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Viral Replication and Interferon Production in Fetal and Adult Ovine Leukocytes and Spleen Cells

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Peripheral blood leukocyte and spleen cell cultures derived from adult sheep and from third-trimester (107 to 145 days of gestation) and second-trimester (70 to 98 days of gestation) fetal lambs were examined for their ability to support viral replication and to produce interferon. Bluetongue virus, *Herpesvirus hominis* type 2, and Chikungunya virus failed to replicate in either leukocyte or spleen cell cultures derived from adult ewes or in cultures from second- or third-trimester fetal lambs. Similarly, peripheral blood leukocytes from adult sheep or third-trimester fetal lambs did not support the replication of Semliki Forest virus, vesicular stomatitis virus, Newcastle disease virus, or vaccinia virus. No major differences were observed in the ability of fetal and adult leukocytes to produce interferon in response to viral infection. In contrast, mean interferon titers induced by bluetongue virus, *H. hominis* type 2, and Chikungunya virus in spleen cells from second-trimester fetuses were 4- to 10-fold greater than those induced in spleen cells from adult ewes. Variations in interferon levels induced on separate occasions with cells from the same donor age group were observed. The antiviral substance induced in both the fetal and adult cell cultures fulfilled the usual criteria for characterization as interferon.

The mammalian fetus is known to exhibit enhanced susceptibility to many different viral infections in that abortion, stillbirth, intrauterine infection, and congenital malformations have been shown to be associated with several benign or asymptomatic viral diseases of the mother (8, 30, 39). Congenital rubella virus (11) and cytomegalovirus (16) infections represent the prototype diseases in the human fetus, while bluetongue virus (BTV) of sheep (19), hog cholera virus of swine (12), and bovine viral diarrhea-mucosal disease virus of cattle (23) are among the agents associated with severe disease of the fetus and newborn in domestic animals. This association between enhanced susceptibility and immature age has been investigated in many experimental viral infections of animals (15, 17, 18, 22, 27, 46, 48, 50).

A number of reports have indicated that many viruses may be closely associated with blood, lymphatic, and reticuloendothelial tissues during the course of an infection (10, 13-15, 21, 22, 24, 26, 29, 33, 34, 52). It may be postulated that age-dependent host resistance to certain viral infections is at least partially determined by an inability of fetal lymphoreticular cells to control viral replication. In this regard rubella virus has been isolated from the spleen and thymus of congenitally infected fetuses (31) and from blood leukocytes of infants with congenital rubella (21) and cytomegalovirus has been recovered from lymphocytes of congenitally infected infants (24). Viral interaction with peripheral blood leukocytes has been felt to be of importance in the pathogenesis of both congenital rubella (21, 43, 49) and congenital cytomegalovirus (16, 24) infections. In addition, several investigations in mice have demonstrated the critical role of macrophages in the age-related resistance to infections with *Herpesvirus hominis* type 1 (HVH-1) (18, 22), mouse hepatitis virus (13), and togaviruses (34).

Several investigators have suggested that the inability of fetal and newborn animals to cope with viral infections may be based on an immature interferon response (3, 4, 9, 17, 18, 20, 25, 32, 46). In an attempt to evaluate the ability of the human fetus to produce interferon, a number of studies have compared the production of interferon in vitro by peripheral blood leukocytes from premature and full-term infants with cells from normal adults (2, 5, 44). An inherent limitation to the human model is the difficulty in determining if the interferon response in vitro reflects that of the intact fetus in vivo. Data from our laboratory have demonstrated that fetal lambs can produce levels of

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circulating interferon equal to or greater than the adult animal after intrauterine inoculation of Chikungunya virus (CV) (38, 40) and nonviral agents (J. C. Overall, Jr., unpublished data).

In this report we have investigated several parameters of the interaction of a number of different deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) viruses with fetal and adult peripheral blood leukocytes and spleen cells in vitro in an attempt to: (i) determine whether viral replication occurs in fetal but not adult cells, (ii) compare the interferon response of fetal with adult cells, and (iii) ascertain whether the interferon response of fetal and adult cells in vitro reflects that previously reported for the intact host in vivo (38, 40).

(This investigation is part of a dissertation submitted by C.R.R. in partial fulfillment of the requirements for the Ph.D. degree from the University of Utah.)

MATERIALS AND METHODS

Cells and media. The cells and cultivation procedures have previously been described (45). All cell cultures were maintained in Eagle minimum essential medium supplemented with 10% (vol/vol) fetal calf serum, 100 U of penicillin per ml, 100 μg of streptomycin per ml, and 25 μg of tylosin tartrate per ml. The cell lines used included fetal lamb kidney cells (passage 10 through 70) and human embryonic lung WI-38 cells (passage 20 through 30), as well as cloned, continuous lines of mouse L-929 and baby hamster kidney-21/C13 (BHK-21/C13) cells (45). In addition, a continuous line of CV-1 monkey kidney cells originally obtained from the American Type Culture Collection cell repository (Rockville, Md.) was also employed. Primary cultures of chicken embryo cells, mouse embryo fibroblasts derived from CD-1 albino Swiss-Webster mouse embryos (Charles River Breeding Laboratories, Brookline, Mass.), and rabbit kidney cells derived from a 4- to 6-week-old albino rabbit were prepared in a similar manner as were the fetal lamb kidney cells.

Viruses. All of the viruses used in these experiments were assayed by the plaque technique and quantitated as the number of plaque-forming units (PFU) per milliliter as previously described (45).

Stock preparations of vesicular stomatitis virus (VSV) titered 1.5×10^7 PFU/ml in CV-1 cells, and the vaccine strain of BTV titered 4×10^6 PFU/ml in L cells. The origin and methodology for preparation of VSV and BTV have been described (45). CV, originally supplied by P. Russell (Walter Reed Army Medical Center, Washington, D.C.), was prepared in BHK-21/C13 cells and titered 3×10^7 PFU/ml in CV-1 cells. Semliki Forest virus (SFV), obtained from S. Baron (National Institutes of Health, Bethesda, Md.) was propagated in an identical manner and titered 1×10^8 PFU/ml in CV-1 cells. The MS strain of HVH type 2 (HVH-2) was supplied by A. Nahmias (Emory University, Atlanta, Ga.), prepared in primary rabbit kidney cells, and titered 2×10^6 PFU/ml in mouse embryo fibroblasts. Vaccinia virus, obtained from the National Institutes of Health, was grown in primary chicken embryo cell monolayers and titered 7×10^6 PFU/ml in mouse embryo fibroblasts. The Herts strain of Newcastle disease virus (NDV), donated by S. Baron, was propagated in embryonated chicken eggs which had been injected by the allantoic route and titered 3.5×10^9 PFU/ml when assayed in primary chicken embryo cells.

Sheep. Mixed-breed, pregnant ewes with a known breeding date, and therefore a known gestation age, and nonpregnant ewes were obtained through A. E. Larsen, University of Utah. For purposes of these studies, the normal 150-day gestation period of the ovine has been divided in 50-day trimesters. The gestational ages of the fetal lambs used in these experiments ranged from 70 to 98 days for second-trimester fetuses and from 107 to 145 days for third-trimester fetuses. Experiments could not be performed on fetuses younger than 70 days gestational age because sufficient number of peripheral blood leukocytes and spleen cells could not be obtained from such small animals (blood volume 2.0 ml or less, spleen very undeveloped).

Preparation of leukocytes. Peripheral blood leukocytes from fetal and adult sheep were prepared in the manner previously described (45). Cell viability, as detected by trypan blue dye exclusion, routinely ranged from 95 to 98% in adult leukocyte preparations and from 70 to 90% in leukocyte cultures derived from fetal lambs. Differential counts made with Wright stain demonstrated that leukocytes of both the peripheral blood and the final cell preparations from adult ewes ranged between 55 and 65% lymphocytes, 25 to 35% neutrophils, 5 to 10% monocytes, 5 to 10% eosinophils, and less than 2% basophils. The blood and leukocyte cultures from second- and third-trimester fetuses contained approximately 67 to 85% lymphocytes, 5 to 23% neutrophils, 5 to 15% monocytes, and less than 2% eosinophils and basophils. Both the blood and the leukocyte cultures from second- and early third-trimester fetuses also contained a small proportion of nucleated erythrocytes.

Preparation of spleen cells. Spleens from adult ewes and from fetal lambs (the same donors used in the leukocyte studies) were aseptically removed. The inner portions of spleens from adult and late third-trimester fetal lambs, containing both the red and white pulp areas, were separated from the capsule and finely minced in a phosphate-buffered saline solution. Due to their relatively small size, whole spleen from second- and early third-trimester fetuses were used in these investigations. The spleen cell suspensions were centrifuged at $15 \times g$ for 10 min at room temperature to remove tissue debris. The cell-rich supernatant was then centrifuged for 10 min at $250 \times g$, and the cell pellet was treated with 0.83% NH_4Cl at 4 C for 15 min to lyse residual erythrocytes. Differential counts made on Wright-stained smears showed that final preparations of spleen cells from adult ewes contained 80 to 90% lymphocytes, 5 to 8% neutrophils, 4 to 12% macro-

phages, and less that 5% fibroblasts. Cells from the spleens of fetal sheep were also predominantly mononuclear (90% of total), the majority of which were lymphocytes. Several forms of immature hematopoietic cells were also observed in the fetal preparations.

Interferon production and viral replication. Adult and fetal leukocytes were suspended to a final concentration of 2×10^6 cells/ml in Eagle minimal essential medium without fetal calf serum, as serum was found to enhance clumping of the leukocytes. Spleen cells from fetal and adult sheep were treated in a similar manner. The cells cultures were inoculated with the particular virus at a specified multiplicity of infection (MOI), dependent upon the titer of the viral pool utilized. The ranges of the MOI used were: 0.1 to 1.7 for BTV, 0.05 to 2.5 for HVH-2, 0.3 to 6.0 for CV, 2.0 to 4.0 for SFV, 2.0 to 3.5 for NDV, 2.0 to 6.0 for VSV, and 0.3 to 0.7 for vaccinia virus. Following a 1-h adsorption period at 37 C in 5% CO_2 and humidity, the cells were washed twice to remove residual, unadsorbed virus by centrifuging at $250 \times g$ for 10 min and suspending the cell pellets in cold Eagle minimal essential medium. After the final wash, the infected cell pellets were resuspended to the appropriate volume in Eagle minimal essential medium with 10% (vol/vol) fetal calf serum. The infected cell cultures were distributed in 1-ml portions into siliconized glass culture tubes and incubated at 37 C in 5% CO_2 and humidity. At timed intervals, samples were resuspended and a portion of the infected cell suspensions was frozen at -70 C for virus (except for BTV samples, which were found to be more stable when stored at 4 C). The remainder of the sample was centrifuged at $400 \times g$ for 10 min, and the cell-free supernatant was stored at -20 C for interferon.

Cell-free portions consisting of virus in Eagle minimal essential medium with 10% (vol/vol) fetal calf serum were incubated concurrently with the virus-infected cell samples as a measure of thermal inactivation of virus. To examine for the presence of autogenous or "spontaneous" interferon production, portions of uninfected leukocyte and spleen cell suspensions at a concentration of 2×10^6 cells/ml were also included in the experiments.

Before assay, viral samples were rapidly freeze thawed in a dry ice-acetone bath to release intracellular virus. Viral plaque counts were made in the appropriate cell system previously indicated for each viral species. The interferon samples were assayed by the 50% viral plaque reduction technique on fetal lamb kidney cells using VSV as the challenge virus (45).

RESULTS

Absence of viral replication in leukocyte and spleen cell cultures. Peripheral blood leukocytes and spleen cells from fetal and adult sheep were examined for their ability to support the replication of a variety of viral agents. BTV, a double-stranded RNA virus, was chosen because it is a natural pathogen of sheep and is a known cause of congenital malformations in fetal lambs (19). HVH-2, a DNA-enveloped virus, was utilized because of the known enhanced susceptibility of human newborn infants and newborn mice to this virus and the demonstrated failure of macrophages from suckling mice to control HVH-1 replication (8, 18, 22, 30, 39). CV, a group A togavirus (RNA), was used because of the previously reported studies (38, 40) concerning interferon production in fetal lambs in vivo after intravenous inoculation with this virus. SFV, a group A togavirus (RNA), was chosen because we have demonstrated that this virus is capable of replicating in both adult sheep and newborn and fetal lambs after intravenous challenge (37). VSV, an RNA rhabdovirus, and NDV, an RNA paramyxovirus, were utilized as challenge viruses because their capacity to induce interferon and replicate in human leukocytes had been well studied (52). Vaccinia virus, a DNA poxvirus, was chosen because of the known enhanced susceptibility of the human fetus to this viral agent (30, 39), and the fact that it has been shown to replicate in human peripheral blood leukocytes (52). Although it should be recognized that virus-cell interaction may result in the initiation of the early steps of the replicative cycle without the production of infectious virions, the present investigation has focused on the replication of fully infectious virus in leukocytes and spleen cells since the capacity of the fetal cell to serve as a permissive host may be an important aspect of the pathogenesis of a viral infection in the mammalian fetus. Evidence of viral replication was considered to be a 10-fold or greater rise in viral titer in the infected cell samples, in comparison with a concurrent thermal inactivation control. No detectable production of infectious virions of BTV, HVH-2, or CV occurred during a 3- to 7-day incubation period in either leukocyte or spleen cell cultures derived from adult sheep or second- and third-trimester fetal lambs. Results of a representative experiment illustrating the failure of BTV replication in leukocyte cultures are illustrated in Fig. 1. Similarly, peripheral blood leukocytes from adult and third-trimester animals were unable to support the replication of SFV, VSV, NDV, or vaccinia virus as evidenced by the absence of an increase in viral titers over a 4- to 7-day incubation period. It should be pointed out that each of the seven viral agents has been demonstrated to replicate to high titers in fetal lamb kidney cells and NDV, VSV, and CV to high titers in adult sheep kidney cells in our laboratory (J. C. Overall, Jr., unpublished observations). These re-

sults would indicate that leukocytes and spleen cells are generally nonpermissive cells in vitro with regard to the production of fully infectious virions and that fetal leukocytes and spleen cells do not demonstrate an enhanced susceptibility as compared with the same cells from adult animals.

Interferon production in fetal and adult leukocyte cultures. Peripheral blood leukocyte cultures derived from fetal and adult sheep were examined for their ability to produce interferon in response to viral infection. Data presented in Table 1 demonstrate that second- and third-trimester fetal leukocytes produced mean peak levels of interferon equal to or in some instances slightly greater than those induced in adult cells after infection with BTV. The kinetics of the interferon response were also similar in both fetal and adult leukocytes. Variation in the MOI of the particular virus within the range indicated in these experiments did not appear to result in significant differences in the amount of interferon produced. Similar results were obtained in experiments challenging leukocytes from second- and third-trimester fetal lambs and from adult sheep with HVH-2 and CV. No difference was observed between adult and third-trimester fetal leukocytes in the titers of interferon induced by SFV, NDV, VSV, or vaccinia virus. Levels of interferon induced by SFV and NDV reached peak amounts of approximately 2,500 and 1,500 U/ml, respectively, by 24 h postinoculation. In contrast, the titers of interferon induced by VSV and vaccinia virus (Fig. 2) were of lower magnitude (<40 to 500 U/ml) and peak levels were reached

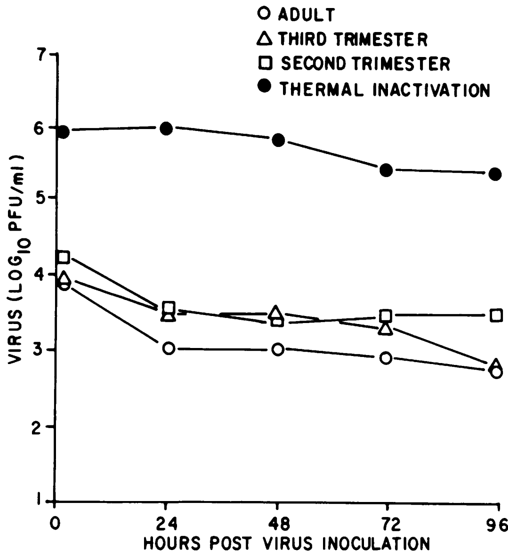

FIG. 1. *Absence of viral replication in BTV-infected leukocyte cultures derived from an adult ewe, a third-trimester fetus (140-day gestation), and a second-trimester fetal lamb (98-day gestation), as compared with a thermal inactivation control.*

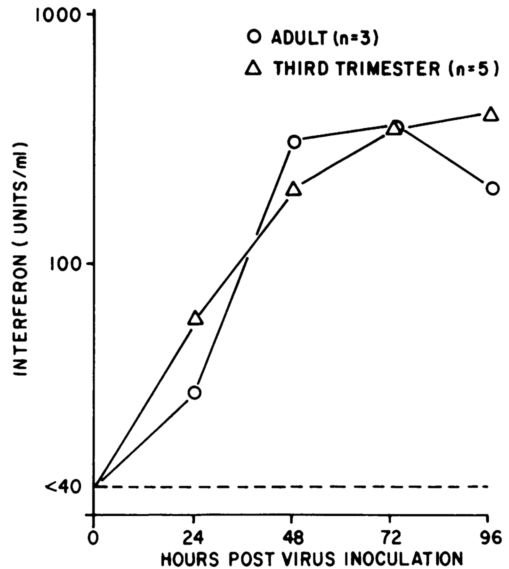

FIG. 2. *Mean interferon levels (units/milliliter) induced by vaccinia virus in ovine leukocyte cultures derived from adult and third-trimester fetal sheep.*

TABLE 1. *Interferon production induced by BTV in cultures of peripheral blood leukocytes from adult sheep and fetal lambs*

Donor age group	No. of cell donors	Range of MOI[a]	Time postinoculation (h)			
			24	48	72	96
Adult ewes	9	0.10–1.70[b]	1,000[c]	1,150	1,400	—[d]
3rd-trimester fetuses	10	0.10–0.50	3,100	3,400	3,400	2,550
2nd-trimester fetuses	9	0.25–1.20	2,050	—	—	3,550

[a] MOI expressed as number of viral plaque-forming units per cell.
[b] Range of MOI between the experiments.
[c] Mean levels of interferon expressed as units per ml.
[d] Insufficient number of samples at this time period.

later (48 h) than with the other five viruses. No spontaneous production of interferon in uninfected leukocyte controls was ever detected throughout the course of these experiments.

Interferon production in fetal and adult spleen cell cultures. Studies were performed to determine the comparative ability of fetal and adult cells from reticuloendothelial tissue, i.e., the spleen, to produce interferon. Data in Table 2 indicate that spleen cells from second-trimester fetuses consistently produced about 10-fold higher levels of interferon after BTV infection, and four- to fivefold higher after HVH-2 and CV inoculation, than did spleen cells from adult ewes. These differences did not correlate with the variation in the MOI between separate experiments. Spleen cells from third-trimester fetuses also appeared to be as competent as, or slightly superior to, adult cells in the interferon response to BTV, HVH-2, and CV. However, it will be necessary to examine a greater number of third-trimester fetal donors before definite conclusions can be made. Low levels of an interferon-like substance (20 to 100 U/ml) were detected in uninfected spleen cell cultures in two experiments with second-trimester fetuses and in one experiment with cell cultures from a third-trimester fetal lamb. Interferon was never detected in uninfected cells from spleens of adult ewes or in leukocyte cultures derived from these same three fetuses. The presence of this spontaneously produced interferon-like substance did not correlate with the ability of the fetal spleen cells to produce enhanced levels of interferon as compared with spleen cells from adult ewes.

Variation in the interferon response. Data from these studies indicated that there was marked animal-to-animal and experiment-to-experiment variation in the interferon response to a given viral inducer. For example, the mean peak interferon response in peripheral blood leukocyte cultures from eight third-trimester fetal lambs inoculated with CV was 2,163 U/ml, with a range of 350 to 3,800 U and a standard deviation of ±1,334 U. There was even variation in the interferon response of peripheral blood leukocytes obtained from the same donor on different occasions. For example, the mean peak interferon response in leukocytes obtained from a single adult ewe on four separate occasions and challenged with BTV was 3,425 U/ml, with a range of 400 to 6,500 U and a standard deviation of ±2,490 U. Similar variations in the interferon response were observed with each of the seven viral inducers, and have previously been noted in this laboratory (45) and by other investigators (41).

In a few experiments there was an opportunity to limit the genetic variability between animals by comparing the interferon response of peripheral blood leukocytes and spleen cells

TABLE 2. *Interferon production induced by BTV, HVH-2, and CV in cultures of spleen cells from adult sheep and fetal lambs*

Viruses	Donor age group	No. of cell donors	Range of MOI[a]	Time postinoculation (h)			
				24	48	72	96
BTV	Adult ewes	6	0.20–0.60[b]	1,050[c]	1,300	1,200	1,150
	3rd-trimester fetuses	2	0.30	3,000	3,700	3,350	3,500
	2nd-trimester fetuses	9	0.25–1.00	14,250	15,700	12,250	10,900
HVH-2	Adult ewes	5	0.06–2.50	2,500	2,450	2,900	—[d]
	3rd-trimester fetuses	2	0.10	3,850	2,850	3,700	—
	2nd-trimester fetuses	9	0.60–2.50	13,500	16,400	13,650	—
CV	Adult ewes	5	0.30–2.00	1,900	1,950	2,050	—
	3rd-trimester fetuses	2	1.00–3.00	2,200	2,900	2,550	—
	2nd-trimester fetuses	8	0.30–3.00	8,250	7,800	10,650	—

[a] MOI expressed as number of viral plaque-forming units per cell.
[b] Range of MOI between the experiments.
[c] Mean levels of interferon expressed as units per ml.
[d] Insufficient number of samples at this time period.

from a pregnant adult ewe with the response of cells from her own fetal lamb. In a representative experiment, the peak level of interferon produced by leukocytes from the ewe after infection with HVH-2 was 950 U/ml and from the 86-day gestational age fetus, 500 U/ml. In contrast, cells from the maternal spleen produced only 550 U of interferon per ml after infection with HVH-2 as compared with 6,650 U/ml induced in the fetal spleen cells. These results would further support the observation that fetal spleen cells have an enhanced capacity to produce interferon after viral challenge in vitro when compared with adult spleen cells.

Characterization of the interferon. The antiviral substance induced by each of the viral agents in the fetal and adult cell cultures was characterized as interferon by the following criteria: (i) inactivation by trypsin treatment, (ii) resistance to pH 2 for 24 h at 4 C, (iii) no detectable activity in mouse L cells, (iv) stability at 56 C for 30 min, (v) activity against both VSV and Sindbis viruses in fetal lamb kidney cells, (vi) inability to inactivate VSV directly, and (vii) lack of activity in fetal lamb kidney cells treated with actinomycin D.

DISCUSSION

The present studies demonstrate that several different DNA and RNA viruses failed to replicate in either adult or fetal ovine peripheral blood leukocytes or spleen cells when replication did occur in both adult and fetal kidney cells. These results fail to support the hypothesis that decreased resistance of the fetus to viral infection is reflected by an increased susceptibility of fetal lymphoreticular cells to direct viral infection. It should be recognized, however, that lymphoid or reticuloendothelial cells from younger fetal lambs (the youngest age tested in these studies was 70 days) might support the replication of fully infectious virus in vitro. A second possibility is that virus could replicate in cells of third-trimester or late second-trimester fetal lambs in vivo, but not in these same cells in vitro.

Previous reports have indicated that the addition of mitogens, such as phytohemagglutinin, to cultures of lymphocytes had increased the capacity of these cells to support viral replication (52) and that fetal lymphocytes exhibit an enhanced responsiveness to phytohemagglutinin and a greater level of spontaneous blastic transformation than adult cells (6, 51). Although it is reasonable to postulate that phytohemagglutinin stimulation of fetal lamb leukocytes and spleen cells might permit greater degrees of viral replication than stimulation of adult cells, we elected not to use mitogens in our investigations so that in vitro manipulation could be kept at a minimum. Further investigation will be required to resolve this issue.

Our data also indicate that peripheral blood leukocytes from second- or third-trimester fetal lambs and spleen cells from third-trimester lambs produce levels of interferon that are similar to or greater than the levels produced by adult leukocytes or spleen cells following in vitro challenge with several different DNA or RNA viruses. Although we were unable to determine the interferon response of cells from first-trimester fetal lambs, these results corroborate the previous reports from this laboratory (38, 40) and others (28) which suggest that inefficient production of interferon may not be the basis of enhanced susceptibility of the intact fetal host to viral infections. In this regard, interferon production by fetal cells in vitro has been utilized as a measure of the ability of the human fetus to mount an interferon response. Carter et al. (7) have presented evidence that skin fibroblast cultures derived from human fetuses showed a fourfold enhanced interferon response to NDV as compared with adult cells. Other investigations have found no consistent differences in the levels of interferon induced by Sendai (2, 5, 44) or rubella (2) viruses in blood leukocytes derived from human fetuses and adults. It is significant that the fetal lamb has been shown to produce 100- to 1,000-fold greater levels of circulating interferon than the adult ewe after intravenous inoculation of CV (38, 40). In contrast, similar titers of interferon were induced by CV in both fetal and adult ovine leukocyte cultures. These data suggest, therefore, that in vitro studies with peripheral blood leukocytes may not accurately reflect the ability of the intact fetal host to produce interferon.

It is of interest that spleen cells from second-trimester fetuses produced 4- to 10-fold higher levels of interferon than adult cells after infection with BTV, HVH-2, or CV, whereas there were no differences in levels produced by peripheral blood leukocytes from the same animals. The basis for and the biological significance of these differences remain unknown at the present time. Recent studies from this laboratory (J. C. Overall, Jr., unpublished data) have demonstrated that spleens from fetal lambs inoculated in utero with CV contain high titers of interferon (e.g., 250,000 U per g of tissue) whereas other fetal tissues (e.g., lung, liver, kidney) have lower levels of interferon. These data indicate that fetal spleen cells may account for a significant portion of the high titers

of circulating interferon induced by CV in fetal lambs (38, 40). The results are in agreement with other reports which have demonstrated that the spleen is a major source of interferon during viral infection of other animal species (1).

There is a possibility that at a certain gestational age the cells of the fetal host are unresponsive to the antiviral effects of interferon, regardless of their capacity to produce it. It has been demonstrated that fibroblastic cell lines derived from the least mature human embryos were less sensitive to interferon than those derived from neonatal tissues (47). Unfortunately, none of the seven viral agents tested in this series of experiments replicated in either fetal or adult leukocytes or spleen cells and this question, therefore, could not be approached with these cell systems. Preliminary investigations with fetal lamb kidney and adult sheep kidney cells demonstrate no significant differences in the sensitivity of these cells to the antiviral protective effect of interferon against VSV and encephalomyocarditis virus in vitro (J. C. Overall, Jr., unpublished observations).

It is of interest that although BTV has been recovered mainly from the buffy coat, spleen, and mesenteric lymph nodes during the course of experimental infection of adult sheep (26, 42) and administration of the vaccine strain of BTV in utero has resulted in severe congenital anomalies of the central nervous system in the fetal lamb (35, 36), we failed to demonstrate any replication of BTV in cultures of either fetal or adult leukocytes or spleen cells. In addition, fetal cells produced equal or greater levels of interferon than did adult cells in response to BTV. These data suggest that increased replication of the virus in fetal leukocytes or spleen cells or impaired production of interferon may not be the basis for enhanced susceptibility of the fetal lamb to BTV infection. Investigations must be performed during the course of BTV infection in utero, however, to provide definitive information on these points.

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