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In Utero Sensitization With Influenza Virus In Man (38918)

FREDERICK L. RUBEN, ALAN WINKELSTEIN, AND RUDY E. SABBAGHA
(Introduced by Harry A. Feldman)

Montefiore Hospital, 3459 Fifth Avenue, Pittsburgh, Pennsylvania 15213, Magee Women's Hospital, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania 15213

The effects on the human fetus of maternal infections with influenza A viruses have not been adequately defined. The virus reportedly has been isolated from the fetal lungs of abortuses of women with severe influenza infections. This suggests that influenza virus may be transplacentally transmitted to the fetus (1, 2).

In contrast, many pregnant women acquire influenza without apparent adverse effects on their fetuses (3). Thus, it may be questioned as to whether the fetus is infected by the virus in such pregnancies (4). The results of the present studies of humoral and cell-mediated immunity which were undertaken to determine whether intra-uterine infection occurs, suggest that transplacental passage of influenza virus to the fetus does occur during pregnancy.

Materials and Methods. Following a community-wide epidemic of influenza A/England/42/72 virus, sera from still pregnant women were collected and examined for serologic evidence of recent infection with this virus. In those with sera having hemagglutination-inhibition (HI) titers of $\geq 1:16$, cord bloods were obtained at the time of delivery. These cord specimens were tested for IgM type antibodies against influenza A/England/42/72 virus using the HI tests both before and after incubation with 2-mercaptoethanol (2-ME) (5). Specimens showing a fourfold or greater reduction in antibody titers after treatment with 2-ME were considered to contain IgM type antibodies against influenza.

A limited number of cord blood lymphocytes were tested for their ability to proliferate in cultures stimulated with influenza A antigens (5). The presence of viable immunologically competent T cells was confirmed by simultaneously measuring reactivity to phytohemagglutinin (PHA).

Results. Influenza, caused by A/England virus, was prevalent in the Pittsburgh area from December, 1972, through March, 1973. Sera from 234 pregnant women were screened in April, 1974, for HI antibody against the A/England strain. None of these women had received influenza vaccine. Among these, 62 (26%) had titers $\geq 1:16$; the cord bloods of 22 were sampled at their delivery (Group I). In addition, 42 cord blood specimens were obtained from deliveries which occurred on the same days as those of Group I and constitute Group II. Thus, the latter were studied without knowledge of possible prior maternal infection with the A/England virus.

Cord blood antibody studies (Table I) showed that 15 of the 22 Group I samples and six of the 42 Group II samples had antibody titers $\geq 1:16$. In Group I, three had 2-ME sensitive antibodies. Their mothers had had influenza infections the first or second trimesters of their pregnancies. One specimen in Group II also had 2-ME sensitive antibodies. Its mother had had an influenza infection during either the second or third trimester of pregnancy. Thus, four cord bloods had significant titers of 2-ME sensitive antibodies which are considered to represent fetally derived IgM type anti-A/England virus antibodies.

Sixteen cord bloods were studied for their lymphoproliferative responses to influenza antigens (Table II). In initial experiments, the influenza A antigens appeared toxic to lymphocytes thereby invalidating the results in several patients. When judged by a two-fold or greater increase in ^{3}H Tdr incorporation, one sample from Group I and two from Group II were responsive to these viral antigens. Responses were not due to the presence of antigen–antibody complexes as the addition of sera with known antibodies to viral stimulated cultures did not increase ^{3}H Tdr incorporation.

TABLE I. CORD BLOOD HI ANTIBODY STUDIES WITH INFLUENZA A/ENGLAND.

	Trimester exposed	# Bloods tested	Titer ≥ 1:16 (No.)	2-ME reduced (No.)
	1	8	7	1 (256 to <16)
	2	11	6	2 (64 to <16)
Group I				(64 to 16)
	3	3	2	0
	Totals	22	15	3
	1	6	1	0
Group II	2–3	36	5	1 (32 to <16)
	Totals	42	6	1

[a] = Numbers in () refer to reciprocal of titers before and after 2-ME treatment.

TABLE II. CORD BLOOD LYMPHOCYTE STUDIES.

	Trimester exposed	Tested (No.)	Positive response (No.)[a]
	1	2	1
Group I	2	6	0
	3	0	0
	Totals	8	1
	1	1	1
Group II	2–3	7	1
	Totals	8	2

[a] = Positive response = twofold or greater increase in uptake of H^3 Tdr in influenza A/England antigen stimulated cultures over untreated controls.

Results in all cord bloods showing a positive test for either serological or cellular evidence of sensitization with influenza A are summarized in Table III. Seven cord bloods showed one positive test; no samples were positive in both assays.

All seven infants with evidence of immunological responses to influenza virus had normal deliveries and all were in good health on follow-up examination 5–9 mo later. At the time of their follow-up visits, sera from six of seven were tested for persisting HI antibodies; four had titers of 1:4 to 1:8.

Discussion. These investigations suggest that the fetus can be transplacentally infected with influenza A virus. Two criteria were employed to detect the fetal immune response to this virus; the presence of 2-ME sensitive antibodies and the *in vitro* proliferative responses of fetally derived lymphocytes. The data indicate that specific immune responses were present in four of 22 cord blood samples from women with known infections during pregnancy and in three of 42 unselected cases.

TABLE III. CORD BLOODS WITH POSITIVE TESTS WITH A/ENGLAND ANTIGENS.

Cord Blood	Fetal IgM HI	Responsive Lymphocytes
1	+[a] (256 to <16)	–
2	+ (64 to <16)	ND[b]
3	+ (64 to 16)	–
4	+ (32 to <16)	ND
5	– (<4)	+
6	– (16)	+
7	– (8)	+

[a] = + indicates a positive test. 2-ME reductions were performed on all sera with HI titers ≥1:16.

[b] = Not done.

The presence of influenza specific antibodies of the IgM type was considered to be serological evidence for fetal infection since maternally derived IgM antibodies do not cross the placental barrier except by occasional leakage. The fetus is capable of IgM synthesis as early as 10.5 wk (6). Of 64 cord bloods examined, four had significant titers of 2-ME sensitive antibodies.

Three samples were reactive in the lymphoproliferative assay using influenza A antigen as the stimulus. Since fetal lymphocytes are immunologically competent by 18 wk of gestation as judged by PHA responsiveness (7), and since lymphocyte responses to influenza are specific (8), the finding of cord lymphocyte proliferation in response to influenza A suggests *in utero* sensitization of these cells to these viral antigens.

As shown in Table III, there was no positive correlation between positive cellular and humoral antibody tests. Such factors as fetal age and the development of the fetal immunologic system at the time of maternal infection, the severity of the maternal infection and the degree of viremia may bear on the character of the fetal immune response.

In addition, the sensitivity of the test utilizing lymphocyte proliferation is not known. Because of these and other variables, the lack of correlation between the two tests is not surprising.

These results, however, indicate that *in utero* infection with influenza virus is not a rare event. In this series, evidence for fetal immunologic responses to such a virus was found in seven of 64 cord bloods tested. Further testing using more sensitive measures may reveal an even higher incidence. Sustained follow up of these infected offspring seems to be warranted.

Summary. Following a community-wide epidemic of influenza A/England virus infection, 64 cord blood samples were evaluated for their fetal serum and lymphocyte responses to this virus. Cord sera were tested for HI antibody vs. A/England with and without 2-ME. A fourfold or greater reduction in titer was observed in four, indicating fetal IgM antibody responses. In addition, lymphocyte samples from three of 16 tested showed proliferative responses to influenza A suggesting fetal lymphocyte sensitization with influenza virus. These data suggest that, in some pregnancies, influenza virus is capable of transplacental sensitization of the fetus.

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