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IMPAIRED IN VITRO CELL-MEDIATED IMMUNITY TO RUBELLA VIRUS DURING PREGNANCY

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Abstract A significant depression in cell-mediated immunity, measured by phytohemagglutinin and mixed-lymphocyte-culture responsiveness was observed in 11 pregnant women. Specific cell-mediated immunity to rubella virus, measured by a ^{51}Cr-release microassay, was also found to be diminished during pregnancy. The mean (± S.D.) specific immune release for 13 seropositive subjects during pregnancy was 4.3 ± 5.9 per cent as compared to a mean of 19.9 ± 0.9 per cent in 14 seropositive nonpregnant women. This impairment in specific cell-mediated immunity to rubella virus was shown to be transient because there was subsequent increase in immunity in each of four subjects studied post partum. Thus, these changes in cell-mediated immunity during pregnancy may contribute to the known increased severity of viral infections in the gravid state. (N Engl J Med 289:604-606, 1973)

A TRANSIENT depression of cell-mediated immunity occurs in pregnancy[1-5] and may represent an adaptive maternal response to protect the fetus from rejection. A number of reports show an increased severity of certain viral infections during pregnancy.[6-12] Since it is now recognized that cell-mediated immunity constitutes an important mechanism of host resistance to viral infections,[13] it seemed of interest to investigate specific immunity to viruses during pregnancy.

In the present study, we measured cell-mediated immunity of pregnant women by phytohemagglutinin and mixed-lymphocyte culture responses, and compared these results with assessment of specific cell-mediated immunity to rubella virus by a ^{51}Cr-release microassay recently developed in our laboratory.[14]

Patients and Methods

Patients were selected from a group of healthy pregnant women attending the ante-partum clinic of Georgetown University Hospital. Healthy nonpregnant women of child-bearing age served as controls.

Rubella antibody titers were measured by the hemagglutination-inhibition technic.

Lymphocytes were separated from 10 to 15 ml of peripheral blood by centrifugation on a Hypaque-Ficoll gradient,[15] washed and resuspended in RPMI 1640 medium containing 10 per cent heat-inactivated fetal bovine serum, 100 U per milliliter of penicillin, and 100 μg per milliliter of streptomycin. All cell cultures were performed in Falcon Plastics Microtest II tissue-culture plates at 37°C in a 5 per cent carbon dioxide atmosphere.

The phytohemagglutinin stimulation studies were performed with test cultures containing 2×10^5 lymphocytes in 0.2 ml of medium and 0.1 per cent phytohemagglutinin; controls were identical except for the addition of phytohemagglutinin. For studies of mixed lymphocyte cultures, stimulating lymphocytes were treated with mitomycin-C at a concentration of 20 μg per milliliter for 1 hour, washed thrice, and resuspended in medium. Test cultures contained 2×10^5 lymphocytes together with an equal number of mitomycin-treated lymphocytes of an unrelated subject; control cultures contained 2×10^5 lymphocytes together with an equal number of mitomycin-treated lymphocytes of the same subject.

Phytohemagglutin cultures were incubated for 3 days, and mixed lymphocyte cultures for 6 days. Six hours before harvesting, 1 μCi of ^{3}H-thymidine was added to each culture. A special apparatus[14] was employed for the separation of the lymphocytes on glass-fiber filter paper, for washing them free of medium with physiologic saline, and for their recovery and quantitation of radioactive uptake. The filter paper was dried in an oven and transferred into vials containing 10 ml of Bray's solution for counting in a Packard Tricarb Liquid Scintillation Spectrometer. The results were expressed as average counts per minute (cpm) of triplicate samples.

A ^{51}Cr microassay procedure for measurement of cell-mediated immunity to rubella virus was performed as previously described.[14] Test target cells consisted of baby hamster-kidney (BHK-21) cell lines chronically infected with rubella virus. The same virus-free cell line served as control target cells. A concentration of 1×10^7 infected or control target cells were labeled with 100 μCi of sodium ^{51}Cr, incubated at 37°C for 1 hour, washed thrice, and resuspended in RPMI 1640 medium. Test cultures consisted of 5×10^5 lymphocytes and 5×10^4 target cells in 0.2 ml of medium, giving an attacker-to-target cell ratio of 100:1. Additional controls consisted of target cells without lymphocytes. Experiments were performed in triplicate. The cultures were incubated on a rocker platform, and harvested at 2 time periods of 18 and 24 hours, by the harvesting apparatus,[13] which separated the released ^{51}Cr medium from the reacting cells.

The per cent release for each cell line was calculated as follows:

$$\%\ \text{release} = \left\{ \frac{\left\{ \begin{array}{l}\text{cpm } ^{51}\text{Cr released from} \\ \text{target cells \& lymphocytes} \\ \text{during incubation}\end{array} - \begin{array}{l}\text{cpm } ^{51}\text{Cr released} \\ \text{spontaneously from target} \\ \text{cells alone during incubation}\end{array} \right\}}{\left\{ \begin{array}{l}\text{Total } ^{51}\text{Cr releasable}\end{array} - \begin{array}{l}^{51}\text{Cr released at 0-time} \\ \text{incubation}\end{array} \right\}} \right\} \times 100.$$

The specific immune release was obtained by subtraction of the percentage release for rubella-infected cells from that for control cells. The highest specific immune release at intervals of either 18 or 24 hours was used as the index of cell-mediated immunity to rubella virus.[14]

Results

The results of lymphocyte stimulation studies on 11 pregnant patients are presented in Table 1 and compared to those on an equal number of controls. The mean responses (±S.D.) to phytohemagglutinin stimulation as measured by ^{3}H-thymidine incorporation was 29,539 ± 6200 cpm in pregnant women, as compared to a value of 51,166 ± 24,500 cpm in controls. The difference was statistically significant ($t = 2.324$, $p<0.05$). The mean responses to mixed-lymphocyte-culture stimulation was 2579 ± 1128 cpm in the pregnant group, compared to a value of 4289 ± 1650 cpm in nonpregnant subjects. This difference was statistically significant ($p<0.05$, two-sample rank test).

The results of rubella hemagglutination-inhibition titers and rubella cell-mediated lymphocytotoxicity for 15 pregnant and 17 nonpregnant women are presented in Figure 1 and Table 2. Seronegative members of both

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Table 1. Lymphocyte Responses to Phytohemagglutinin (PHA) and Mixed-Lymphocyte-Culture (MLC)* Stimulation in Pregnant and Nonpregnant Women.

Subjects	No. Studied	PHA	MLC
		cpm	
Pregnant	11	29,539 ± 6,200†	2,579 ± 1,128†
Nonpregnant	11	51,166 ± 24,500	4,289 ± 1,650

*Mean ± SD. †Significant difference from nonpregnant controls ($p<0.05$).

groups (two pregnant and three nonpregnant) showed no significant specific immune release. In seropositive women, no significant differences in rubella hemagglutination-inhibition titers were observed between pregnant and nonpregnant groups (geometric mean titers 1:70 and 1:49, respectively). However, striking differences in specific immune release were observed between groups (Fig. 1 and Table 2). Of 13 seropositive pregnant women, eight showed no significant specific immune release; in contrast, 14 seropositive nonpregnant women all showed significant release. The mean (± S.D.) specific immune release was 4.3 ± 5.9 per cent for the seropositive pregnant and 19.9 ± 9.0 per cent for the seropositive nonpregnant group ($t = 3.9$, $p<0.001$).

In addition, four of the pregnant subjects were recalled three days to six weeks post partum for subsequent studies (Table 3). There was no history of exposure to rubella during the interim period, and no significant changes in titers of rubella hemagglutination-inhibition antibody were observed. Lymphocytotoxicity to rubella, however, was found to be significantly increased over ante-partum levels for each of the four subjects studied ($p<0.05$, paired t-test). In one

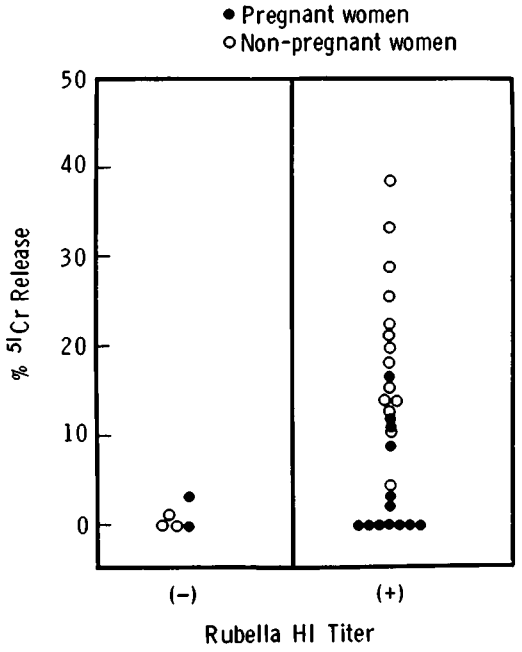

Figure 1. Correlation between Rubella Hemagglutination-Inhibition (HI) Titer and ^{51}Cr Release in Pregnant and Nonpregnant Women.

Table 2. Comparison of Humoral and Cell-Mediated Immunity to Rubella Virus in Pregnant and Nonpregnant Women.

Subjects	No. Studied	Rubella Hemagglutination-Inhibition Titer*	Specific Immune Release† (%)
Pregnant	15	No. positive 13 (1:70)	4.3 ± 5.9‡
		No. negative 2 (<1:4)	Negative
Nonpregnant	17	No. positive 14 (1:49)	19.9 ± 9.0
		No. negative 3 (<1:4)	Negative

*Geometric-mean titers shown in parentheses. †Mean ± SD.
‡Significant difference from nonpregnant controls ($p<0.001$).

woman, this increase was observed as early as three days postpartum.

Discussion

The mechanism (or mechanisms) by which the fetus escapes homograft rejection during pregnancy, although not well understood,[16] appears to be multifactorial, involving the interplay of fail-safe responses such as blocking antibody[17,18] and placental barrier,[19-22] as well as suppression of maternal cell-mediated immunity. The findings of Finn[4] and Purtilo[5] and their co-workers of impaired lymphocyte responsiveness to pytohemagglutinin during pregnancy were confirmed and extended by the present study, which showed a depressed maternal cell-mediated immunity by the three measurements of phytohemagglutinin response, response of mixed lymphocyte culture and specific cell-mediated immunity to rubella virus. In contrast, Watkins[23] found no differences between the phytohemagglutinin response of pregnant and nonpregnant women. Other investigators have also presented data to support the role of plasma factors in the suppression of maternal cell-mediated immunity[24,25] during pregnancy. The present experiments were all performed without the addition of plasma, and although no special studies were carried out to determine if lymphocytes were free of plasma factors (i.e., antibody), we interpret our findings to suggest an intrinsic dysfunction of T-lymphocytes during pregnancy. Impaired cell-mediated immunity in pregnancy is consistent with the purported increased severity of certain viral infections during the gravid state.[6-12]

Host defense against viral infections appears to be a complex phenomenon, and includes interferon,[26] cell-

Table 3. Comparison of Humoral and Cell-Mediated Immunity to Rubella Virus during and after Pregnancy.

Subject No.	Ante Partum		Post Partum		Interval
	Rubella Hemagglutination-Inhibition Titer	Specific Immune Release (%)	Rubella Hemagglutination-Inhibition Titer	Specific Immune Release (%)	
1	1:64	0	1:32	31.4	5 wk
2	1:32	9.4	1:32	20.6	6 wk
3	1:128	16	1:256	21.2	4 wk
4	1:64	5.6	1:64	19.0	3 days

mediated immunity[13] and secretory[27] and circulating antibody responses. Regarding generalized viral infections, such as rubella, serum antibody is believed to play a primary part in defense. However, several studies have shown that reinfection with rubella can occur in persons with adequate serum antibody titers; in those with naturally acquired immunity, the reinfection rate can range from 2 to 10 per cent,[28-31] whereas, in persons with vaccine-induced immunity, the range is 50 to 80 per cent.[28,31-36] The absence of local IgA-associated immunity in vaccinated persons may in part account for this difference,[37] a situation analogous to secretory antibody production in measles immunity.[38]

The crucial question in maternal rubella reinfection is whether viremia supervenes, with resultant fetal hazard. In that regard, most rubella reinfections are clinically inapparent, and viremia is believed to occur only rarely in both clinical[31,39] and experimental reinfections.[30] However, the magnitude of the antibody response after reinfection suggests extensive viral multiplication rather than limited replication at the portal of entry, and the possibility of viremia exists. Indeed, fetal infections have occurred in some cases of maternal rubella reinfection.[40-43] Although the importance of cell-mediated immunity in host resistance to viral infections is becoming well accepted, its role in rubella immunity is not known, especially regarding prevention of viremia and fetal infection. The transient depression of specific cell-mediated immunity to rubella virus during pregnancy demonstrated in the present study increases the need for further definition of the role of cell-mediated immunity not only in rubella but also in other viral infections.

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