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Cell-Mediated Immunity in Humans During Viral Infection

I. Effect of Rubella on Dermal Hypersensitivity, Phytohemagglutinin Response, and T Lymphocyte Numbers

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Phytohemagglutinin-induced lymphocyte deoxyribonucleic acid synthesis, dermal hypersensitivity, and peripheral blood thymus-derived lymphocyte numbers were assessed in nine men with experimentally induced rubella infection. Five of these men and two additional volunteers received treatment with tilorone dihydrochloride, an antiviral drug. Response to phytohemagglutinin was not changed during rubella; T lymphocyte numbers in peripheral blood were not influenced by the viral illness. However, dermal hypersensitivity was markedly impaired in all volunteers during the height of the illness. Tilorone alone, or with rubella, had no effect on any of the parameters studied.

The effect of rubella virus infection on cell-mediated immunity (CMI) has been probed most extensively in children with congenital rubella. Lymphocytes from these children show a diminished response to in vitro stimulation with nonspecific mitogens and specific antigens; dermal reactivity to various antigens remains normal (15, 16, 24). However, these children are a unique population with regard to viral persistence, and findings in this group may not necessarily be applicable to the normal population (3). With the exception of Debre and Papp's findings that three of eight children with rubella had transient anergy to tuberculin (2), little is known about the effect of rubella infection on the cellular immune system in the normal person. Several recent studies have documented a depression of skin test reactivity and in vitro lymphocyte function during vaccination with attenuated rubella virus (10, 12). However, Lalla et al. were unable to confirm these findings (9). The present report documents the effect of experimentally induced rubella infection on several parameters of CMI in humans. In addition, the combined effects of rubella and treatment with an antiviral agent, tilorone {2,7-bis[2-(diethylamino)ethoxyl]fluoren-9-one} dihydrochloride are reported.

MATERIALS AND METHODS

Nine fully informed rubella-susceptible volunteers from the Lebanon Correctional Institution, Lebanon, Ohio, were challenged intranasally with 1.0 ml of 3×10^4 to 4×10^4 mean tissue culture infective doses of the Howell strain of rubella virus which had been maintained through five passages in African green monkey kidney tissue culture. Five of these men received 1,000 or 1,500 mg of tilorone orally (Merrell-National Laboratories, Cincinnati, Ohio) immediately before or immediately after the viral challenge. The remaining four men received no antiviral therapy. Signs (fever, lymphadenopathy, rash) and symptoms (malaise, myalgia, headache) of infection were assessed daily. Two healthy physicians received 1,500 mg of tilorone alone.

Rubella hemagglutination inhibition antibody was determined in a microtiter system using complete inhibition as an end point (11). Parameters of CMI were assessed prechallenge, on day 12 after viral challenge during the expected period of viremia and leukopenia, on day 16 during the height of the illness, and on day 40 after convalescence. The individuals receiving tilorone alone were studied before administration of the drug and serially on days 12, 16, and 40.

Intradermal skin tests were performed with 7 U of streptokinase-streptodornase (Lederle, Inc., Pearl River, N.Y.). Results were read by one observer and were considered positive if induration greater than 10 mm was present at 48 h. Thymus-derived (T) lymphocytes were determined by spontaneous red blood cell (E) rosette formation by the method of Jondal et al. (7). In vitro lymphocyte response to phytohemagglutinin (PHA) was assessed by a semimicro method modified from that of Fernald (4). Briefly, peripheral blood leukocytes were obtained by sedimentation of heparinized blood, washed twice in Eagle minimum essential media, and suspended in a final concentration of 12.5×10^6 mononuclear cells per ml in minimum essential media with 20% fetal calf serum, 2 mM L-glutamine, and 100 μg of streptomycin per ml. A 0.2-ml amount of the above cell

suspension was added to each glass culture tube (6 by 50 mm). PHA-M (Difco Laboratories, Detroit, Mich.) was added to each tube in amounts varying from 10 μliters of a 1:10 dilution to 30 μliters of an undiluted solution. Control cultures contained no mitogen. All cultures were prepared in triplicate.

After 72 h of incubation at 37 C in a humidified 5% CO_2-95% air atmosphere, one μCi of [*methyl*-^{3}H]thymidine (specific activity 6 Ci/mmol) was added to each culture. After a 2-h labeling period, the cell button was successively treated with 5% trichloroacetic acid, 100% methanol, and finally hydroxide of hyamine. The solubilized material was added to scintillation fluid containing 0.3% 2,5-diphenyloxazole and 0.01% 1,4-bis[2-(5-phenyloxazolyl)]benzene in toluene. Radioactivity was assayed in a beta liquid scintillation counter. Results were expressed as the ratio: (counts of PHA-treated cultures per minute)/(counts of non-stimulated cultures per minute).

RESULTS

All men inoculated with rubella irrespective of the presence of the antiviral drug developed four-fold or greater rises in hemagglutination inhibition antibody to rubella. All manifested clinical illness, characterized by fever, malaise, myalgia, lymphadenopathy, and rash. The height of the illness was from days 15 to 17. The five men given tilorone had slightly milder clinical illness than those not receiving this agent (19).

Skin test reactivity was diminished markedly in all persons during the height of the illness, irrespective of whether tilorone had been given (Fig. 1). Six of nine men had completely negative skin tests on day 16, and the other three had a decrement ranging from 18- to 68-mm induration during the illness. The two controls given tilorone alone had no change in dermal reactivity.

The number of T lymphocytes measured by E rosette formation was not changed during rubella infection (Table 1). Maximal response to PHA was not diminished during the period of viremia (day 12) or during the height of the illness (day 16) in persons given rubella alone or rubella plus tilorone (Fig. 2). Dose response curves with PHA also were not altered in the infected or the infected and treated volunteers. The two controls given tilorone alone had no alteration in PHA response.

The antiviral agent tilorone appeared to have no effect on dermal hypersensitivity, PHA response, or T cell numbers in the controls given the drug alone or in the volunteers given both rubella and tilorone.

DISCUSSION

A large body of information has accumulated on the effect of various viral infections on CMI in humans (14, 18). However, few patients with rubella have been studied (2). Most interest has focused on CMI in children with persistent rubella infection acquired in utero (15, 16, 24). Defects in CMI observed during this period of prolonged viral excretion do not necessarily reflect the situation found during acute rubella infection (3).

Rubella virus is closely related to the paramyxoviruses, such as measles and mumps,

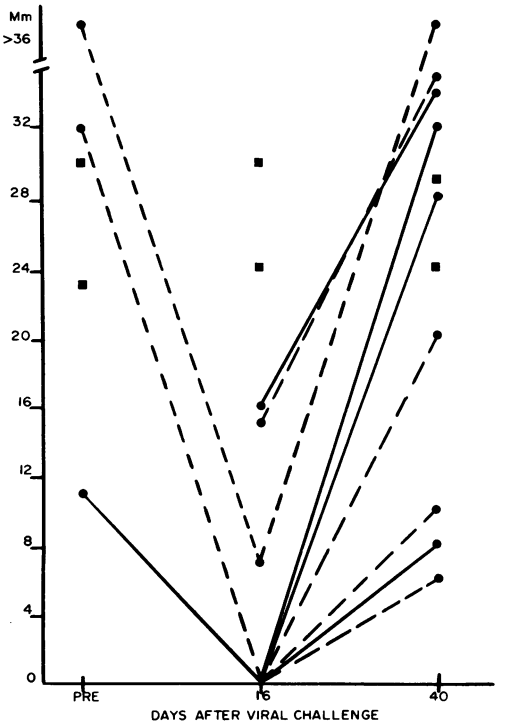

FIG. 1. *Skin test responses to streptokinase-streptodornase during rubella. Symbols:* ——, *rubella alone;* – –, *rubella and tilorone;* ■, *tilorone alone. Prechallenge skin test results were available for only three out of nine men.*

TABLE 1. *Lack of effect of rubella on number of T lymphocytes in peripheral blood*

Days after viral challenge	T cells (mean %)[a]		
	Rubella (3)[b]	Rubella plus tilorone (2)	Tilorone (2)
Prechallenge	70	61	73
12	78	65	73
16	68	74	76
40	76	70	77

[a] Mean ± 1 standard deviation (80 normal controls) 71.7 ± 6.2%.

[b] Numbers in parentheses indicate number of persons studied.

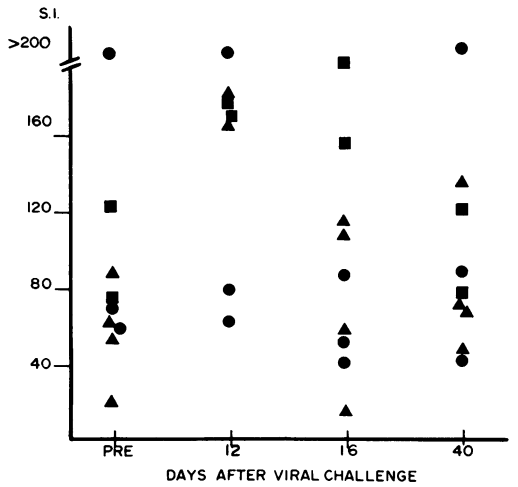

FIG. 2. *PHA-induced lymphocyte deoxyribonucleic acid synthesis during rubella. Symbols:* ●, *persons given rubella alone;* ▲, *persons given rubella and tilorone;* ■, *persons given tilorone alone. SI (counts of PHA-treated cultures per minute)/(counts of unstimulated cultures per minute). Data are not available on day 12 for two persons given rubella and tilorone.*

which have been associated with depressed CMI in humans (5, 8, 22). Viremia occurs from 2 to 7 days prior to the development of the rash in rubella, and the virus can be recovered from leukocytes during this phase of the illness (20).

Our studies in experimentally induced rubella have shown a transient mild defect in CMI at the height of the illness. The most marked abnormality was the depression of skin test reactivity. Six of nine persons demonstrated a complete loss of dermal hypersensitivity to streptokinase-streptodornase, and three showed a marked decrease in the amount of induration. Although there was a clear-cut change in skin test responsiveness, rubella infection did not appear to influence the in vitro lymphocyte proliferative response to PHA.

E rosette-forming T lymphocytes were not reduced during rubella. This is in accord with the findings of Aiuti et al., who studied a variety of viral illnesses (1). However, these results differ from those of Wybran (25) and Niklassen (13), who studied a few cases of unspecified viral illness. Differences in technique for the detecting of E rosettes or differences in the viral diseases studied may explain these discrepancies.

These results contrast with those found in children with congenital rubella, in whom skin tests and dinitrochlorobenzene sensitization were normal and PHA stimulation was depressed (15, 16, 24). Decreased dermal hypersensitivity with intact mitogenic stimulation has been found previously in other viral infections (5, 17) and is similar to the results reported by Midulla after vaccination of children with attenuated rubella virus (12).

Many complex interactions occur during the response to dermal challenge with specific antigens. In this case, the inability to mount a delayed hypersensitivity skin reaction was not related to a decrease in the number of T lymphocytes or to a diminished proliferative response of the lymphocytes to a mitogenic stimulus. However, other parameters of CMI possibly involved in skin test responses, such as release of migration inhibitory factor or chemotactic factors, were not assessed in this study and may be diminished during viral infection (6). Alternatively, the diminished skin test response during rubella may reflect viral interference with macrophage/monocyte function rather than lymphocyte function (21, 23) or may be related to dermal changes associated with this exanthem.

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