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In vivo study of interferon-alpha-secreting cells in pig foetal lymphohematopoietic organs following in utero TGEV coronavirus injection

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SUMMARY

Non-infectious UV-inactivated transmissible gastroenteritis virus (TGEV) was previously shown to induce interferon alpha (IFNa) secretion following in vitro incubation with blood mononuclear cells. In this study, pig foetuses at different stages of gestation were injected in utero with (a) partially UV-inactivated wild TGEV or (b) fully UV-inactivated wild or dm49-4 mutant TGEV coronavirus. Nucleated cells from foetal liver, bone marrow, spleen and blood were isolated 10 or 20 h after injection and assayed ex vivo for IFNa secretion by ELISPOT and ELISA techniques. The administration of TGEV induced IFNa-secreting cells in foetal lymphohematopoietic organs at mid-gestation. In contrast, IFNa was not detected in control sham-operated foetuses. A specific point mutation in the amino acid sequence of the viral membrane glycoprotein M of TGEV mutant dm49-4 was associated with lower or absent IFNa in utero inducibility by mutant virus as compared with wild virus. Flow cytometry analysis did not show differences in leukocyte surface marker expression between control and TGEV- or between dm49-4 and wild virus-treated foetuses, with the exception of a reduction in percentages of polymorphonuclear cells in TGEV-treated lymphohematopoietic tissues, which is probably due to IFNa secretion. The present data provided in vivo evidence of IFNa secretion at the cell level in foetal lymphohematopoietic organs. Such IFNa-secreting cells in lymphohematopoietic tissues may be the source of IFNa detected during foetal infections.

Key-words: Coronavirus, Transmissible gastroenteritis virus, IFNa; ELISA, ELISPOT, Foetus, Pig.

INTRODUCTION

Interferon-α (IFNα) is a critical component of early, host non-specific immune defence against viral infections. It acts as both an antiviral agent and an immunomodulator as well as a cell growth inhibitor. Several leukocyte populations are able to secrete IFNα, in response to virus stimuli, depending on the virus used and on whether viral infection of leukocytes is necessary or not.
Monocytes are most often associated with production of IFNα in response to infectious viruses (Roberts et al., 1979; Saksela et al., 1984), whereas a distinct population of leukocytes among peripheral blood mononuclear cells (PBMC), referred to as "natural interferon-producing cells" (NIPCs), is able to secrete IFNα following exposure to non-infectious viral structures (Lebon et al., 1982a; Capobianchi et al., 1985; Charley and Laude, 1988). Human and porcine NIPCs have been characterized as highly infrequent, non-phagocytic, non-adherent, non-B, non-T, MHC II+ and CD4+ cells (Cederblad and Alm, 1990; Charley and Lavenant, 1990; Sandberg et al., 1990; Fitzgerald-Bocarsly, 1993; Nowacki and Charley, 1993).

Viral glycoproteins were suggested as being responsible for triggering synthesis of IFNα in NIPC. Monoclonal antibodies (mAbs) to viral glycoproteins of herpes simplex virus type I, transmissible gastroenteritis virus (TGEV) and Aujeszky’s disease virus were shown to block virus-induced IFNα secretion (Lebon, 1985; Charley and Laude, 1988; Artursson, 1993). However, the precise nature of NIPCs as well as of the interactions between NIPCs and virus leading to IFNα production remains to be elucidated.

TGEV is an enteric coronavirus which causes acute and fatal diarrhoea, as well as intense and early IFNα production in newborn piglets (La Bonnariède and Laude, 1981). In vitro studies on IFNα induction by TGEV have shown that IFNα-secreting cells (IFNα-SCs) were detected among non-adherent porcine PBMCs after exposure to UV-inactivated TGEV or glutaraldehyde-fixed TGEV-infected cells (Charley and Lavenant, 1990; Nowacki and Charley, 1993). A specific point mutation in the amino acid sequence of the viral membrane glycoprotein M in dm49-4 mutant TGEV was shown to be associated with a defect in in vitro induction of IFNα (Laude et al., 1992).

The epitheliocorial nature of placentation in pig prevents transfer of immunoglobulins or antigens from mother to foetus, which precludes any immune activation of foetuses (Sterzl et al., 1966). This type of placentation together with multiparity, relatively long term of gestation and size of foetuses makes this species suitable for studies on the development of the immune system. In an earlier study on the prenatal ontogeny of porcine IFNα-SCs, we detected in vitro inducible IFNα-SCs in pig foetal liver and other lymphohaematopoietic organs, at very early stages of gestation (Šplíchal et al., 1994). The present study was undertaken to evaluate in utero viral induction of IFNα-SC in pig foetuses by TGEV, either partially or fully UV-inactivated, at different stages of gestation. We found that 10 or 20 h after in utero injection of TGEV in the umbilical cord of pig foetuses, IFNα-SCs were detected ex vivo in lymphohaematopoietic tissues by the ELISPOT assay.

MATERIALS AND METHODS

Animals

Healthy pregnant gilts of miniature pig bred in the Laboratory of gnotobiology in Nový Hrádek were used in our experiments. They had free access to water but were starved 12 hours before first surgery. The gilts were subcutaneously (s.c.) premedicated with 1 mg of atropin sulphate (Hoechst-Biotika, Slovakia) per 25 kg of body weight, and they were anaesthetized with 1.5-2.5% of halothane (Léciva, Czech Republic) mixed with O₂ and N₂O. HCG (1,500 U) (Léciva, Czech Republic) and acetate

| FCM | = flow cytometry (analysis). |
| FCS | = foetal calf serum. |
| FSC | = forward scatter. |
| HCG | = human chorionic gonadotropin. |
| IFN | = interferon. |
| IFNα-SC | = IFNα-secreting cell. |
| mAb | = monoclonal antibody. |
| MOC | = mononuclear cell. |
| NIPC | = natural interferon-producing cell. |
| PBL | = peripheral blood leukocyte. |
| PBMC | = peripheral blood mononuclear cell. |
| PBS | = phosphate-buffered saline. |
| PFU | = plaque-forming unit. |
| PMN | = polymorphonuclear (cell). |
| SSC | = side scatter. |
| SWC3 | = an antigen common for myelomonocytic lineages. |
| TGEV | = transmissible gastroenteritis virus. |
medroxyprogesterone (50 mg per 25 kg of body weight) (Upjohn, Netherlands) were intramuscularly (i.m.) injected. In the first series of experiments, fourteen foetuses of 52, 82 and 101 days of gestation were injected with partially UV-inactivated TGEV (500 PFU/ml in 50, 300 and 500 μl of saline, respectively) via the umbilical vein when the umbilical cord was exteriorized after laparotomy and uterotomy of the gilt. Control foetuses were subjected to the same surgery but treated with equivalent volumes of the saline only. The uterine and abdominal walls were sutured, and gilts were placed in a postsurgical care unit. They had free access to water but a limited amount of food. Animals were treated with 1,500,000 U penicillin G (Sopfa, Czech Republic) s.c. and 0.5 g streptomycin (Medexport, Russia) i.m. per 25 kg of body weight.

In the second series of experiments, twenty-one foetuses at 75, 77, 91 and 105 days of gestation were injected via the umbilical vein (300-1,500 μl) with a fully UV-inactivated wild or dm49-4 mutant TGEV (initial titre of 6x10^7 PFU per ml before inactivation). The injected volume was proportionally adjusted to expected body weight. The treatment of animals was the same as described above.

**Cell suspensions**

The second uterotomy was performed 20 or 10 hours later, in the first or second series of experiments, respectively. The sows were anaesthetized, foetuses were bled via umbilical cord arteries, and blood samples containing 20 U of heparin/ml of blood (Lécliva, Czech Republic) were collected. Cell suspensions from liver, spleen, both femurs and sternum were prepared by cutting these organs with scissors in cold PBS. Supernatants containing cells were collected after 10 min sedimentation at 1 g to remove debris and clumps. PBMCs were prepared by centrifugation of diluted blood on “Ficoll” density gradient (Pharmacia, Sweden), and red cells were depleted by hypotonic lysis with deionized water as described (Śplichal et al., 1994). In the case of a non-adherent cell fraction isolation, cells were incubated for 90 min in RPMI-1640 with 20% foetal calf serum (FCS) (Seromed, Germany), L-glutamine (2 mM), penicillin (100 U/ml) and streptomycin (100 μg/ml) (Gibco, UK) in 5% CO₂ atmosphere at 37°C to remove plastic-adherent cells. The number of nucleated cells and cell viability were calculated before assays.

**IFNα assays**

Cell suspensions (200 μl) in RPMI with 10% FCS and antibiotics were log₂ diluted in 3-6 wells of 96-well cell culture microplate (Costar, UK). ELIS-POT was performed as described (Nowacki and Charley, 1993). Cells in ELISPOT plates or in tissue culture microplates were incubated in 5% CO₂ atmosphere for 16 and 18 h, respectively. IFNα-SC frequency was estimated by an ELISPOT assay using peroxidase-labelled anti-pig IFNα mAb F17. Spots were counted under binocular microscopy, and the frequency was calculated from the total number of living nucleated cells and number of spots. IFNα titres in supernatants from tissue culture microplates incubated for 18 h, and in plasma, were estimated by pig IFNα-specific ELISA using the same antibodies as in the case of ELISPOT (De Arce et al., 1992; Nowacki and Charley, 1993). Results are expressed as IFNα unit/ml. Production of IFNα/IFNα-SC (yield) was estimated from the IFNα titre and the number of IFNα-specific spots.

**Flow cytometry analysis**

The following mouse mAbs directed against porcine leukocyte surface markers were used: K252.1E4 (anti-CD45), 74-22-15 (anti-SWC3), an antigen common for myelomonocytic lineages, 10-2H2 (anti-CD4) and MSA3 (anti-SLA-DR). Erythrocytes were removed from cell suspensions by hypotonic lysis of the cell pellet with water. Leukocytes were washed and stained as described recently (Cukrowska et al., 1996): briefly, cells were treated with a primary mouse mAb and then with fluorescein-conjugated swine anti-mouse Ig polyclonal antibodies (Sevac, Czech Republic); for double staining, biotinylated mAbs were revealed by streptavidin-phycocerythrin conjugate (Immunotech, France). Cells were divided into two major populations on the basis of their size and internal complexity: polymorphonuclear cells (PMN) with higher SSC parameter gated separately from mononuclear leukocytes (MOC) with lower internal complexity. Flow cytometry data were obtained using a “FACSort” flow cytometer (Becton-Dickinson, CA). Propidium iodide was added to cells just before cytometry to prevent counting of dead and damaged cells, and at least 10,000 events were collected. Data were analysed using PC-LYSYS 1.0 software (Becton-Dickinson, CA).

**Virus**

High-passage Purdue 115 “wild strain” of TGEV and dm49-4 mutant TGEV (Laude et al., 1992) were used as virus sources. Procedures for virus preparation have been described previously (Char-ley and Laude, 1988). Viruses were inactivated by UV irradiation. In the first series of experiments, TGEV was partially UV-irradiated to obtain a residual infectivity titre of 5×10⁴ PFU/ml. In the second series of experiments, both wild and dm 49-4 mutant virus, at initial titres of 6×10⁷ PFU/ml, were fully inactivated before injection.
RESULTS

Induction of IFNα-SC following in utero injection of partially inactivated wild TGEV

The optimal amount of partially inactivated wild TGEV allowing foetus survival after intravenous injection at 54 days of gestation was determined in preliminary experiments. Foetuses were thereafter infected with this amount of partially inactivated TGEV, and other foetuses were sham-operated as controls. At 52 days of gestation only three foetuses (two infected and one control) survived till the next day; dead foetuses infected by virus did not exhibit any macroscopic pathological lesions when compared with the dead control foetus. Only cell suspensions and plasma of surviving foetuses were used for IFNα determinations. IFNα-SCs or IFNα were detected in liver and bone marrow cells or plasma in only one of two TGEV-treated foetuses already at mid-gestation (day 52) (table I). In the spleen they were found at later stages of gestation. The highest IFNα-SC frequency was observed in foetal liver. A slightly higher IFNα-SC frequency was detected in non-adherent cells compared with that in total cells (table I, 101 days). The absence of IFNα-SCs in spleen cells at 52 days may be due to the limited number of cells. The highest IFNα-SC frequency was observed by 101 days of gestation, whilst IFNα yield (production of IFNα per cell) was roughly constant (table II). No IFNα secretion in bone marrow cell culture supernatants could be detected at 52 days of gestation (table II), although low numbers of IFNα-specific spots were detected in one stimulated foetus (table I).

Table I. IFNα-secreting cells in pig foetal lymphohaematopoietic organs, and plasma IFNα levels 20 h after experimental in utero injection of partially UV-inactivated wild TGEV coronavirus.

| Day of gestation | Liver | IFNα-SC frequency (spots per 10^2 cells) | Bone marrow | IFNα level (units per ml) |
|------------------|-------|----------------------------------------|-------------|--------------------------|
| 52nd (n = 2)     | 26 ; 0 | 0(b)                                   | 2.1 ; 0     | 4 800 ; 0                |
| 82nd (n = 4)     | 84.5 ± 57.7  | 2.2 ± 1.1                             | 14.8 ± 13.5 | 9 200 ± 2 870           |
| 101st (n = 3)    | 47.3 ± 23.4  | 6.3 ± 0.2                              | 7.7 ± 4.7   | 10 190 ± 2 920          |
| 101st(a) (n = 3) | 66.3 ± 23.7  | ND                                    | ND          |                          |

Results are expressed as individual data or as means ± standard deviation. Day of gestation = day of stimulation by TGEV; (a) non-adherent fraction; (b) low number of cells. ND = non-detected.

Numbers of sham-operated controls on 52nd, 82nd and 101st day of gestation were 1, 2 and 2, respectively. No IFNα-SC or IFNα titres were detected in lymphohaematopoietic organs or plasma of controls.

Table II. IFNα yield per cell in pig foetal lymphohaematopoietic cell cultures 20 h after experimental in utero injection of partially UV-inactivated wild TGEV coronavirus.

| Day of gestation | Liver | IFNα secretion (units per IFNα-SC) | Bone marrow |
|------------------|-------|-----------------------------------|-------------|
| 52nd (n = 2)     | 0.7 ; 0 | 0(b)                              | 0           |
| 82nd (n = 4)     | 1.9 ± 0.8  | 0.3 ± 0.1                         | 0.9 ± 0.8   |
| 101st (n = 3)    | 0.3 ± 0.2  | 0.2 ± 0.1                         | 0.2 ± 0.1   |
| 101st(a) (n = 3) | 0.2 ± 0.1  | ND                                | ND          |

(a) Non-adherent fraction; (b) low number of cells. ND = non-detected.

No IFNα titres were detected in cell culture supernatants of lymphohaematopoietic organs of controls.
As controls, IFNα titres were determined in the plasma of TGEV-injected foetuses. High IFNα titres were found in foetal plasmas at 82 and 101 days of gestation (table I). Only one TGEV-injected foetus had plasma IFNα at 52 days of gestation.

No IFNα-specific spots or IFNα secretion was found in control sham-operated foetuses, which were subjected to in utero injection of saline only (data not shown).

Flow cytometry (FCM) analysis was performed in 52- and 101-day-old foetuses. SSC/FSC dot plot analysis showed a lower percentage of PMN cells in virus-injected foetal pig organs (fig. 1). FCM of leukocyte cell markers did not show significant differences between control and TGEV-injected foetuses (data not shown).

After evaluation of the results from this first series of experiments, we reduced the length of in utero stimulation from 20 to 10 hours in order to analyse IFNα secretion at its expected time of maximal production.

**Induction of IFNα-SC following in utero injection of fully inactivated wild and dm49-4 mutant TGEV**

In a second series of experiments using fully inactivated virus, differences in IFNα induction in pig foetuses stimulated by wild or dm49-4 mutant TGEV were analysed. In order to exclude any possible IFNα secretion by cells of monocytic/macrophage lineage, only non-adherent cells were used (as performed with liver cells at 101 days: table I). The main differences observed between the two series of experiments were a much lower plasma IFNα level, a reduced IFNα-SC frequency in liver and spleen and the absence of IFNα-SCs in foetal bone marrow (table III compared with table I). IFNα yields per cell were similar (tables II and IV). Neither plasma IFNα nor IFNα-SCs in organs were found in dm49-4-injected foetuses, with the exception of very low IFNα-SC numbers in liver and spleen of 75-day-old foetuses (tables III and IV).

Flow cytometry showed lower percentages of PMN cells in liver and bone marrow of wild TGEV-infected foetuses than in dm49-4-treated foetuses, with one exception — the liver of 105-day-old foetuses (fig. 2). No significant changes in leukocyte marker expression were observed (data not shown).

**DISCUSSION**

Intraamniotic infections provoke abortion, infertility, foetal death and abnormal foetal development and are associated with increased levels of amniotic inflammatory cytokines (Gravett et al., 1994; Romero et al., 1994; Dudley et al., 1996). IFN was found not only in human foetuses during intraamniotic rubella infection (Lebon et al., 1985) but also in the amniotic fluid of pregnant women without clinical signs of congenital virus infection (Lebon et al., 1982b). Following our previous demonstration that in vitro inducible IFNα-SCs were present at early stages of gestation in porcine foetal lymphohaematopoietic organs (Splichal et al., 1994), the aim of the present study was therefore to evaluate, at the cell level, the secretion of IFNα following in utero intravenous injection of TGEV in pig foetuses at different stages of gestation. The study was divided into two parts: (a) induction of IFNα secretion by partially inactivated wild TGEV, for which IFNα secretion was analysed in total cell populations (first series of experiments) and (b) induction of IFNα secretion in the non-adherent IFNα-SC fraction (presumably NIPC-like cells) after exposure to non-infectious TGEV. Wild and dm49-4 mutant TGEVs were compared in order to evaluate the influence of an amino acid point mutation in protein M on the IFNα-inducing properties in vivo (second series of experiments).

The experimental approach using “open” surgery is an efficient procedure for in vivo infection of foetuses via the umbilical cord vein. It has, however, several disadvantages such as loss of amniotic fluid, damage of foetal membranes and possible damage of the umbilical cord (Kovářová et al., 1971). In the present study we observed high mortality of pig foetuses at 52 days of gestation.
Fig. 1. FCM of umbilical cord blood, bone marrow and liver cells of 52- and 101-day-old foetuses after *in utero* injection of control medium or TGEV.

Dot plot analysis of foetal bone marrow cells (101 days of gestation) from control (a) or TGEV-injected (b) foetuses. Percentages of PMN cells in the different cell suspensions at 52 and 101 days of gestation (c). Number of foetuses as indicated in table 1.

Even in the case of one control foetus treated by saline only, which could be due to easier damage of the umbilical cord at this period of gestation than with older foetuses.

The main finding of the present study was that IFNα-SCs were found as early as 52 days of gestation in foetal liver and bone marrow following *in utero* injection of partially UV-inactivated
IN UTERO INDUCTION OF IFNα-SECRETING CELLS

### Table III. IFNα-secreting cells in pig foetal lymphohaematopoietic organs and plasma IFNα levels 10 h after experimental in utero injection of UV-inactivated wild and dm49-4 mutant TGEV coronavirus.

| Day of gestation (wild/mutant) | IFNα-SC frequency (spots per 10^3 cells) | IFNα levels (units per ml) |
|-------------------------------|------------------------------------------|---------------------------|
|                               | Liver Wild virus Mutant virus            | Spleen Wild virus Mutant virus |
|                               |                                           | Plasma Wild virus Mutant virus |
| 75th (n = 3/3)                | 3.4 ± 2.9 0.1 ± 0.1                       | 1.9 ± 1.4 0.1 ± 0.1         | 990 ± 120 0 |
| 77th (n = 2/1)                | 0.7 ± 1.7                                    | 0 0 : 2.4 0                | ND 0 |
| 91st (n = 3/3)                | 3.7 ± 1.4                                    | 28.8 ± 11.5 0             | 1480 ± 1180 0 |
| 105th (n = 3/3)               | 0.1 ± 0.1                                    | 18.1 ± 1.7 0              | 1400 ± 780 0 |

IFNα ELISPOT assay was performed with non-adherent cells. ND = non-detected.

### Table IV. IFNα yield per cell in pig foetal lymphohaematopoietic organs 10 h after experimental in utero injection of UV-inactivated wild and dm49-4 mutant TGEV coronavirus.

| Day of gestation (wild/mutant) | IFNα secretion (units per IFNα-SC) |
|-------------------------------|-----------------------------------|
|                               | Liver Wild virus Mutant virus     | Spleen Wild virus Mutant virus |
| 75th (n = 3/3)                | 0.8 ± 0.4 0.1 ± 0.1               | 0.1 ± 0.1 0                  |
| 77th (n = 2/1)                | 0.2 ± 1.2 0                       | 0 0 : 3 0                   |
| 91st (n = 3/3)                | 0.8 ± 0.4 0                       | 0.4 ± 0.1 0                 |
| 105th (n = 3/3)               | 0.1 ± 0.1 0                       | 0.3 ± 0.1 0                 |

IFNα ELISPOT assay was performed with non-adherent cells.

Virus. This is, to our knowledge, the first demonstration of the presence of IFNα-SCs in foetal lymphohaematopoietic tissues after in vivo viral induction. The fact that IFNα-SCs were found in only one virus-injected foetus may be due to an unsuccessful virus inoculation. At 52 days of gestation, a low frequency of spots but no IFNα in supernatants were observed in bone marrow cells of one foetus. This discrepancy may be due to a lower sensitivity of ELISA in comparison with that of ELISPOT.

Haematopoiesis in the bone marrow of pig foetuses starts at around 50 days of gestation (Šterzl and Kovářů, 1977), and the finding of IFNα-SCs at that time indicates the early appearance of these cells in lymphohaematopoietic organs. This finding is in accordance with our previous data on in vitro inducible foetal IFNα-SCs (Špíchal et al., 1994) and supports the likely haematopoietic origin of IFNα-SCs (Charley et al., 1995).

At later stages of gestation, in utero TGEV injection induced IFNα-SCs in foetal liver, spleen and bone marrow. When fully UV-inactivated TGEV was used, IFNα-SCs were not found in bone marrow, which may reflect the inability of non-replicating virus to reach that organ. A much lower IFNα production was observed after injection of fully inactivated virus compared with that after injection of infectious TGEV, as evidenced by both lower plasma IFNα titres and IFNα-SC frequency. This lower IFNα induction very probably reflects a lower amount of virus available when non-infectious TGEV is injected.

IFNα-SCs or IFNα was never found in blood cell suspensions (negative data not shown), but
Fig. 2. Percentages of PMN cells in bone marrow and liver cell suspensions of wild or dm49-4 mutant TGEV-injected foetuses of different ages. Numbers of foetuses as indicated in table III.

due to the low number of PBMC obtained, we cannot conclude whether these negative results could only be due to an insufficient number of cells tested or to a real absence of IFNα-SCs.

The lower IFNα inducibility by the UV-inactivated dm49-4 TGEV mutant characterized by a mutation in glycoprotein M, in comparison with inducibility by wild virus, has already been shown in in vitro experiments (Laude et al., 1992). In the present work, IFNα was induced by the dm49-4 mutant to very low levels at only one period of gestation (75 days), while IFNα was observed in liver and spleen of foetuses at all ages tested, after induction by wild virus. This result implies that in utero IFNα induction by inactivated TGEV largely depends upon the presence of a native envelope protein M, as expected from in vitro studies (Laude et al., 1992).

The presence of IFNα-SCs in pig lymphohaeematopoietic tissues, together with plasma IFNα, confirms recent data obtained in mice within a broader framework: briefly, early and transient serum IFNα production was detectable as well as the presence of IFNα-producing cells localized in specific areas of spleen and lymph nodes, but not in bone marrow, after in vivo inactivated virus injection (Eloranta et al., 1996; Riffault et al., 1996).

The aim of FCM was to study changes in cell populations during induction of IFNα production. NIPC size and granularity as well as surface markers have been described (Sandberg et al., 1990; Charley and Lavenant, 1990; Cederblad and Alm, 1990; Fitzgerald-Bocarsly, 1993; Nowacki and Charley, 1993). A more comprehensive characterization of human NIPC surface markers has recently been published (Svensson et al., 1996). The latter study concluded that NIPC could be immature dendritic cells. In the present study, cells isolated from foetal lymphohaeematopoietic organs were analysed on the basis of
their size, internal complexity (SSC/FSC) and surface markers. PMN with a higher SSC parameter could be distinguished from mononuclear leukocytes (MOC) with lower internal complexity. The PMN/MOC ratio was found to be reduced in virus-treated foetal organs (figs. 1a, b and c). In most cases studied, PMN percentages were lower in wild-virus-treated than in dm49-4-treated foetuses (fig. 2). Because dm49-4 did not induce IFNα in vivo, it is likely that TGEV-induced reduction of PMN percentages is IFNα-mediated. Previous studies also showed that immunization of pig foetuses with corpuscular antigen resulted in depletion of myeloid cells from foetal lymphohematopoietic organs (Šterzl and Kovářů, 1977).

No significant differences in leukocyte surface marker expression between stimulated and non-stimulated animals, including SLA-DR and CD4 previously described on adult pig blood IFNα-SCs (Nowacki and Charley, 1993), were observed in any tested cell suspensions.

In conclusion, our present results provide in vivo evidence of IFNα secretion, at the cell level, in pig foetal lymphohematopoietic organs, at early stages of lymphohematopoietic development. IFNα detected in foetal infected tissues (Lebon et al., 1985) may indeed originate from NIPCs localized in foetal lymphohematopoietic tissues. In addition, our experimental model of in utero induction of IFNα in pig foetuses will prove to be useful for future studies on prenatal cytokine network development.

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L'injection in utero de coronavirus VGET induit la sécrétion d'interféron alpha dans les organes lymphohématopoïétiques de fœtus de porc

Nous avons montré précédemment que l'incubation in vitro de cellules mononucléées sanguines avec du coronavirus de la gastroentérite transmissible (VGET) inactif par irradiation UV, induisait la sécrétion d'interféron alpha (IFNα). Le travail présenté ici a consisté à réaliser sur des fœtus de porcs à différents stades de gestation une injection in utero (a) de VGET sauvage partiellement inactif par les UV, ou (b) de virus sauvage ou de mutant dm49-4 totalement inactivés. La sécrétion d'IFNα par les cellules nucléées isolées, 10 ou 20 h après injection, de la rate, du foie, de la moelle osseuse et du sang fœtal a été analysée ex vivo par technique ELISPOT et ELISA. L'injection de VGET a induit la présence de cellules sécrétrices d'IFNα dans les organes lymphohématopoïétiques fœtaux à mi-gestation. Une sécrétion d'IFNα plus faible, voire absente, a été observée à la suite de l'injection du mutant viral dm49-4 caractérisé par une mutation ponctuelle dans la séquence de la glycoprotéine M. L'étude en cytométrie de flux n'a pas permis de montrer de différences d'expression des marqueurs de surface leucocytaires entre les cellules de fœtus traitées par le VGET ou par du milieu, ou traitées par le virus sauvage ou le mutant dm49-4, à l'exception d'une réduction du pourcentage de polynucléaires, après injection par le VGET, probablement due à l'IFNα sécrété.

Ces résultats montrent, in vivo, la sécrétion au niveau unicellulaire d'IFNα dans les tissus lymphohématopoïétiques fœtaux. De telles cellules sécrétrices d'IFNα localisées dans les tissus pourraient constituer la source de l'IFNα qui est détecté au cours d'infections fœtales.

Mots-clés: Coronavirus, Virus de la gastroentérite transmissible, IFNα; ELISA, ELISPOT, Fetus, Porc.
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