods

Virus

Bovine rotavirus strains S14 and S77, both isolated from stools of calves dead from diarrhœa (Dagenais *et al.*, 1981a), were used after four (S77) or six (S14) passages on GBK (Georgia Bovine Kidney) cells. Virus was titrated following the method of Matsuno *et al.* (1977).

Experimental procedure

Eleven colostrum-deprived newborn calves were used in the experiments reported here. They were isolated immediately after birth in individual containers in thoroughly decontaminated stable and inoculated orally with different doses of one of the two rotavirus strains studied.

The experimental procedure has been described in detail in a separate paper (Schwers *et al.*, 1983b) and is briefly summarized in figure 1. To allow direct correlation of the data, identical numbering of the calves is used in both papers.

Plasma and stool samples were collected before and twice a day for at least ten days after inoculation; sera were taken on days 0 and 15.

Virus isolation and counter-immuno-electroosmophoresis (CIEOP)

Virus isolation from stool samples have been performed for one sample per calf and per day, following the method of Babiuk *et al.* (1977).

The CIEOP technique described by Middleton *et al.* (1976) has been used for detection of rotavirus antigen in stools and of antirotavirus antibodies in sera.

Interferon titration

The plasma were maintained at − 85 °C until interferon titration. The circulating interferon activity was determined in GBK cells, grown in MEM containing non essential amino-acids, penicillin, streptomycin and 10 % foetal calf serum.

The technique of reduction of cytopathic effect of VSV as challenge virus was used according to Stewart (1979).

Results

The kinetics of interferon response to experimental infection of newborn calves with rotavirus in different conditions were correlated with virus excretion in the faeces. They are schematically presented for all the calves studied in figure 1, and are detailed, for some of them, in figure 2.

With a first group of animals (calves 1 to 5) receiving within two hours after birth high doses of rotavirus, a similar profile of interferon production was observed.

An early peak of activity was detected beginning on day 1 after infection. After total clearance from the circulation, two other peaks of antiviral activity appeared around days 6 and 10.

The different waves of interferon production correlate well with the periods of virus excretion. None of the animals of this group showed any sign of diarrhœa.

In calves 7 and 8, inoculated 3 days after birth, a first peak of interferon production appeared in the circulation in less than 24 hours; the second wave of interferon production was delayed until day 9 to 12 (6 to 9 days after viral inoculation). The calves of this group did not show any symptom either.

The third group of animals received immediately after birth a much lower dose of rotavirus S77: in this case, also multiple peaks of interferon activity appeared in the circulation. The antiviral activity reached a similar level with these virus doses as with the higher dose used before. The kinetics of interferon induction was however quite different: the antiviral activity did not appear before the third day of life. This delay of 24 to 48 h was observed for the three waves of interferon production.

In these conditions, a severe but transient diarrhœa was observed 48 h after viral infection, before any interferon could be detected in the circulation. No sign of diarrhœa persisted at the time interferon appeared in the plasma. No other general symptom was observed. Finally (data not shown), an uninfected control calf (10), isolated in the same conditions, was also studied; a contact calf (9) not inoculated, but kept in the same stable as the infected calves 7 and 8, showed a very minor virus excretion at day 7; soft stools rich in mucus were observed from day 2 to day 4. In neither of these calves, was interferon detected.

Discussion

The data reported here indicate a possible role of interferon in the control of viral infections in the bovine species.

Our results show that a threshold level of infecting virus is necessary to observe an early interferon response. With the lower dose of virus used here, the pathogenicity of the virus is expressed transiently. The replication of the virus occurs in the epithelial cells of duodenum and jejunum and (Mebus *et al.*, 1971) interferon is subsequently produced. With very high doses of rotavirus (in fact much higher than what a calf may ingest in natural conditions), interferon is produced immediately and no symptoms were observed.

However, using a sensitive ELISA test, La Bonnardière *et al.* (1981) detected in young animals inoculated with bovine rotavirus a weak, but significant viraemia. Whether the interferon appearing in the circulation is produced by intestinal cells or induced in blood cells by these circulating viral antigens is not yet known and deserves further investigation.

A mechanism of interference was suggested to explain the early and non-specific protection observed in calves after oral vaccination with an attenuated rotavirus strain (Mebus *et al.*, 1972) and in mice successively inoculated with two different strains of rotavirus (La Bonnardière *et al.*, 1979), but the induction of interferon has not been demonstrated in these conditions.

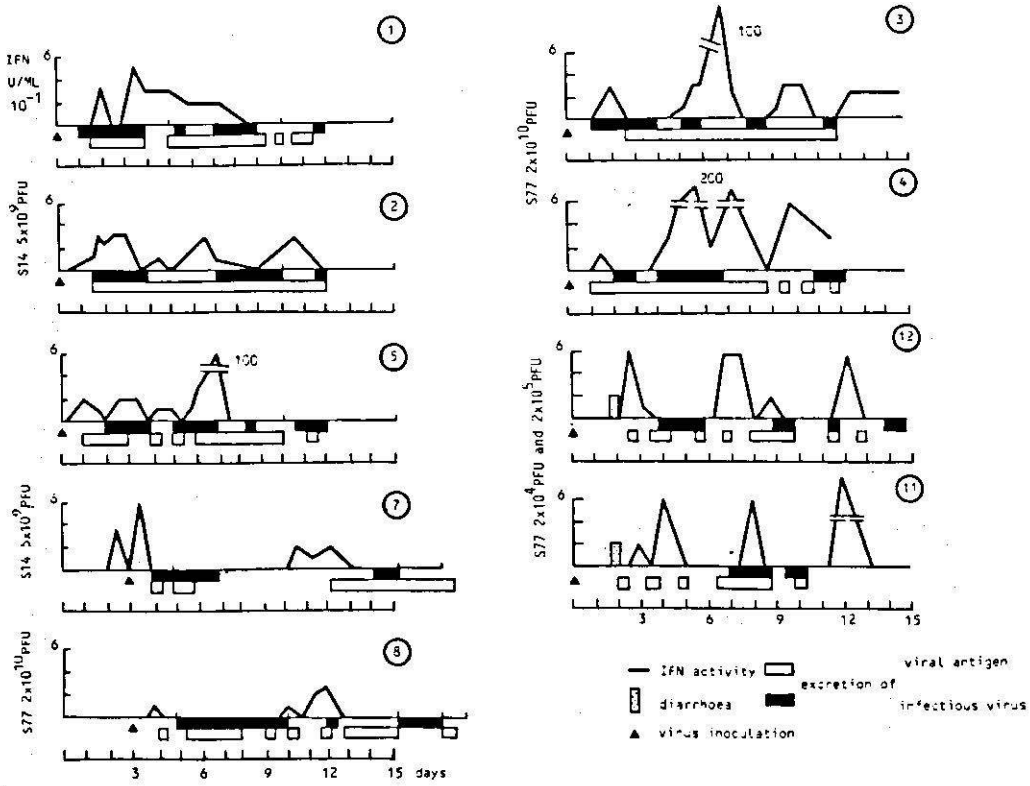

Fig. 1. — Correlation between virus excretion and interferon production. The kinetics of interferon production are presented for three groups of animals. High dose of virus given immediately after birth (5×10^9 PFU S14 for calves 1, 2 and 5 or 2×10^{10} PFU S77 for calves 3 and 4). Same high doses of virus S14 (calf 7) or S77 (calf 8) given on day 3. Low doses of virus S77 at birth (2×10^4 PFU for calf 12; 2×10^5 for calf 11). Viral excretion (infectious virus or viral antigen detected by CIEOP) is presented schematically in parallel.

As interferon production in calves inoculated with high doses of virus is so early and so well correlated with virus excretion, our data are strongly indicative of a protection mediated by interferon. Moreover, if lower doses of virus are used, the interferon appears much later after the onset of diarrhœa, which stops when the level of antiviral activity becomes significant in the circulation.

If this interpretation is correct, one should conclude that with a high dose of infecting virus, interferon is produced immediately when particles reach the target cells. With a lower virus dose, at least one multiplication cycle appears necessary before interferon could be significantly induced. During this later process, the pathogenicity is transiently expressed.

The periodicity of interferon production results probably from the progression of the infectious process along the intestinal tract (Mebus *et al.*, 1971): the virus reaches regions where the antiviral state is not yet established. By infecting cells at these sites, a new wave of interferon production is observed, which limits the infectious process again. A similar process has recently been described in calves after experimental infection with bovine respiratory syncitial virus (El Azhary *et al.*, 1981). In this case however, interferon production could not be correlated with virus production.

Our data indicate that interferon plays an important role in the resistance of the calf to viruses. It appears as a key factor in the natural recovery from viral infections; it may also block completely the development of symptoms. Such involvement of endogenous interferon in host resistance to various viral diseases has been directly demonstrated in the mouse by showing that anti-interferon antibodies increase mortality

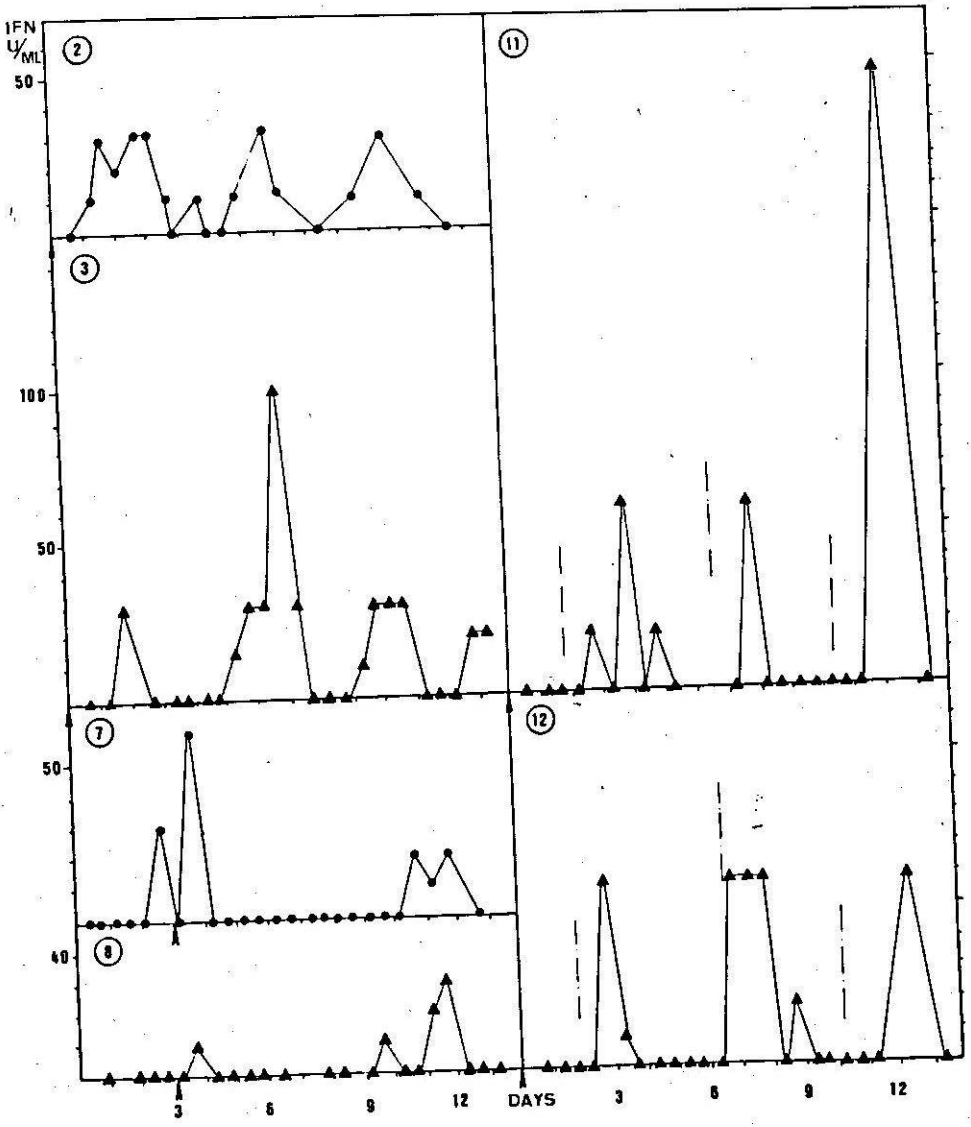

Fig. 2. — Kinetics of interferon production induced by rotavirus. For some of the calves studied, the data are reported in detail. Calves 2 and 3 : high dose of virus (respectively S14 5×10^9 PFU and S77 2×10^{10} PFU) given immediately after birth ; Calves 7 and 8 ; same dose of the same virus given on day 3 ; Calves 11 and 12 : Low doses of rotavirus S77 (respectively 2×10^5 PFU and 2×10^4 PFU). The vertical interrupted line shows in these two pannels the corresponding position of the peaks of interferon in the calves infected with high dose of virus.

(Fauconnier, 1971 ; Gresser *et al.*, 1976 a,b).

Moreover, interferon permits a limited multiplication of rotavirus, and seroconversion may appear (Schwers *et al.*, 1983b). This observation indicates that interferon is able to protect an animal against viral infection without blocking the

establishment of the specific long term resistance mechanisms.

The protection we report here is linked to the sensitivity of the rotavirus strains used (Dagenais *et al.*, 1981b). As La Bonnardière *et al.* (1980) reported a weak *in vitro* susceptibility of another rotavirus strain, our *in vivo* study should be extended before it could be generalized. However, as the multiplication in cell culture of Bovine herpesvirus 1 and Suid herpesvirus **1**, two other economically important viruses, is inhibited by interferon (Goossens *et al.*, 1983), we may expect that exogenous interferon will be active against many viral diseases in cattle.

The recent demonstration of an efficient protection against experimental vaccinia infection using human interferon (Hu-IFNα2) (Werenne *et al.*, 1984) opens therefore interesting perpectives for interferon in veterinary medicine.

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Summary

Colostrum-deprived newborn calves were experimentally infected with cell-culture rotavirus. A similar process of infection was observed when the animals were inoculated immediately after birth or at the age of three days, with a corresponding delay in the onset of virus excretion and interferon production in the later case. With high doses of virus, interferon was produced very early and no symptoms were observed. With lower doses of virus, interferon production was delayed and preceded by a severe but transient diarrhœa. In all cases, several waves of interferon production were observed. Our data indicate that interferon plays an important role in the control of viral diseases in calves and in their natural recovery.

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