Mouse interferon produced *in vivo* does not inhibit the development of preimplantation mouse embryos

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**Summary.** Mouse embryos were cultured *in vitro* in medium with serum containing interferon which had been induced *in vivo* by intravenous administration of polyinosine-polycytidylic acid. Two-cell and blastocyst-stage embryos were incubated for 72 and 24 h respectively before embryo transfer, or fixation to determine cell number. Further, blastocysts were outgrown on coverslips in embryo culture medium with fetal calf serum and interferon. Expression of an intermediate filament protein (Mr 55 000) in blastocyst outgrowths was examined with a monoclonal antibody. Embryos appeared morphologically normal and after treatment the mean cell number did not differ from that of controls. Implantation was unaffected by any of the treatments, but culture before transfer in medium containing mouse serum reduced the number of normal fetuses recovered on Day 14 of gestation compared to those cultured in medium without serum. Exposure to interferon did not modify the expression of filaments in the outgrown blastocyst. It is therefore unlikely that interferon induced by viral infection during pregnancy is responsible for preimplantation embryonic loss.

**Introduction**

Mouse interferons inhibit the proliferation of many cell lines of murine origin (for a review see Taylor-Papadimitriou, 1980), including primary cultures of mouse embryo cells (Lindahl-Magnusson, Leary & Gresser, 1971). When 2-cell mouse embryos were exposed *in vitro* for 72 h to 3.4 × 10^3 units of interferon/ml, no significant difference was noted in the number scored morphologically as morulae or blastocysts, although the [3H]thymidine incorporation was lower in the interferon treated group than in the controls (Drasner, Epstein & Epstein, 1979). Drasner et al. (1979) also found a significant difference in cell proliferation and thymidine incorporation between interferon-treated and control groups of primary embryonic cells derived from 8- and 9-day-old mouse embryos. Barlow, Randall & Burke (1984) found that the developing mouse embryo is first capable of initiating interferon production when stimulated by an interferon inducer at about 8 days after fertilization. A more subtle effect, an increase in surface antigen expression in L1210 cells after treatment with mouse interferon, has also been demonstrated (Lindahl, Leary & Gresser, 1973). Tam, Wang, Lansberger & Pfeffer (1983) have shown that interferon can alter the organization of actin-containing microfilaments in Hela and mouse L929 cells.

It is known that interferons differ according to their cell source and mode of induction (Maehara, Ho & Armstrong, 1977). Using interferons induced *in vivo* we examined the development of embryos exposed to interferon *in vitro* from the 2-cell to the hatching blastocyst stage. This includes the period when any subtle change in surface expression of the trophoblast cells or modulation of expression of cytoskeletal proteins might lead to failure of implantation or development even though morphologically the embryos appear normal. This also exposes the developing embryo to interferon at a stage when rapid cell proliferation is occurring. Brinster (1967) has shown
that there is a 25% decrease in protein synthesis during the cleavage of the fertilized egg up to the morula stage, whereas there is an increase in protein synthesis during cell proliferation from the morula to the blastocyst stage.

It is well known that viral infections during pregnancy can cause growth retardation or death, as in the case of murine cytomegalovirus in mice (Neighbour, 1978) or Sendai virus in rats (Coid & Wardman, 1971) and that the virus cannot be recovered directly from the fetus. It seems that there must be an indirect effect of the virus infection on the embryo due to maternal ill health or placental malfunction. Here we are examining the possibility that interferon, which is induced during viral infections, may have a direct pathological effect on early embryonic development and subsequent implantation.

Materials and Methods

Preparation and titration of mouse interferon. Mouse interferon was induced in randomly bred MF1 female mice by the intravenous injection of 100 µg polynosinic-polyribotidyl acid (poly I.C., Sigma, Poole, Dorset U.K.) per mouse into the tail vein. After 2 h the mice were exsanguinated by cardiac puncture and serum was collected aseptically. Control serum (mock interferon) was prepared in the same manner except that blood was collected 30 min after intravenous injection of poly I.C., i.e. before interferon is induced (Finter, 1973). Serum samples were titrated for interferon activity on mouse L929 cells against mouse hepatitis virus (MHV) strain A59. MHV A59 is grown in NCTC 1469 cells, but when used to infect L929 cells it forms large syncytiata 18–20 h after infection. Dilutions of serum which gave 50–70 syncytia per field at a magnification of 100 in 96-well microtitre plates. The interferon-containing samples completely inhibited the formation of syncytia (as judged after staining with haematoxylin) and the test is very sensitive. Using this assay, one mouse interferon unit is equal to 1–2 NIH units using the G002, 904 standard obtained under the antiviral programme from the National Institutes of Health, Bethesda, Maryland, U.S.A. Serum samples prepared by induction with poly I.C. 2 h after intravenous injection routinely contained 2000–6000 units mouse interferon/ml, whereas the control samples prepared 30 min after intravenous injection contained < 100 units/ml.

Collection and in-vitro culture of mouse embryos. Randomly bred MF1 female mice were superovulated at 3–4 weeks of age by intraperitoneal injection of 5 i.u. PMSG (Folligon: Intervet, Cambridge, U.K.) and 5 i.u. hCG (Chorulon: Intervet, Cambridge, U.K.) administered 48 h apart. After injection of hCG the mice were paired with C57BL/6J × CBA/CaLac F2 hybrid males and inspected the next morning for copulation plugs (Day 1 of pregnancy). Embryos were flushed from the oviducts on Day 2 (2-cell) or from the uterus on Day 4 (blastocysts) in a Hapes-buffered medium (M2; Quinn, Barros & Whittingham, 1982). At each collection the embryos were pooled and washed through two changes of bicarbonate-buffered embryo culture medium (No. 16; Whittingham, 1971). Groups of embryos were finally washed through 1 ml Medium 16 containing 10% mouse serum with the appropriate titre of mouse interferon (666 u/ml) or mock interferon (< 10 u/ml) before being placed in drops of the same medium under paraffin oil and cultured at 37°C in an atmosphere of 5% CO2 in air. Control embryos were cultured in Medium 16 without interferon or mock interferon (i.e. no mouse serum) or in Medium 16 supplemented with 10% MF1 female mouse serum collected from mice not injected with poly I.C. (serum control). After culture in vitro for 72 h (2-cell) or 24 h (blastocysts) the embryos were assessed morphologically before transfer to foster mothers or fixation to determine cell number.

Embryo transfer. Samples of embryos were transferred to the uterine horns (4–6 embryos/horn) of CBA/Nimr × C57BL/Nimr F1 hybrid (CBBF1) recipients at Day 3 or Day 4 of pseudopregnancy. Pseudopregnancy was induced in the CBBF1 females by mating with sterile males.
Cell counts. Embryos were fixed by the method of Tarkowski (1966). Briefly the embryos were placed in 1% sodium citrate at room temperature for 5–15 min. Up to 5 embryos at a time were pipetted in a small volume of the hypotonic solution onto a slide and fixed with a solution of glacial acetic acid:ethanol (1:3, v/v). The slides were stained with Giemsa and the nuclei counted.

Detection of intermediate filaments in blastocysts outgrown in vitro in the presence of interferon. Blastocysts collected on Day 4 of pregnancy were pooled and allowed to outgrow for 72 h on glass coverslips in Medium 16 supplemented with 10% fetal calf serum and containing interferon (666 u/ml) or mock interferon. After washing in phosphate-buffered saline the outgrowths were fixed in 95% ethanol for 30 min before staining by the immunoperoxidase technique with a monoclonal antibody to a mouse filament protein of Mr 55 000 (P. Cartew, unpublished data). Outgrowths were counterstained with haematoxylin, mounted in aqueous mounting media and examined with the light microscope.

Results

Embryos cultured for 72 or 24 h from the 2-cell or blastocyst stage respectively in Medium 16 containing interferon, mock interferon or normal mouse serum did not differ morphologically from controls. Day 2 embryos (2-cell stage) cultured for 72 h before fixation had about 50% fewer cells than did blastocysts collected on Day 4 of pregnancy and cultured for 24 h (Tables 1 & 2). When embryos cultured for the same period were compared, the mean cell number was similar for control and treated embryos.

Of control embryos cultured in Medium 16, 76% implanted after transfer to pseudopregnant recipients. The addition to the culture medium of interferon, mock interferon or normal mouse serum did not inhibit implantation (Tables 1 & 2). Compared to controls, the culture of blastocysts in Medium 16 containing mouse serum for 24 h before embryo transfer significantly reduced the number of normal fetuses recovered on Day 14 of gestation (71% vs 40%; $\chi^2_{[1]} = 9.09, P < 0.01$). Treatment with interferon or mock interferon before transfer did not further inhibit fetal development (Table 2). When 2-cell embryos were cultured for 72 h in Medium 16 supplemented with interferon, mock interferon or mouse serum, fewer treated embryos than controls cultured in Medium 16 only formed fetuses after transfer (Table 1) but overall the difference did not reach an acceptable level of statistical significance ($\chi^2_{[2]} = 8.1, P > 0.05$). When the 4 groups of embryos were analysed statistically by partition analysis there were no significant differences within the serum.

Table 1. Effect of interferon (666 units/ml) on cell number and development of 2-cell mouse embryos after 72 h culture in vitro and subsequent development 11 days after transfer at 14 days gestation

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of 2-cell embryos cultured</th>
<th>No. of normal embryos after 72 h* (%)</th>
<th>Mean ± s.d. cell no. (no. of embryos counted)</th>
<th>No. of embryos transferred to ♀♂ maintaining pregnancy†</th>
<th>Autopsy on Day 14 of gestation</th>
<th>No. of fetuses implanted (%)</th>
<th>No. of fetuses (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interferon</td>
<td>149</td>
<td>99 (66)</td>
<td>$57 \pm 14.6 (17)$</td>
<td>38</td>
<td>29 (76)</td>
<td>10 (26)</td>
<td></td>
</tr>
<tr>
<td>Mock interferon</td>
<td>149</td>
<td>103 (69)</td>
<td>$50 \pm 12.5 (16)$</td>
<td>28</td>
<td>20 (71)</td>
<td>5 (18)</td>
<td></td>
</tr>
<tr>
<td>Serum control</td>
<td>144</td>
<td>111 (77)</td>
<td>$66 \pm 19.5 (15)$</td>
<td>35</td>
<td>27 (77)</td>
<td>9 (26)</td>
<td></td>
</tr>
<tr>
<td>Medium No. 16 control</td>
<td>131</td>
<td>103 (79)</td>
<td>$64 \pm 10.9 (14)$</td>
<td>45</td>
<td>34 (76)</td>
<td>22 (49)</td>
<td></td>
</tr>
</tbody>
</table>

Values are for 2 replicates.
* Blastocysts, expanded and hatched blastocysts, outgrowing embryos.
† Two recipients did not become pregnant.
containing groups ($\chi^2_{21} = 6.62$) but these groups were significantly different from the control group without serum ($\chi^2_{11} = 9.11, P < 0.01$).

Embryos collected at the blastocyst stage and outgrown in the presence of interferon did not differ from controls in their ability to spread on glass and form outgrowths. Subsequent staining of the trophoblast cells in the outgrowths for the presence of the filament protein of $M_f$, 55 000, which is normally expressed at this stage, demonstrated the normal filamentous distribution of this protein when compared to controls outgrown in media without interferon.

**Discussion**

The failure of many embryos to implant and develop normally in the uterus is a phenomenon about which little is known. Interferon is induced by a wide range of microorganisms (Merigan, 1973). It is possible that the interferon produced during disease could retard development of the embryo by its growth inhibitory properties (Lindahl-Magnusson, Leary & Gresser, 1971) or could inhibit implantation by altering the expression of surface antigens on the embryo (Lindahl et al., 1973) or intracellular proteins important in normal development such as the cytoskeletal filament proteins known to be produced by mouse embryos.

Preimplantation embryos form morphologically normal morulae and blastocysts when cultured in interferon preparations induced by stimulating C-234-3 cells with Newcastle's disease virus (Drasner et al., 1979). However, C-234-3 cell-induced interferon lacks a component produced by macrophage stimulation (Maehara et al., 1977).

We attempted to stimulate the interferons circulating in vivo during disease by collecting serum from mice 2 h after intravenous injection of poly I.C. It is clear from our results that exposure in vitro to interferon induced in vivo does not retard the subsequent development of preimplantation mouse embryos. The percentage of 2-cell embryos that formed morphologically normal blastocysts after 72 h culture was not affected by the presence of interferon in the medium (Table 1). These results are similar to the findings of Drasner et al. (1979) for interferon induced in vitro. By transferring the embryos treated with interferon to pseudopregnant foster mothers we also examined the possibility that it produced changes in surface antigen expression which would inhibit implantation. However, implantation and development of morulae and blastocysts to Day 14 fetuses was unaffected when interferon treatment was discontinued at the time of embryo transfer.

Even when blastocysts are exposed to interferon for 24 h and cultured to more than 100 cells per blastocyst, a stage at which subtle changes in surface expression of the trophoblast cells might lead

### Table 2. Mean cell number and outcome of transfer of embryos cultured for 24 h from the blastocyst stage in Medium 16 containing interferon (666 units/ml), mock interferon, or mouse serum

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of blastocysts cultured</th>
<th>No. of expanded blastocysts after 24 h*</th>
<th>Mean ± s.d. cell no. (no. of embryos counted)</th>
<th>No. of embryos transferred to ♀♀ maintaining pregnancy†</th>
<th>Autopsy on Day 14 of gestation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>No. of embryos implanted (%)</td>
</tr>
<tr>
<td>Interferon</td>
<td>136</td>
<td>123 (90)</td>
<td>116 ± 27-7 (18)</td>
<td>60</td>
<td>40 (66)</td>
</tr>
<tr>
<td>Mock interferon</td>
<td>124</td>
<td>118 (95)</td>
<td>107 ± 31-1 (21)</td>
<td>54</td>
<td>38 (70)</td>
</tr>
<tr>
<td>Serum control</td>
<td>133</td>
<td>131 (98)</td>
<td>110 ± 29-9 (14)</td>
<td>72</td>
<td>54 (75)</td>
</tr>
<tr>
<td>Medium No. 16 control</td>
<td>128</td>
<td>126 (98)</td>
<td>112 ± 27-8 (24)</td>
<td>42</td>
<td>32 (76)</td>
</tr>
</tbody>
</table>

Values are for 2 replicates.
* Including hatched and outgrowing blastocysts.
† Two recipients did not become pregnant.
to failure of implantation, there was no effect on mean cell number, implantation, or subsequent development (Table 2). The results show that any inhibition of fetal development observed in treated samples compared to controls is due to the serum content of the culture medium and not due to exposure to interferon per se. Interferon had no effect on the expression of the trophoblast cell filaments recognized by the monoclonal antibody prepared against mouse blastocyst. Unlike Hela and L929 cells treated with interferon (Tam et al., 1983), there was no increase in the organization of filaments in the trophoblast cells of outgrown embryos after interferon treatment. It therefore seems unlikely that, in the mouse, interferon induced in vivo due to viral infections plays any pathological role in the early preimplantation stages of pregnancy, probably due to the lack of specific receptors necessary for the mediation of interferon activity (Aguet, 1980).

Viral infections can cause growth inhibition in vivo by delaying the normal regenerative response of the liver after partial hepatectomy (Carthew, 1981a, b). The mediator of this inhibition could be interferon, since it is known to be induced during viral infections and is also growth inhibitory in the regenerative response after partial hepatectomy (Frayssinet, Gresser, Tovey & Lindahl, 1973). It can therefore be questioned whether interferon plays a role in the inhibition of normal development of the fetus in utero, so that congenital malformations due to viral infections in utero are not only caused by the direct cytopathic effect of viral infection, but also by a temporary delay in development at a crucial stage in embryogenesis. Further work on the culture of post-implantation embryos with interferons in vitro may provide an answer.

References


Brinster, R.L. (1967) Protein content of the mouse embryonic during the first five days of development. J. Reprod. Fert. 13, 413-420.


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