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CHEMICALLY INDUCED VIRAL RESISTANCE IN SINGLE PREIMPLANTATION BOVINE EMBRYOS[1]

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SUMMARY: Viral mRNA encoding vesicular stomatitis virus glycoprotein sequences was detected and quantitated using a DNA-hybridization dot-blot technique. This assay was employed to determine if the synthetic double-stranded polynucleotide complex of polyriboinosinic-polyribocytidylic acid would elicit viral resistance in vitro in single day 9 bovine embryos. The levels of viral mRNA were assayed in 4 groups of bovine embryos: unexposed, virus-exposed, polynucleotide-treated, and virus plus polynucleotide treated. Reduced quantities of viral mRNA in single polynucleotide treated embryos demonstrated that resistance to viral infection was induced in day 9 bovine embryos.

Cells of bovine embryos have been shown to be susceptible to a variety of viral pathogens resulting in embryonic death (1-3). Reports of the inability of early embryos to respond to exogenous IFN (3) and of their ability to synthesize their own IFN-like activity

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Abbreviations Used: VSV, vesicular stomatitis virus, VSV-NJ, VSV-New Jersey serotype, polyI:C, polyriboinosinic-polyribocytidylic acid, IFN, α-interferon, oTP-1, ovine trophoblast protein 1, bTP-1, bovine trophoblastic protein 1, PFU, plaque forming units, PBS, phosphate-buffered saline, FBS, fetal bovine serum, CPM, radioactive counts per minute, SD, standard deviation.

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(4) are however, conflicting. Hatched bovine blastocysts, cultured for 24 hr in the presence of IFN and subsequently challenged with VSV or bluetongue virus were not able to resist viral infection (3). In contrast, oTP-1, a polypeptide produced by early embryos, has been shown to possess high specific antiviral activity to VSV (4). The ability of early embryos to express antiviral activity and the earliest period during development when this can occur are still unclear.

The potential to produce IFN-like activity in early bovine embryos could be demonstrated indirectly if treatment with an IFN-inducer was able to protect embryonic cells that are subsequently exposed to virus. The synthetic nucleic acid, polyI:C, has been shown to be an effective inducer of IFN production in some cells in culture (5). The objective of the current study was to evaluate the ability of polyI:C to protect single cultured day 9 bovine embryos from VSV infection. VSV is both cytopathic for bovine embryos (3, L.H. Laurerman, unpublished results) and sensitive to the antiviral effects of IFN (2). This report represents the first demonstration of biological IFN-like activity in vitro in single early bovine embryos.

MATERIALS AND METHODS

Virus and Cell Culture - VSV-NJ was obtained from E.G. Erckson, National Veterinary Services Laboratory, Ames, Iowa, and stored at -70°C. Stock VSV-NJ was propagated in Vero cell cultures at a titer of $1.2X10^6$ PFU/ml (6).

Bovine Embryos - Twenty-one day 9, zona pellucida-free, bovine embryos of excellent quality were collected from superovulated donor cows. Embryos were washed as described (6,7), and single embryos were assigned to 1 of 4 groups: no treatment and no exposure to VSV (group 1), exposed to VSV (group 2), treated with polyI:C (group 3), and treated with polyI:C and subsequently exposed to VSV (group 4). Group 1 embryos were washed 10 times in PBS containing 2% v/v FBS, 1 time in PBS alone, and then harvested. Group 2 embryos were exposed to VSV for 1 hr ($8X10^8$ PFU/ml) in growth medium (HEPES-buffered Ham's F10 nutrient mixture with 10% v/v FBS, 0.11 mg/ml sodium pyruvate, and 100 ng/ml epidermal growth factor), washed as described for group 1 and resuspended in growth medium until harvest. Two embryos were harvested at 1, 10, and 14 hr after viral exposure. Group 3 embryos were treated with polyI:C (50 μg/ml) in growth medium for 45 min and washed and resuspended as described for group 2. PolyI:C was prepared as previously described (8). Group 3 embryos were maintained in growth medium until harvested at 8 and 26 hr after polyI:C treatment (1 embryo/time point). Group 4 embryos were treated with polyI:C as described for group 3, then exposed to VSV for 1 hr in growth medium and washed and resuspended as described for group 2. Group 4 embryos were harvested either immediately (3 embryos) or maintained in growth medium and harvested at 10 hr (4 embryos) or 14 hr (3 embryos). In all groups the condition of each embryo was observed at 50X magnification immediately prior to each treatment and at the time of harvest by phase contrast microscopy.

Preparation of Plasmid Clones - Plasmids containing cloned copies of the VSV glycoprotein gene pG1 (9) were introduced into *E. coli* strain TB-1, amplified, collected and purified as previously described (10). Plasmid DNA was labeled by nick-translation with ^{32}P-dCTP to approximately $4X10^8$ CPM/μg.

RNA Preparation and Blotting - RNA was purified from infected and uninfected embryos as previously described (11). Cells were lysed in tissue culture wells by addition of lysis buffer followed by homogenization. A post-mitochondrial supernatant was prepared (10,000Xg for 20 min) and total cytoplasmic RNA was purified by proteinase K digestion in the presence of 1% sodium dodecyl sulfate followed by phenol/$CHCl_3$ extraction and ethanol precipitation (11). RNA concentration was determined spectrophotometrically at 260 nm.

Dilutions of RNA from bovine embryos were analyzed by dot-blots on nitrocellulose (12). Blots were prehybridized overnight at 42°C in 10 ml of hybridization buffer containing 50% v/v formamide, 5XSSC (1XSSC is 0.15 M NaCl and 0.015 M sodium citrate, pH 7.2), 0.02% w/v Ficoll, 0.02% w/v polyvinyl pyrrolidone, 0.1 mg/ml yeast tRNA, 0.1 mg/ml polyadenylic acid, 10 mM Tris-HCl, pH 7.2, and 0.1% sodium dodecyl sulfate (11). Blots were hybridized to $2X10^6$ CPM of labeled plasmid DNA in hybridization buffer (10 ml) for 48 hr at 42°C. Blots were washed of excess probe as previously described and exposed to X-ray film for autoradiography (11). Autoradiograms were quantified with a scanning microdensitometer (Hoefer Scientific). This technique has previously been shown to be linear in response with respect to input RNA mass (13).

RESULTS AND DISCUSSION

Single VSV infected bovine embryos (group 2) could be differentiated from noninfected embryos (group 1) on dot-blots of total cytoplasmic RNA hybridized to labeled VSV pG1 DNA (Fig.1). Approximately 2.5 fold more density was present in dots derived from VSV infected cells compared to the background density in uninfected cells. Comparable levels of VSV expression were detected (per μg input RNA) at both 1 and 10 hr post-infection. Pretreatment of embryos with polyI:C (group 3) resulted in a reduction in hybridization levels, when compared to untreated control embryos (group 1), by approximately half. Untreated (group 1) embryos were of excellent quality at the time of

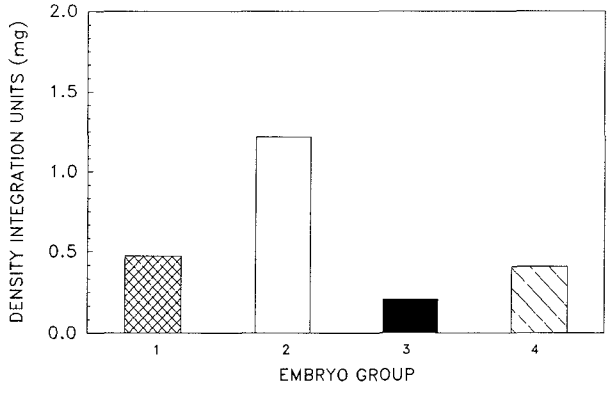

Figure 1. PolyI:C provides resistance to VSV infection in early bovine embryos. Mean relative levels of VSV pG1 RNA (mean autoradiographic density integration units per μg input RNA) in total cytoplasmic RNA from day 9 bovine embryos. Group 1: unexposed (SD=0.14), Group 2: VSV exposed (SD=0.16), Group 3: polyI:C treated (SD=0.14), Group 4: polyI:C treated and VSV exposed (SD=0.19). The density observed in group 1 represents the background level observed in the absence of virus.

harvest and showed no signs of deterioration. In contrast, VSV infected embryos (group 2) had undergone obvious degenerative changes including increased visual cell density, as observed by phase contrast microscopy, at 10 hr post-infection, severe cell damage with vesiculation and sloughing of cells from the surface of the embryo by 14 hr. Treatment of embryos with polyI:C alone caused no visible changes in group 3 embryos suggesting that viability was unaffected.

Pretreatment of embryos with polyI:C dramatically reduced the ability of VSV to induce cell damage. Dot-blot density was comparable to that of untreated control embryos (within 15%) and no visible evidence of viral damage was apparent up to 14 hr post-infection. Pretreatment of the embryos in group 4 with polyI:C had induced measurable resistance to VSV infection. While even low levels of polyI:C have been shown to effectively induce IFN production in a variety of species and cell lines (5,14-16) this is the first report of polyI:C induced resistance to viral infection in very early (day 9) preimplantation embryos.

The effectiveness of IFN on disruption of the infectious cycle of VSV is thought to be due in part to selective inhibition of the function or level of expression of the VSV glycoprotein which encodes the glycoprotein projections from the viral envelope (17). The result is release of fewer virions from infected cells treated with IFN and the viral particles which are produced appear to have fewer projections. Expression of VSV pG1, which encodes the VSV glycoprotein, was reduced in the presence of infected bovine embryos which had been pretreated with polyI:C. This could be due to lower numbers of virus present or to a depression of VSV glycoprotein mRNA levels in infected cells. The most likely mechanism responsible for such an effect is the induction of an IFN-like activity in treated embryos. Proteins (oTP-1 and bTP-1) with significant homology (45-70%) to mammalian IFNs have been identified between days 16 and 25 of development, are capable of high levels of specific antiviral activity and may be involved in the maternal recognition of pregnancy (4,18,19). Whether polyI:C treatment elicited bTP-1, IFN or another antiviral activity in early bovine embryos is presently speculative. It is clear, however, that very early bovine embryos do possess the capability to resist infections by otherwise lethal viruses in the presence of an appropriate inducer.

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