INFECTION AND IMMUNITY, July 1979, p. 352-356
0019-9567/79/07-0352/05$02.00/0

Vol. 25, No. 1

Immunosuppression and Isolation of Rubella Virus from Human Lymphocytes After Vaccination with Two Rubella Vaccines

ELENA BUIMOVICI-KLEIN* AND LOUIS Z. COOPER

Virus Research Laboratory, Pediatric Service, The Roosevelt Hospital, and Department of Pediatrics, College of Physicians and Surgeons of Columbia University, New York 10019

Received for publication 6 February 1979

Two groups of young rubella-susceptible women were vaccinated with two rubella vaccines. Heparinized blood samples were taken from all individuals the day of vaccination and 5, 7, 15, 21, 30, 35, and 42 days later. Purified lymphocytes from these samples were cocultivated with AGMK cells for rubella virus isolation. Parallel samples of lymphocytes were stimulated with phytohemagglutinin, and the rate of [^{14}C]thymidine incorporation was determined. Rubella virus was isolated from lymphocytes collected on days 7, 15, and 21 after RA27/3 vaccination in contrast to days 7 to 35 after HPV$_{77}$ vaccination. The lymphocyte response to phytohemagglutinin was markedly suppressed from day 5 to 15. Normal lymphocyte responses were restored within 1 month after vaccination with RA27/3, but even later (1 week) after HPV$_{77}$ vaccine. Lymphocytes from rubella-susceptible persons infected in vitro with rubella virus vaccines and stimulated with phytohemagglutin displayed a decrease in their responsiveness to the mitogen similar to that observed with lymphocytes from vaccinees. The transient immunosuppression observed in vaccinees is probably due to virus-induced functional damage of the lymphocytes since no direct cytocidal effect of rubella vaccine has been demonstrated on human lymphocytes.

Rubella virus decreases blastogenic response to phytohemagglutinin (PHA) in lymphocytes from patients with congenital (8, 20) or postnatally acquired rubella (16) or after rubella vaccination (11, 18). The immunosuppression was found to be only transitory in some studies (18), whereas others observed that it lasted several weeks (26). Similar depression of PHA responsiveness was observed in lymphocytes infected in vitro with live rubella virus (17).

The mechanism of immunosuppression in rubella infection has not been defined, and it continues to be the subject of much speculation. Comparisons have been made with measles infection, in which virus receptors have been described on T lymphocytes (25). Measles virus suppresses T-cell helper activity (15). Measles antigen (21) and rubella virus (23) have been found in lymphocytes of patients during the period of active infection. These observations suggest a direct role of the virus, whereas other findings may indicate a more indirect role, such as production of a factor(s) with an inhibitory action. Mahler et al. (14) noticed that immunosuppression to PHA in patients with natural rubella was more pronounced in autologous serum than in pooled homologous control serum. The inhibitory factor might be an antibody-antigen complex, the antibody being in the immunoglobulin M class, as Lee and Siegel noticed in their in vitro experiment (13).

In the present study, we monitored the lymphocyte responses to PHA and attempted to isolate rubella virus from purified lymphocytes after vaccination with two rubella vaccines (RA27/3 and HPV$_{77}$). Lymphocytes from rubella-susceptible donors were also infected (in vitro) with the rubella vaccines and then were stimulated with PHA. Results of in vivo versus in vitro experiments are compared, and the significance of rubella virus isolation in relation to PHA response is discussed.

MATERIALS AND METHODS

Study population. Fifteen young women (22 to 29 years old) were enrolled in the study. Six were vaccinated with HPV$_{77}$, and eight were vaccinated with RA27/3 (the rubella vaccines were kindly supplied by Merck Sharp & Dohme, West Point, Pa.). All recipients were rubella susceptible before vaccination. Each had a rubella hemagglutination inhibition (HAI) titer of less than 1:8, and no traces of rubella immunoglobulin G were detectable in their serum specimens tested by sucrose gradient fractionation (3). Each individual

was counseled to avoid pregnancy for three months after vaccination. Heparinized samples of venous blood were obtained on the day of vaccination and 7, 15, 21, 30, and in some instances, 35 and 42, days thereafter for monitoring of cell-mediated immunity and virus isolation. Another sample of blood was taken 2 months after vaccination solely for rubella antibody testing.

Lymphocyte stimulation with PHA. Purified lymphocytes were obtained from heparinized blood using a Ficoll-Hypaque gradient as described previously (3). Establishment of lymphocyte cultures (LC); stimulation with purified PHA (Wellcome-Burroughs Co.); addition of [^{14}C]thymidine to the LC; and harvest of cultures were accomplished also as previously described (26). The PHA was used in concentrations of 0.2, 1.0, and 10 μg per ml by using three LC for each concentration. Three other LC not stimulated with PHA served as controls. Lymphocyte cultures were incubated for 48 h at 37°C in a CO_2 incubator (5% CO_2).

Isolation of rubella virus from lymphocytes. Attempts to isolate rubella virus from the subjects' lymphocytes were initiated concurrently with studies of PHA stimulations. Lymphocytes were cocultivated on primary AGMK cells grown in tubes at a ratio of 50 to 1 (4). After 10 to 12 days of incubation, the AGMK cell layer was washed, and the presence of rubella virus was determined by using the echovirus 11 interference method. The confirmation of interfering agents in the culture fluid as rubella virus was accomplished with control and hyperimmune rabbit anti-rubella sera.

PHA stimulation of lymphocytes infected in vitro with rubella virus vaccine. Lymphocytes from four susceptible individuals were infected in vitro with various doses of rubella virus vaccine, two of them with RA27/3, the other two with HPV_{77}. The vaccine viruses had been passaged twice in tissue culture: RA27/3 in human foreskin fibroblast cells and HPV_{77} in Vero cells. From each susceptible individual, 5×10^6 lymphocytes were infected with vaccine virus at a multiplicity of infection (MOI) of 0.01, 0.1, or 1. The infected lymphocyte suspensions were incubated for 2 h at 37°C, and afterwards 15 tubes were established, each containing 3×10^5 infected lymphocytes per one LC in 1 ml of medium (minimum essential medium + 10% autologous plasma). The LC were stimulated with PHA in concentrations ranging from 0.1 to 1 μg per ml by using three LC tubes per dilution; three unstimulated cultures served as controls. Uninfected LC containing the same number of lymphocytes, incubated for 2 h at 37°C and then stimulated with the same concentrations of PHA, were also established as controls. All cultures were incubated, treated with [^{14}C]thymidine, and harvested as described previously. Results were expressed as a stimulation index (SI): the mean count of stimulated cultures divided by the mean of unstimulated control cultures.

Serological techniques. Rubella HAI testing was performed by the Center for Disease Control protocol (5). Small amounts of rubella immunoglobulin G were detected by an HAI test done with serum fractions obtained by sucrose gradient fractionation (3).

RESULTS

Lymphocyte response to PHA stimulation. PHA concentration has a definite effect on the SI of lymphocytes. The optimal dose appears to be 1 μg/ml. High doses of PHA lead to a less pronounced decrease in the SI than do low doses of PHA. The SI obtained with 10 μg of PHA represents 60%, whereas that obtained with 0.1 μg of PHA only represents 10% of the value of the SI observed with the optimal dose of 1 μg (Fig. 1 and 2, results of day 0).

After RA27/3 vaccination, a low point of the immunosuppression was detected 5 days after vaccination when high doses of PHA (1 and 10 μg) were used. With the low dose, this low point was detected only after 15 days (Fig. 1).

After HPV_{77} vaccination, the low point of immunosuppression was detected after 15 days, irrespective of the dose of PHA used in the test. The immunosuppression induced by RA27/3 vaccine lasted from 5 to 15 days after vaccination. By day 21, marked recovery was observed. Normal SI values were reestablished 30 days after vaccination (Fig. 1).

After HPV_{77} vaccination, the lowest point of immunosuppression was observed after 15 days, and the unresponsive phase lasted somewhat longer (from day 7 to 21) than after RA27/3 vaccination. Recovery of the SI to 50% of normal values occurred between days 30 and 35, and normal values were attained again 6 weeks after vaccination (Fig. 2). Low SIs were not caused by higher background counts in unstimulated LC,

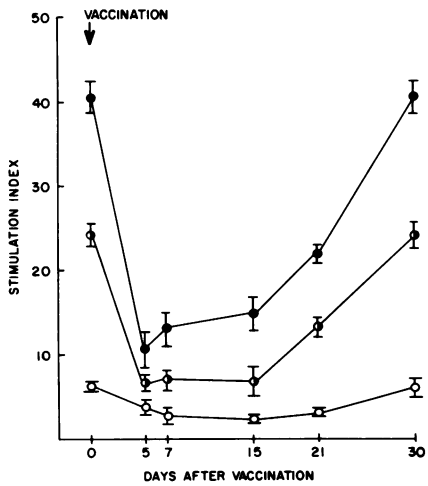

FIG. 1. *Evolution of lymphocyte responsiveness after rubella vaccination with RA27/3 vaccine. [^{14}C]thymidine incorporation stimulated by high (◑, 10 μg/ml), low (○, 0.2 μg/ml), and optimal (●, 1 μg/ml) doses of PHA. Points and bars represent mean ± standard deviation from eight vaccinated persons.*

at any interval after vaccination with either one of the vaccines. In all experiments, the counts in unstimulated LC were low, ranging from 76 to 102.

Rubella virus isolation. Rubella virus can be isolated from persons vaccinated with either RA27/3 or HPV_{77} vaccine. After RA27/3 vaccination, the virus was detected from days 7 to 21 (Table 1), whereas after HPV_{77} vaccination, virus was isolated from days 7 to 30 and was still present in one individual after 35 days (Table 1). The overall isolation frequency after RA27/3 vaccination (12 isolates out of 43 attempts, or 28%) was not significantly different from that observed after HPV_{77} vaccination (15 out of 40 attempts, or 37%). However, the high isolation frequency observed on day 21 in recipients of HPV_{77} vaccine (six out of six, or 100%) is significantly different ($P < 0.005$) from that obtained on the same day in recipients of RA27/3 vaccine (one out of seven, or 14%).

When rubella virus was detected, the interfering agent was present in the initial cocultivation. Blind second passage failed to yield additional virus. Because of the large number of lymphocytes required for cocultivation (3×10^6 lymphocytes per tube), no more than three tubes were ever inoculated from a patient. In each virus-positive specimen, interference to echovirus 11 challenge was observed in all three culture tubes. No attempt was made to estimate the infectivity titer of virus present.

Response of in vitro rubella-infected lymphocytes to PHA. Rubella vaccine-infected lymphocytes showed a marked decrease in responsiveness to stimulation with PHA. This decrease in SIs can be best demonstrated by using an optimal concentration of PHA (1 μg/ml), although the differences are noticeable with suboptimal doses as well (Fig. 3). The degree of lymphocyte unresponsiveness was dependent on the MOI. An MOI of 0.01 had no effect, whereas an MOI of 1.0 induced an approximately sixfold decrease when infected lymphocytes were stimulated with PHA at a concentration of 1 μg/ml. It appears from our data that the lowest MOI able to induce a significant decrease of lymphocyte responsiveness is 0.1. Studies with both RA27/3 and HPV_{77} vaccines gave similar results. The viability count of lymphocytes at the end of the 48-h incubation period showed no loss of viability in infected versus uninfected cells.

TABLE 1. *Isolation of rubella virus from lymphocyte cultures after vaccination with RA27/3 and HPV_{77} rubella vaccines*

Vaccine	No. of isolates/no. of attempts on day after vaccination:						
	5	7	15	21	30	35	42
RA27/3	0/5	4/8	7/8[a]	1/7[b]	0/7	0/5	0/3
HPV_{77}	0/6	2/6	4/6[c]	6/6	2/6	1/5[d]	0/5

[a] Including three individuals from which virus was isolated also on day 7.
[b] From an individual in which virus was detected on day 15.
[c] Including the two individuals shedding virus on day 7.
[d] From an individual shedding virus on day 30.

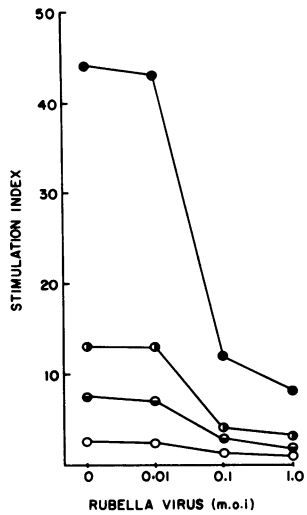

FIG. 3. *Decrease of SI elicited by various PHA concentrations in the presence of increasing doses of rubella virus. PHA concentrations of 1 (○), 0.4 (◑), 0.2 (◒), and 0.1 μg/ml (○).*

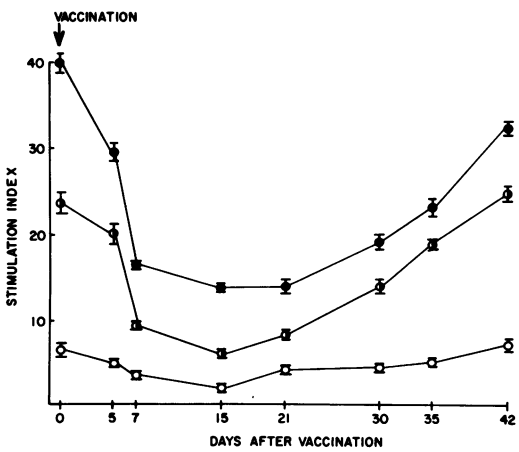

FIG. 2. *Evolution of lymphocyte responsiveness after rubella vaccination HPV_{77} vaccine. [^{14}C]thymidine incorporation stimulated by high (◑, 10 μg/ml), low (○, 0.2 μg/ml) and optimal (●, 1 μg/ml) doses of PHA. Points and bars represent mean ± standard deviation from six vaccinated persons.*

DISCUSSION

Live rubella virus has been isolated from the pharynx (3), skin (9), cervix (22), cerebrospinal fluid (24), synovial fluid (19), and lymphocytes (23) at various intervals after the onset of natural viral infection. After vaccination, attenuated rubella virus is usually present in small amounts and for a short period of time in the pharynx (6, 7). Occasionally, it may be detected in the urine (E. B. Klein and L. Z. Cooper, unpublished data) or milk of vaccinated mothers (1). Previously, Cappel (4) recovered the Cendehill strain of rubella vaccine virus from the lymphocytes of rabbits up to 25 days after vaccination. This study reports the first isolations of rubella vaccine virus from lymphocytes in humans.

In the lymphocytes from RA27/3 vaccinees, rubella virus was isolated (Table 1) 7 and 15 days after vaccination. It appears that the PHA response paralleled virus isolation (Fig. 1). The SI was significantly lower at all PHA concentrations from day 5 to 15, with a significant but not complete recovery on day 21 (when one out of eight vaccinees still shed virus). The SI returned to normal 30 days after vaccination when the virus was no longer detected in the lymphocytes of any of the vaccinees. Among the HPV_{77} vaccinees, rubella virus was detected in the lymphocytes from day 7 (Table 1) until day 35 (in one of the cases) after vaccination. The PHA response of lymphocytes from this group of vaccinees was likewise depressed during the period of rubella virus isolation. Five days after vaccination when rubella virus was not yet detected, the response to PHA was only slightly lower than normal. On day 7, when the virus was present in two out of six vaccinees, the PHA response was significantly lower, especially when higher concentrations of PHA were used. The lowest response to all PHA concentrations was observed on day 15 when virus was present in most of the vaccinees. The PHA response was affected up to 35 days after HPV_{77} vaccination, when rubella virus was still isolated from one vaccinee. Normal PHA response and no virus isolation were noticed 42 days after vaccination.

The clinical consequences of virus persistence in lymphocytes for several days or weeks after rubella vaccine is not known. Two circumstances deserve consideration and for the moment are probably best dealt with by avoidance of vaccination. A placenta may be at risk of infection from rubella vaccine for at least 35 days after HPV_{77} and 21 days after RA27/3 administration. Furthermore, the effectiveness of other viral vaccines given 7 to 25 days after rubella vaccination could conceivably be reduced by viral interference.

Several studies have described periods of immunosuppression of various length in patients with rubella infection (2, 8, 20) or in recipients of rubella vaccines (11, 26). This immunosuppression was reflected as reduced tuberculin skin sensitivity (12) or hyporesponsiveness of patients' purified lymphocytes to PHA stimulation in vitro (8, 11, 26). Reproducible demonstration of decreased lymphocyte responsiveness was obtained when different concentrations of PHA were used (2). The mechanism of this transitory immunosuppression is not yet fully elucidated. Our results show that the presence of rubella vaccine virus in the lymphocytes is associated with their decreased response to PHA stimulation.

The relation between virus-infected lymphocytes and low responsiveness to PHA stimulation was further substantiated by our in vitro experiments. Lymphocytes from susceptible donors infected in vitro with rubella virus and stimulated with PHA showed a decrease in the SI similar to that displayed by lymphocytes from vaccinated individuals.

It therefore appears that the presence of rubella virus in lymphocytes after rubella vaccination may, at least partly, explain the transitory immunosuppression observed in vaccinees. This low unresponsiveness is probably due to virus-induced functional damage of the lymphocytes because a direct cytocidal effect of the virus has not been detected.

ACKNOWLEDGMENTS

We are grateful to Karen E. Weiss and Thomas Byrne for their enthusiastic support in following the patients and helping in the laboratory work. We thank Willie Cheeks for her skillful technical assistance. The excellent secretarial help of Deborah Robinson is highly appreciated.

This work was supported by an in-aid grant from Merck Sharp & Dohme, West Point, Pa.

LITERATURE CITED

1. **Buimovici-Klein, E., R. L. Hite, T. Byrne, and L. Z. Cooper.** 1977. Isolation of rubella virus in milk after postpartum immunization. J. Pediatr. **6**:939–941.
2. **Buimovici-Klein, E., T. Vesikari, C. F. Santangelo, and L. Z. Cooper.** 1976. Study of the lymphocyte in vitro response to rubella antigen and phytohemagglutinin by a whole blood method. Arch. Virol. **52**:323–331.
3. **Buimovici-Klein, E., K. E. Weiss, and L. Z. Cooper.** 1977. Interferon production in lymphocyte cultures after rubella infection in humans. J. Infect. Dis. **135**:380–385.
4. **Cappel, R.** 1975. Cell mediated immunity in experimental rubella infections. Arch. Virol. **47**:375–379.
5. **Center for Disease Control.** 1970. A procedural guide to the performance of the standardized rubella hemagglutination-inhibition test. Center for Disease Control, Atlanta, Ga.
6. **Detels, R., K. S. W. Kim, J. I. Gale, and J. T. Grayston.** 1971. Viral shedding in Chinese children following vaccination with HPV_{77} and Cendehill-51 live attenuated rubella vaccines. Am. J. Epidemiol. **3**:473–478.
7. **Dudgeon, J. A., W. C. Marshall, and C. S. Peckham.**

1969. Rubella vaccine in adults and children: comparison of three attenuated vaccines. Am. J. Dis. Child. **118**:237–242.

8. **Fucillo, D. A., R. W. Steele, S. A. Hensen, M. M. Vincent, J. B. Hardy, and J. A. Bellanti.** 1974. Impaired cellular immunity to rubella virus in congenital rubella. Infect. Immun. **9**:81–84.
9. **Heggie, A. D.** 1978. Pathogenesis of the rubella exanthem: distribution of rubella virus in the skin during rubella with and without rash. J. Infect. Dis. **1**:74–77.
10. **Heggie, A. D., and F. C. Robbins.** 1969. Rubella in naval recruits: a virologic study. N. Engl. J. Med. **3**: 231–234.
11. **Honeyman, M. C., J. M. Forrest, and D. C. Dorman.** 1974. Cell-mediated immune response following natural rubella and rubella vaccination. Clin. Exp. Immunol. **17**:665–671.
12. **Kauffman, C. A., J. P. Phair, C. C. Linnemann, Jr., and G. M. Schiff.** 1974. Cell-mediated immunity in humans during viral infections: T effect of rubella on dermal hypersensitivity, phytohemagglutinin response, and T lymphocyte numbers. Infect. Immun. **10**:212–215.
13. **Lee, J. C., and M. M. Siegel.** 1974. A differential effect of IgM and IgG antibodies on the blastogenic response of lymphocytes to rubella virus. Cell. Immunol. **13**:22–31.
14. **Mahler, R., and L. Soren.** 1977. In vitro effects of rubella virus, strain RA27/3 on human lymphocytes. Acta Pathol. Microbiol. Scand. Sect. C **85**:49–56.
15. **McFarland, H.** 1974. The effect of measles virus infection on T and B lymphocytes in the mouse. T suppression of helper cell activity. J. Immunol. **113**:1978–1983.
16. **McMorrow, L. E., T. Vesikari, S. R. Volman, J. P. Giles, and L. Z. Cooper.** 1974. Suppression of the response of lymphocytes to PHA in rubella. J. Infect. Dis. **130**:464–469.
17. **Montgomery, J. R., M. A. South, W. E. Rawls, J. L. Melnick, G. B. Olson, P. B. Dent, and R. A. Good.** 1967. Viral inhibition of lymphocytes response to phytohemagglutinin. Science **157**:1068–1070.
18. **Morag, A., B. Morag, J. M. Bernstein, K. Beutner, and P. L. Ogra.** 1975. In vitro correlates of cell-mediated immunity in human tonsils after natural or induced rubella virus infection. J. Infect. Dis. **131**:409–416.
19. **Ogra, P. L., and K. Herd.** 1971. Arthritis associated with induced rubella infection. J. Immunol. **3**:810–813.
20. **Olson, G. B., M. A. South, and R. A. Good.** 1967. Phytohemagglutinin unresponsiveness of lymphocytes from babies with congenital rubella. Nature (London) **214**:635–696.
21. **Osunkova, B. O., G. J. Adeleye, T. A. Adejumo, and L. S. Salimonu.** 1974. Studies on leukocyte culture in measles. II. Detection of measles virus antigen in human leukocytes by immunofluorescence. Arch. Gesamte Virusforsch. **44**:323–329.
22. **Seppala, M., and A. Vaheri.** 1974. Natural rubella infection of the female genital tract. Lancet **i**:46.
23. **Simons, J. J., and M. G. Fitzgerald.** 1968. Rubella virus and human lymphocytes in culture. Lancet **2**:937–940.
24. **Squadrini, F., F. Taparelli, B. De Rienzo, and G. Giovaninni.** 1977. Rubella virus isolation from cerebrospinal fluid in postnatal rubella encephalitis. Br. Med. J. **11**:1329–1330.
25. **Valdimarsson, H., G. Agnarsdottir, and P. J. Lachman.** 1975. Measles virus receptor on human T lymphocytes. Nature (London) **255**:554–556.
26. **Vesikari, T., and E. Buimovici-Klein.** 1975. Lymphocyte responses to rubella virus antigen and phytohemagglutinin after administration of the RA27/3 strain of live attenuated rubella vaccine. Infect. Immun. **11**: 748–753.