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Clinical and Laboratory Studies of Live Cytomegalovirus Vaccine Ad-169 (40382)

BEVERLY J. NEFF,[1] ROBERT E. WEIBEL,[2] EUGENE B. BUYNAK,[1] ARLENE A. McLEAN,[1] AND MAURICE R. HILLEMAN[1]

Division of Virus and Cell Biology Research,[1] Merck Institute for Therapeutic Research, West Point, Pennsylvania 19486 and the Department of Pediatrics,[2] University of Pennsylvania, School of Medicine, Philadelphia, Pennsylvania 19104

Cytomegalovirus, a member of the herpesvirus family of viruses, is an important cause for disease in human beings. Infection *in utero* may cause fetal death but is more often associated with low birth weight, hepatitis, severe brain damage and mental retardation (1–3). Infection acquired postnatally following interpersonal contact, fresh blood transfusion or organ transplantation is often asymptomatic but may cause fever, hepatosplenomegaly, atypical lymphocytosis, pneumonia, and increased graft rejection (4–12).

Two live cytomegalovirus vaccines have been developed to date, and each has been tested in human beings. Plotkin *et al.* (13) prepared a vaccine from the Towne strain of cytomegalovirus that was isolated in and passed 125 times in WI-38 strain human diploid fibroblasts in cell culture. Elek and Stern (14) prepared a vaccine using the Ad-169 strain of virus isolated by Rowe *et al.* (15) from human adenoidal tissue that was passed 53 times in several kinds of human cells in culture.

The Ad-169 vaccine virus was obtained from Dr. Stern, and a vaccine was prepared in these laboratories. The vaccine was tested for safety and infectivity in the laboratory and was then injected into 24 adult male priests and seminarians who were observed for clinical reactions, for viral excretion and contagious spread, and for development and retention of antibody. The findings are given in the present report.

Materials and methods. Vaccine. Cytomegalovirus vaccine strain Ad-169 with passage history as recorded by Elek and Stern (14) was received from Dr. Stern in cell culture passage 53 and was passed five additional times in these laboratories in WI-38 strain diploid human fibroblasts monitored in accord with the Revised Standards for Karyology of Human Diploid Cell Cultures (16). The vaccine was prepared using virus from infected WI-38 cells that had been maintained for 4 days in serum-free Eagle's minimal essential medium (EMEM) supplemented with neomycin. The virus was freed by circulating the infected cell suspension through a Branson sonifier (Branson Labs, Plainview, NY) at 150 ml per min. Clarification was accomplished by filtration through a 15 micron sintered glass filter. The final vaccine contained 25% sorbitol and was preserved in the frozen state at −70°. The infectivity titer, as measured in WI-38 cells, was $10^{-3.4}$ per 0.1 ml. Appropriate *in vivo* and *in vitro* tests, including tests in monkeys, were carried out on viral and on uninoculated control culture fluids to rule out the presence of extraneous microbial and viral contaminants by procedures that are consistent with current Bureau of Biologics, USA, Food and Drug Administration standards for safety of live virus vaccines (17).

Clinical tests. Forty-three male Roman Catholic priests and seminarians ranging in age from 19 through 50 years participated in the studies that were carried out in full compliance with the Investigational New Drug Regulations of the U.S. Food and Drug Administration. The subjects were in five different locations in the suburban Philadelphia area. However, insofar as possible, vaccine and control persons were matched for each area so that nearly equal numbers would be present in the two groups at each site. Twenty-four of the subjects received 0.5 ml of vaccine subcutaneously on one occasion, and 19 of the subjects were unvaccinated contact controls. Blood samples for serologic testing and for enzyme measurements were collected from the subjects at the time periods indicated in the text. Urine specimens, throat washings, and heparinized blood samples for virus isolation purpose were taken from the

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subjects prior to and 2, 4, and 8 weeks after the time the vaccine was given. These samples were tested on receipt in the laboratory and a portion of each was supplemented with 25% sorbitol for storage at −70° in the event that retests were required. All the subjects were observed by qualified medical personnel for local and/or systemic reactions 2, 4, and 8 weeks after the time vaccine was given and in addition, the vaccinated individuals were seen one week after vaccination. Each subject was contacted by telephone by medical personnel on a weekly basis during the 8-week follow-up period if he could not be seen in person.

Laboratory tests. Virus isolation. The throat washings, urine, and peripheral leukocytes obtained from the heparinized blood samples were tested for presence of cytomegalovirus using MRC-5 human diploid fibroblast cell cultures. The cultures were maintained with EMEM containing 2% fetal calf serum at 36° for 4–6 weeks and observed microscopically for viral cytopathology.

Enzyme assays. SGOT, SICD, and SGPT assays of the serum samples were by standardized procedures.

Serology. Complement fixation (CF) tests for cytomegalovirus antibody were carried out by ordinary procedures employing two units of antigen, two units of complement, and incubation at 4° for 18 hr prior to addition of the hemolytic system.

Immune adherence (IA) assays were performed by the method of Dienstag *et al.* (18).

Serum neutralization tests were performed in MRC-5 cells employing 20–60 $TCID_{50}$ of Ad-169 cytomegalovirus mixed with equal volumes of serial twofold dilutions of inactivated serum to which guinea pig complement in a final serum concentration of 1:20 was added. Incubation of the virus–serum mixtures was at 37° for 1 hr, and cytopathology was observed after 10–12 days incubation at 36°. In all serologic tests, the titer was the greatest initial serum dilution giving complete fixation (CF), IA, or neutralization.

Results. Prevaccination antibody in volunteers. Table I shows the distribution of cytomegalovirus antibodies, according to titer, in the 43 human subjects prior to vaccination. There was excellent agreement in the three assays, excluding one serum neutralization test result in which a titer of 1:2 was obtained. Thirty of the 43 subjects, 70%, were without detectable antibody before the time when the vaccine was given, and there was no marked difference in the percentage of seropositive persons in the different age groups.

Antibody responses in vaccinated persons. Twenty-four persons were vaccinated, and of these four were seropositive before vaccination. Fig. 1 summarizes the antibody titers, according to time following vaccination, among the 20 persons who were initially seronegative. All the individuals developed IA

TABLE I. DISTRIBUTION, ACCORDING TO AGE, OF CYTOMEGALOVIRUS ANTIBODIES AMONG THE SUBJECTS.

			Serologic findings				
Age (years)	No. of persons	Assay	<4	4	8	16	≥32
19	8	CF	6		2		
	8	IA	6			1	1
	8	Neut.	7[a]	1			
20–29	17	CF	14	1			2
	17	IA	14		1		2
	17	Neut.	14		1	1	1
30–39	11	CF	6		1	2	2
	11	IA	6			1	4
	11	Neut.	6			2	3
40–50	7	CF	4			2	1
	7	IA	4				3
	7	Neut.	4		1		2

[a] The initial serum dilution tested in serum neutralization assays was 1:2. One person, age 19, (who was in the control group) was seropositive at 1:2 dilution, and all the remaining sera with titers <1:4 were seronegative at 1:2 dilution.

and CF antibody, and these were positive by one month after vaccine was given. There was a neutralizing antibody response in all but one of the subjects (95%), and this was slow to appear since only half the individuals had neutralizing antibody by one month. The IA assay appeared to be the most sensitive since the geometric mean titers were highest when this assay was used. Antibody titers appeared to decline in many of the subjects by one year after vaccination, and this was especially noted in the CF assays. It was of importance that one of the total group who had developed neutralizing antibody was without detectable antibody one year following vaccination. There was no significant increase in antibody in any of the four initially seropositive persons who received the vaccine.

Clinical reaction in the total group. Table II shows that the most frequently occurring reaction was local soreness, induration, and erythema at the injection site. These were most evident 8–16 days after vaccination and usually persisted for about 1 week. Fever (100° or >) occurred in a small portion of the vaccinated subjects and was more frequent than among the unvaccinated controls. Four of 20 of the initially seronegative vaccinees and one initially seropositive had temperatures between 100° and 101° on day 11. Tender axillary adenopathy was observed in one vaccinated person on days 8–13. Vague complaints of chills, fatigue, headache, and myalgia were reported during the second week after vaccination in six of the vaccinated group and may have been vaccine related since they were not seen in the control group. No vaccine related rash other than erythema at the injection site was observed or reported. The reactions were transiently annoying but of no clinical importance.

Serum enzyme (SGOT, SGPT, and SICD) determinations were performed on blood samples taken from controls and vaccinees prior to and 2, 4, and 8 weeks after vaccine was given. Samples taken from vaccinees one week after vaccination were also tested. Two controls and one vaccinee showed minor elevations in enzyme levels, and these were not considered to be of significance.

Cytomegalovirus isolation attempts. Cytomegalovirus isolation was attempted from the urine, throat washings, and peripheral leukocytes collected from the subjects prior to and 2, 4, and 8 weeks after vaccine was given.

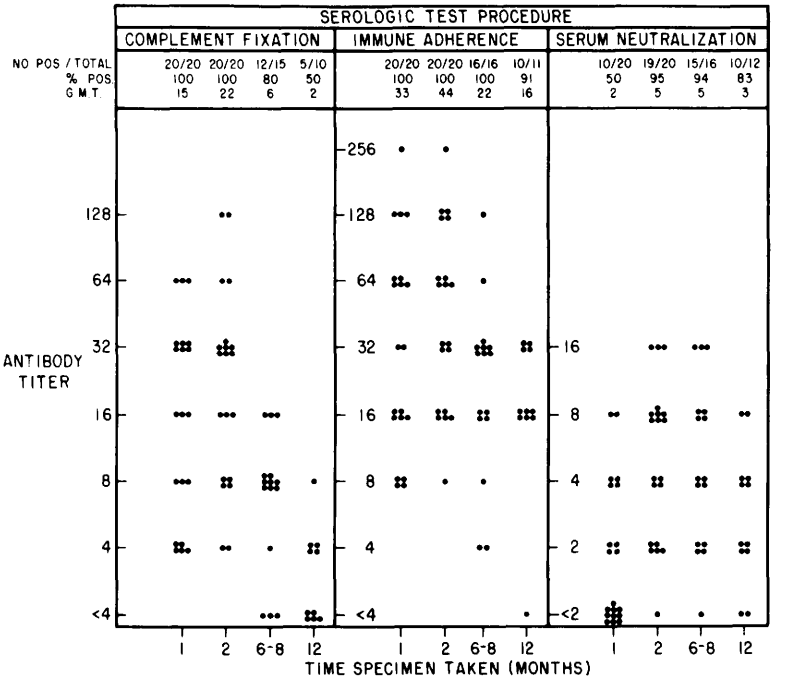

FIG. 1. Antibody titers in initially seronegative volunteers, according to time following vaccination.

TABLE II. CLINICAL SIDE EFFECTS AMONG VACCINATED PERSONS COMPARED WITH UNVACCINATED CONTROLS.

	Vaccinated persons (24 total, 20 initially seronegative)					Controls (19 total, 10 initially seronegative)				
	Days after vaccination					Days after vaccine was given				
Complaint or Reaction	0–7	8–16	17–28	29–49	50–60	0–7	8–16	17–28	29–49	50–60
Signs										
Local arm reaction[a]	7	19	4	1						
Lymphadenopathy		1								
Fever (°F)										
99–99.9	7	3	5	7	5	4	7	11	6	
100–100.9	1	4[b]		2			1		1	
101		1							1	1
Enzyme elevations	1								1	1
Symptoms										
Headache		2	3	1						
Chills		1								
Fatigue	1	4	1		1				2	1
Myalgia		1								

[a] Arm reaction included erythema, induration, pruritus, pyrexia, and soreness at the injection site.
[b] One of these was initially seropositive.

Not all subjects were available for all specimen collections, but a total of 474 of the theoretical total of 516 were taken and tested. All samples were virus-negative except for one of the initially seropositive persons in the control group that received no vaccine. Virus was recovered from his throat 2 weeks and from his urine 2 and 4 weeks after the time that vaccine was given. This individual was strongly seropositive (CF 32; IA 128; and Neutralization 16). The isolate was identified as a cytomegalovirus. The virus appeared to have been the result of a carrier state and was unrelated to the vaccine that was given to the 24 persons in the vaccinated group.

Cytomegalovirus antibody in contact controls. Serum samples taken from the 10 initially seronegative contact controls at the time of and 2, 4, and 8 weeks after the vaccine was given were tested for CF, IA, and neutralizing antibody. All sera were without antibody indicating lack of cross-infection with the virus during the time sequence covered. The antibody levels in the nine initially seropositive persons remained constant or declined slightly by 6–8 months.

Discussion. Development of a safe and effective means for preventing cytomegalovirus infections in human beings is worthy of substantial effort in the contemporary period because of death or fetal CNS damage to the unborn (1–3), because of the dangers of transfusion of infected blood, i.e., posttransfusion syndrome (6–8), and because of the contribution of cytomegalovirus to organ transplant rejection (9–12). The live Ad-169 cytomegalovirus vaccine developed by Elek and Stern (14) and studied by us offers sufficient promise in early human trials to justify the further extensive testing needed to measure its safety and protective effectiveness. The findings in the present study that was carried out in Catholic priests and seminarians are consistent with the findings in earlier studies of this vaccine by Elek and Stern (14) employing Ad-169 virus and by Just *et al.* (19) and by Plotkin *et al.* (20) employing Towne strain cytomegalovirus vaccine.

All the initially seronegative persons in the present study developed antibodies against cytomegalovirus. Such antibody was most easily detected by the IA test that appeared more sensitive than either the CF or serum neutralization test. There was small decline in IA and neutralizing antibody by the end of one year following vaccination. CF antibodies declined far more rapidly, and this might have been due to measurement of a different antibody from that detected by the IA and neutralization tests. Decline in antibody, if continued, might indicate lack of persistence of infection by the modified vaccine virus. The finding of lack of antibody response in persons with preexisting antibody is consistent with that following other parenterally administered live virus vaccines,

such as those of measles, mumps, and rubella. Plotkin *et al.* (20), in studies of Towne strain vaccine, did observe low titer antibody responses in two persons who were initially seropositive, and that may have been due either to reinfection or to booster antibody response to the viral antigen present in the live vaccine. The failure to detect virus in the leukocytes, urine, and throat specimens of initially seronegative persons who were vaccinated suggested a very low level hazard, if any, of vaccinated persons to susceptible individuals in contact with them. The failure of development of cytomegalovirus antibody in seronegative contacts in the present study gave support for the lack of contagiousness of the vaccine virus.

Clinical reactions in man, as anticipated from the previous studies by Elek and Stern (14), were minor. The most frequent reaction was local induration, soreness, and erythema at the injection site that was slow to develop and was most likely due to local proliferation of the virus. Axillary lymphadenopathy occurred in one seronegative vaccinee and was probably vaccine related. Systemic reactions, including headache, chills, myalgia, and fever occurred among some of the seronegative vaccinees but there was no generalized lymphadenopathy indicative of the mononucleosis syndrome (5). Importantly, there was no indication of liver involvement as evidenced by lack of elevation in serum enzymes.

The findings in the present study of cytomegalovirus vaccine, as well as those published by others (14, 19, 20) using Ad-169 and Towne strain vaccine viruses, represent only early probes of vaccines that must cross many hurdles before they can be accepted for general use in man. The pros and cons have been discussed at great length by others (21–24) in earlier publications. The principal remaining problems relate to proof of vaccine safety (early reactions to infection, theoretically possible oncogenicity and the consequences of persistent infection) and protective efficacy (protection for the vaccinated person and protection of the fetus against wild virus, cross-protection between strains, and the duration of protective effect). Answers to answerable questions will be sought in careful and stepwise continuing investigations.

Summary. Live strain Ad-169 vaccine was prepared in human diploid cell strain WI-38 and studied clinically in 43 adult male priests and seminarians. All seronegative persons who were vaccinated developed antibody, and the immune adherence and neutralizing antibodies persisted at high levels for at least one year. Clinical reactions were minor consisting mainly of soreness, induration, and erythema at the injection site and mild systemic reaction including headache, chills, fatigue, myalgia, and fever in a few persons. It was not possible to recover virus from the peripheral leukocytes, urine, or throat of seronegative persons who were vaccinated. Susceptible persons who were in contact with the vaccinated persons failed both to excrete virus and to develop antibody indicating lack of contagious spread of the vaccine virus. Continuing investigations to measure the safety and efficacy of the vaccine seem highly justified in view of the importance of the virus in fetal damage, transfusion disease, and organ transplantation.

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