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Role of interferons in infectious diseases in the bovine species : Effect on viruses and rickettsias

TOTTÉ (Ph.), DE GEE (A.L.W.), WÉRENNE (J.). Le rôle des interférons dans les maladies infectieuses du bovin : leurs effets sur les virus et les rickettsias. *Revue Élev. Méd. vét. Pays trop.*, 1993, 46 (1-2) : 83-86

Le traitement aux interférons (IFN) fournit une protection chez le bovin contre certaines infections virales expérimentales. L'efficacité du traitement a été démontrée contre des infections par le virus de la vaccine et par un rotavirus. En revanche, des infections par le virus de l'herpès bovin, BHV1 (cause de la rhinotrachéite et partiellement du complexe de la fièvre de transport), ne sont pas inhibées par IFN. Le rôle d'IFN dans la résistance des bovins à *Cowdria ruminantium* a été étudié au Zimbabwe. Une bonne corrélation a été trouvée entre la production d'IFN par l'animal après l'infection et sa résistance contre la rickettsie. Cela pourrait indiquer un rôle d'appui des interférons et d'autres cytokines.

Mots clés : Bovin - Interféron - Rickettsiales - Virus - Rotavirus - Herpèsvirus bovin - *Cowdria ruminantium* - Résistance aux maladies.

INTRODUCTION

The authors were, about 10 years ago, able to evaluate the protective activity of recombinant interferons against viral infections in calves (2, 3, 7, 8, 9). Success was obtained in vaccinia and rotavirus infections. But as observed by us and confirmed by others, Interferon was shown to be unable to inhibit the replication of bovine herpes virus 1, even if immunomodulatory effects of the injected interferon appeared to reduce the mortality of superinfection with Pasteurella.

The observation of the authors with rotavirus indicates that the infection itself is able in some situations (infection with extremely high amount of virus (5)) to induce endogenous interferon that exerts an inhibitory effect against the pathogenic effect of the virus. This was the first indication that an interplay of cytokines could play an important role in the natural resistance of cattle against infections.

As interferons have been shown in mouse and human systems to play an important role in the resistance against obligate intracellular parasites, including Rickettsiales (1) the authors undertook an *in vitro* study some years ago to evaluate the importance of this system in cowdriosis.

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MATERIALS AND METHODS

In vivo Cowdria infection, observation of symptoms and of interferon induction

During a vaccination campaign, a group of heifers was infected with *Cowdria ruminantium*, blood from infected goats, injected intravenously, without any antibiotic treatment. Rectal temperature was recorded daily and blood was collected for determination of interferon activity by the classical method of reduction of the cytopathogenic effect of VSV (4).

2-5A Synthetase titration

2-5A Synthetase activity was assayed in the cytoplasmic fraction of the cells. The cytoplasmic fraction was prepared as follows :

- the cells were washed twice with buffer A (NaCl 140 mM, Tris HCl pH 7.5, 35 mM) after trypsinization (all steps at 4 °C) ;

- cells were broken with a Dounce homogeniser in buffer C (10 mM Tris HCl pH 7.5, 1.5 mM magnesium acetate, 1mM DTT, 1 mM benzamidine, 100 µM PMSF) with glycerol 10 % and Triton X100, 0.5 % ;

- lysates were centrifuged for 15 min at 13,000 g in an Eppendorf microcentrifuge and the supernatant containing the cytoplasmic fraction of the cells was stored at -70 °C ;

- 2-5A Synthetase was assayed by the procedure reported before (6). Essentially, incubation of the extract was performed in a reaction mixture containing (in 15 ml volume), 35 mM magnesium acetate, 2 mM fructose-1.6 biphosphate, 1mM DTT, 8 mM ATP, 8 µCi³H ATP, 15 mM Hepes buffer and 15 µg/ml dsRNA was added to the sample (15 µl of reaction mixture and 10 µl of sample) ;

- the tritiated 2-5(A)n are separated from ³H ATP by ion exchange on DEAE Paper (DE 81 from Whatman) after treatment of the mixture reaction with the bacterial alkaline phosphatase.

The radioactivity of the 2-5A synthesized is determined by liquid scintillation. The results are expressed in pmoles of ATP incorporated per hour and per µg of protein .

Interferons used

Recombinant bovine interferons used for *in vitro* were kindly supplied to us by Dr A. Shafferman, Israel Institute of Biological Research (BoIFN α C and BoIFN α D), or by Dr R. STEIGER, CIBA-GEIGY (BoIFN α 1, and BoIFN γ). The human interferon, IFN α 2 was a gift from Dr. C. WEISSMANN, Zürich University.

RESULTS AND DISCUSSION

Correlation between resistance to *Cowdria ruminantium* and IFN induction

In the experimentation undertaken with a group of animals that were infected with the rickettsia and not treated with antibiotics, we observed, as expected, a number of death from the disease. Some animals however resisted the infection without treatment. All of these showed an early induction of an antiviral activity corresponding to interferon, soon after infection, before the rise of temperature. This is in contrast to what was observed with animals which did not survive the infection : they did not produce significant amounts of interferon before the rise in temperature.

The figure 1 presents in function of time after the *Cowdria* infection, the production of interferon as measured by its antiviral effect and the daily temperature as symptom of the infection, in one surviving animal and in one animal that died of the infection, compared to the control. All the animals in each group present the same pattern, and the data presented here are typical. The nature of the interferon has been further characterized. Most of the antiviral activity could be ascribed to IFN α using antibodies (the data, not shown here will be published elsewhere). However we cannot exclude the induction of other cytokines: some evidence indicating that a small part of the antiviral activity is not "species specific", is in favor of such an interpretation. Moreover IFN γ which is not maintained in the circulation for a long period, would not be easily detected if produced locally.

Induction of 2-5A Synthetase by interferons

Interferon is acting on cells through its interaction with membrane receptors activating a signal transduction mechanism which ends up by turning on a number of genes coding for proteins playing a role in its mechanism. Twenty proteins have been detected, and among the best known of these proteins, there is a protein kinase and the 2-5A Synthetase. An inhibition in translation of viral message could result from the activity of those proteins. The kinase is involved at the level of initiation while it has

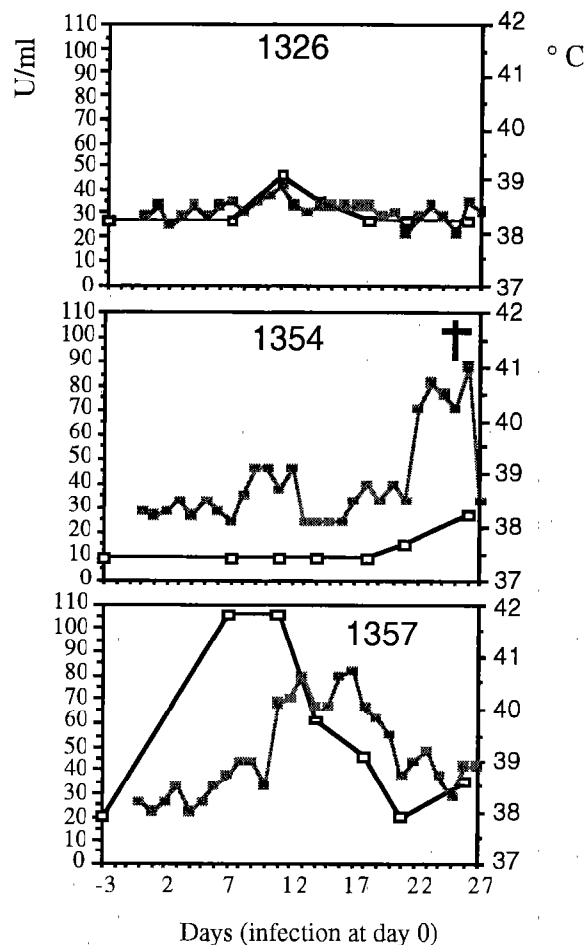


Figure 1 : Circulating antiviral activity (\square) and rectal temperature (\blacksquare) of *C. ruminantium* infected cattle. Animal no. 1326 was included as a non infected control. Animal no. 1354 died of heartwater and animal no. 1357 survived the infection.

been shown that 2-5A Synthetase, by the product of its enzymatic activity 2-5A is activating an RNase. It was shown for some viruses that one of those enzymes is the main factor in their inhibition. Both of these induced proteins require the presence of dsRNA to have their pathway activated. It should therefore be pointed out that other mechanisms, not yet well understood exist.

However, we have shown that 2-5A Synthetase is a good marker to show that interferon has transmitted its message to the cell. When elevated in the cell the activity of 2-5A Synthetase indicates that the interferon system was activated.

Figure 2 shows the kinetics of induction *in vitro* of synthetase in bovine kidney cells by bovine interferons (2 IFN α and 1 IFN γ). There is some difference between the interferons, in the level of activity induced for a given antiviral

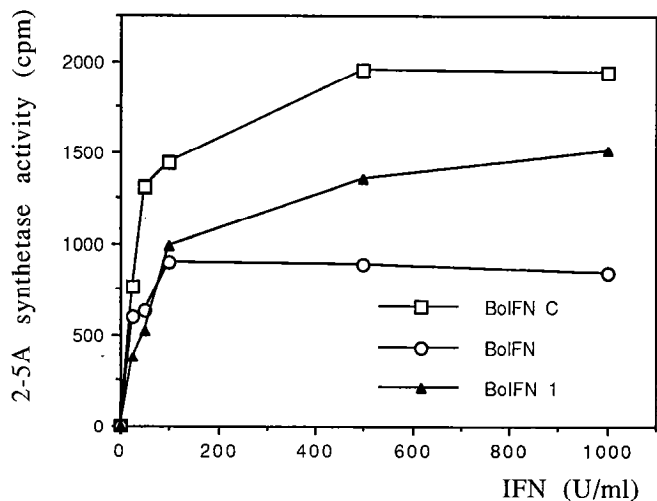


Figure 2 : Kinetics of 2-5A Synthetase induction by bovine interferons in bovine kidney cells.

activity, but they are all active (maximum activity being reached with an amount of interferon as low as 100 U/ml).

Figure 3 shows the 2-5A Synthetase inducing activity in the same cells of the plasma from calves that have been deliberately infected, compared to the activity of different recombinant IFNs. This confirms that the antiviral activity observed in the infected animals is most probably interferon for a large part.

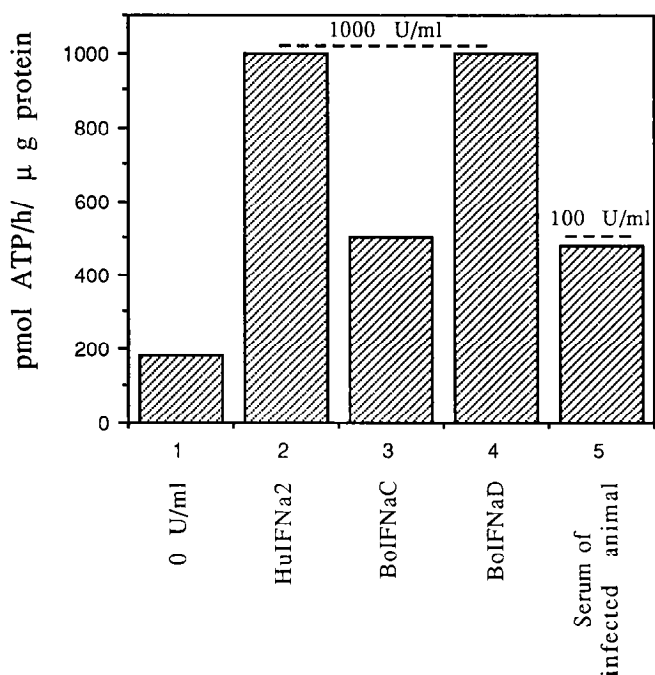


Figure 3 : Induction of 2-5A Synthetase by recombinant interferons and by the serum of an infected animal.

The authors do not imply however that the anti-*Cowdria* activity of interferon results from the activity of 2-5A. It should indeed be pointed out that bovine umbilical endothelial cells (BUEC) are insensitive to the anti-*Cowdria* activity of IFN, while bovine microvasculature endothelial cells (BMC) are very sensitive, both cells responding similarly to IFN for the antiviral activity and for the 2-5A Synthetase induction (see the other paper, "Inhibition of *Cowdria ruminantium* infectious yield by interferons alpha and gamma in endothelial cells").

CONCLUSION

The observations of the authors, taken together, indicate strongly that the interferon system, and probably other cytokines as well, play a key role in the natural resistance to the infection by *Cowdria ruminantium* as it does for other intracellular parasites.

While we do not imply that interferon will be part of the medication one could apply on a large scale to fight Cowdriosis, they pave the way towards understanding the mechanisms of resistance against the rickettsia, which could be of value to develop more adequate vaccines. An attenuated variant of *Cowdria* which would be a good inducer of interferon could be a good candidate for a vaccine. This is a hypothesis which would be worthwhile to test since we possess the necessary tools for such a venture.

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TOTTÉ (Ph.), DE GEE (A.L.W.), WÉRENNE (J.). Role of interferons in infectious diseases in the bovine species : Effect on viruses and rickettsias. *Revue Élev. Méd. vét. Pays trop.*, 1993, **46** (1-2) : 83-86

Successful protection was obtained with interferon treatment in experimental viral infections in the bovine species in a number of cases. The efficacy of the treatment against vaccinia virus infection and against rotavirus infection have been demonstrated. On the contrary, bovine herpes virus 1 (BHV 1- causing rhinotracheitis and part of the shipping fever complex) infections were not inhibited by interferon (IFN). The authors have undertaken a study in cattle in Zimbabwe to assess the role of interferon in the resistance of the animals to *Cowdria ruminantium*. A good correlation between production of interferon by the animal following the infection, and the resistance of the animals against the rickettsia was demonstrated. This pointed out the possible "adjuvant" role of interferons and other cytokines.

Key words : Cattle - Interferon - Rickettsiales - Virus - Rotavirus - Bovine herpes virus - *Cowdria ruminantium* - Disease resistance.

TOTTÉ (Ph.), DE GEE (A.L.W.), WÉRENNE (J.). Papel del interferón en las enfermedades infecciosas en las especies bovinas : efecto sobre virus y rickettsias. *Revue Élev. Méd. vét. Pays trop.*, 1993, **46** (1-2) : 83-86

En varios casos de infecciones virales experimentales en especies bovinas, se obtuvo una protección adecuada con el tratamiento con interferón. Anteriormente se demostró la eficiencia del tratamiento contra la infección por el virus vaccinia y contra la infección por rotavirus. Por el contrario, las infecciones por herpesvirus bovino 1 (BHV 1, agente causal de la rinotraqueítis y parte del complejo de fiebre de transporte ("shipping fever")), no fueron inhibidas por el interferón (IFN). En Zimbabwe, se llevó a cabo un estudio en ganado bovino, con el fin de demostrar el papel del interferón en la resistencia de los animales a *Cowdria ruminantium*. Se demostró una buena correlación entre la producción de interferón por parte del animal después de la infección y la resistencia a la rickettsia. Esto indica un posible papel de colaboración por parte del interferón y otras citoquinas.

Palabras claves : Bovino - Interferón - Rickettsiales - Virus - Rotavirus - Herpesvirus bovino - *Cowdria ruminantium* - Resistencia a la enfermedad.