The *In Vitro* Growth and Serial Passage of RA 27/3 Rubella Vaccine Virus in Cord Blood Mononuclear Leukocytes from Normal Babies

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ABSTRACT

Because of our inability to isolate rubella virus from peripheral blood lymphocytes from patients with chronic symptoms after rubella immunization we performed a series of experiments with cord blood mononuclear cells (CBL) to determine whether and to what extent these cells supported the growth of rubella virus *in vitro*. CBL were stimulated with phytohemagglutinin for 48–72 h, washed, and inoculated with 1000 tissue culture 50% infection dose of rubella virus and placed in RPMI 1640 medium with 10% fetal bovine serum and IL-2. Approximately every 9 d the volume was halved, and new CBL were added. Aliquots at 2- to 9-d intervals were obtained and quantitatively cultured in

African green monkey tissue culture. Rubella virus growth was determined by echovirus 11 challenge. Controls consisted of rubella virus in RPMI 1640 medium. Rubella virus persisted in CBL culture longer than in control medium in seven of 11 experiments, and in three instances there was evidence of productive infection. (*Pediatr Res* 37: 623–625, 1995)

Abbreviations

CBL, cord blood lymphocyte
TCID₅₀, 50% tissue culture infective dose

Arthralgias are common in postpubertal women after immunization with rubella vaccine (1). Infrequently chronic or recurrent arthralgias, sometimes with arthritis and/or varied neurologic manifestations have been noted in vaccinees (2). Several studies have suggested that these chronic illnesses are the result of persistent rubella vaccine virus infection involving mononuclear leukocytes (3–5). Because of these reports our group studied a number of adults with chronic symptoms after rubella immunization, and we were unable to isolate rubella virus from their peripheral blood lymphocytes (6).

Because of our inability to duplicate the findings of others, we have attempted to infect CBL *in vitro* with RA 27/3 vaccine virus. The purpose of the presently reported studies was to determine under controlled conditions whether rubella vaccine virus would persist and grow in immunologically naive mononuclear cells.

METHODS

Preparation of CBL. Umbilical cord blood specimens from normal term newborns, at the UCLA Medical Center, were obtained in 10-mL tubes containing heparin. CBL were obtained by

centrifugation with Lymphoprep (Nycomed Pharmaceuticals, Oslo, Norway) and resuspended in RPMI 1640 medium (Irvine Scientific, Santa Ana, CA) with phytohemagglutinin (Difco, Detroit, MI) for 48–72 h at 36°C. After incubation, cells were washed twice in phosphate-buffered saline and resuspended in 2 mL of RPMI medium. Viable cells were counted using trypan blue exclusion, and two pellets were prepared with 5×10^6 cells in each.

Experimental design. An outline of the experimental design is presented in Figure 1. The rubella vaccine virus was Meruvax II (Merck, Sharp & Dohme, West Point, PA). Penicillin/streptomycin/Fungizone and fetal bovine serum were purchased from Irvine Scientific, Santa Ana, CA, and IL-2 was obtained from Boehringer Mannheim, Indianapolis, IN.

Viral cultures. Cultures were performed in African green monkey tissue culture cells obtained from Bio Whittaker, Walkersville, M.D.. Aliquots (0.5 mL) for culture were diluted from 10^0 to 10^{-3} and 0.5 mL of each dilution were inoculated into single African green monkey kidney tissue culture tubes. In some experiments dilutions were extended to 10^{-4} and duplicate African green monkey kidney tissue culture tubes were used.

Cultures were incubated for 7 d at 36°C and the presence of rubella virus determined by challenge with a 100 TCID₅₀

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Figure 1. Experimental design. Chronology of events in individual experiments.

infective dose of echovirus 11 (7). All cultures were blind passed once and after 7 d of incubation were rechallenged with echovirus 11. Presence of rubella virus infection was assumed if typical enteroviral cytopathic effect did not occur.

When rubella virus was recovered from CBL for a longer duration of time than from the controls, we considered this evidence of virus persistence. Virus growth and productive infection was considered to have occurred if the titer of virus

from the CBL was found to be greater than that of the original inoculum and if it did not decrease over time due to specimen dilution associated with the addition of new CBL every 9 d.

RESULTS

Eleven experiments were performed, and the results are summarized in Table 1. Rubella virus persisted in CBL longer than in controls in 7 of 11 experiments (experiments 4–10) and in three instances there was evidence of productive infection (experiments 6–8). In two experiments (1 and 11) infection did not occur and in two others (experiments 2 and 3) the studies were terminated without the addition of new CBL.

The longitudinal events in experiment 6 are presented in Figure 2. In this experiment the virus titer increased 100-fold and productive infection persisted for 145 d. Similar findings were noted in experiments 7 and 8.

DISCUSSION

After the report of the isolation of rubella virus in 1962 (7) it was found that infants with congenital rubella had prolonged infections (7–14); viremia occurred and leukocytes were found to be infected. In contrast, persistent infection was not noted in postnatal illnesses due to rubella virus. In 1965 Mellman *et al.* (15) noted that leukocytes from a seronegative woman had only a limited ability to support the replication of rubella virus when compared with other viruses such as measles, echovirus 9, and herpes simplex (15).

In recent years there has been a renewed interest in the possibility of persistent rubella virus infection (2) due to a series of publications relating arthritic and other chronic symptoms in previously vaccinated adults with persistent rubella virus in peripheral blood lymphocytes of the affected patients (3–5). These studies led us to search for persistent rubella virus infections in peripheral blood lymphocytes from subjects with

Table 1. The growth and persistence of RA 27/3 rubella vaccine virus in CBL: summary of 11 experiments

Exp. no.	Days virus culture attempted	Maximum titer* of rubella virus in CBL	Last day rubella virus isolated from CBL	Titer* on last day rubella virus isolated from CBL	Last day rubella virus isolated from controls	Titer* of rubella virus in controls on last day of isolation	Evidence of persistence of rubella virus in CBL	Evidence of growth of rubella virus in CBL
1	2, 4, 7	320	4	3.2	7	3.2	No	No
2	0, 3, 5	320	5	3.2	5	3.2	No	No
3	2, 4, 5, 6	32	6	3.2	6	3.2	No	No
4	0, 5, 7, 9, 11, 13	320	13	320	5	3.2	Yes	No
5	0, 5, 12	320	5	3.2	0	3200	Yes	No
6	1, 7, 14, 19, 26, 31, 40, 47, 55, 66, 75, 83, 89, 103, 117, 130, 145	32 000	145	3200	0	320	Yes	Yes
7	0, 7, 12, 20, 27, 33, 42, 50, 63, 70, 78, 84	3200	84	3200	0	320	Yes	Yes
8	0, 7, 12, 20, 27, 33, 42, 50, 63, 70, 78, 84	3200	84	3200	0	320	Yes	Yes
9	0, 7, 13, 18	320	13	3.2	0	320	Yes	No
10	0, 5, 13	3200	5	32	0	320	Yes	No
11	0, 5, 13	320	0	320	0	320	No	No

* TCID₅₀.

Figure 2. The longitudinal events in experiment 6. ■ = virus titer in CBL culture; ▲ = virus titer in control medium; ◆ = calculated decrease in viral titer by dilution associated with removal of old and addition of new CBL.

chronic illnesses following immunization (6). Although we were able to isolate virus from two vaccinees shortly after immunization and from one patient with natural infection, we were unable to isolate virus from any patients with chronic illnesses. In addition, since we also isolated rubella virus from the pharynx and from polymorphonuclear leukocytes of recent vaccinees we questioned the tropism of rubella virus for lymphocytes and therefore initiated the present study.

As noted in Table 1 and Figure 2, productive rubella virus infection was demonstrated in three of 11 experiments.

Since rubella virus can infect and replicate in CBL *in vitro*, it is possible that persistent rubella virus infection of peripheral blood lymphocytes can occur. Therefore in spite of our previ-

ous negative studies in patients with chronic illnesses it seems reasonable to prospectively study adult vaccinees who develop arthralgias and other symptoms for persistent rubella virus infections.

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