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Enhancement of Herpesvirus Type 2 Infection in Pregnant Mice (40461)

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Clinical observations in poliomyelitis (1–3), pandemic influenza (4, 5) and primary infection with herpes simplex type-2 (HSV-2) (6) have suggested that pregnant women may have more severe disease than nonpregnant women of the same age. Numerous studies have shown that pregnant mice are more susceptible to poliomyelitis (7), encephalomyocarditis virus (8) and intravaginal HSV-2 (9), however the exact mechanism for this enhanced susceptibility remains unknown.

This paper summarizes studies on HSV-2 infection of pregnant mice using different routes of infection, and suggests that the enhancement of infection may result from alterations in local defense factors rather than a generalized depression of resistance.

Materials and methods. Animals. Pregnant and nonpregnant adult female C3H mice (14–18 g) were obtained from L. C. Strong Research Foundation, San Diego, CA. Pregnancy was timed, and virus inoculation was made between days 12 and 14 of gestation.

Viruses. HSV-2 strain 196 was obtained from Dr. Richard Courtney of Baylor College of Medicine. A stock virus pool was prepared by infecting confluent monolayers of Vero cells and harvesting the virus at the time of maximal cytopathic effect. The cells were then subjected to sonication for 30 sec at 10 kc, centrifuged at 10^3 rpm for 15 min at 5°, and the supernatant fluid dispensed in aliquots which were frozen at −70°. This stock virus, used throughout, had a titer of 5×10^7 plaque forming units (PFU/ml). The PR-8 strain of influenza-A virus (H_0N_1) was obtained from Dr. Thomas Cate, Influenza Research Center, Baylor College of Medicine and was grown in the allantoic cavity of 11 day embryonated eggs. The quantity of virus that could kill 50% of inoculated mice (LD_{50}) was determined by preparing tenfold dilutions of virus in phosphate buffered saline (PBS) containing 1% fetal calf serum and inoculating 0.05 ml of each dilution intranasally into groups of four nonpregnant adult mice. The number of mice surviving or dead at the end of a 16-day observation period was recorded and the LD_{50} endpoint calculated using the formula of Reed and Meunch (10). The LD_{50} for the stock virus was $3 \times 10^3/0.05$ ml.

Virus inoculations. Intravaginal (IVAG) infection was produced by inserting sterile cotton tampons saturated with virus (0.1 ml) diluted in PBS containing 1% fetal calf serum deep into the vagina of unanesthetized mice. Intraperitoneal (IP) infection was produced by injecting 0.1 ml of diluted virus into unanesthetized mice. Intravenous (IV) infection was produced by injecting 0.1 ml of diluted virus into the tail vein of unanesthetized mice. Intranasal (INAS) infection was produced by allowing mice, lightly anesthetized with ether, to inhale 0.05 ml of diluted virus. Following infection, animals were examined daily for signs of infection and cumulative mortalities were recorded over a 16- to 20-day period.

Statistical analysis. Cumulative mortality distributions were compared using the Wilcoxson Rank Sum test on the days that animals died in each group. Cross sectional analysis were made using the X^2 test (or Fisher exact test where the sample size was small) for 2×2 contingency tables.

Results. Figure 1 compares mortality rates in pregnant and nonpregnant mice infected with HSV-2 by the IVAG route. Signs of local infection, primarily a vaginal discharge, began on day 3 and deaths due to encephalitis first occurred on day 5. At the higher concentration (10^5 PFU/ml) 95% of pregnant mice and 68% of nonpregnant mice died. Because of the high mortality in the nonpregnant controls a second group of animals was infected with a lower concentration of virus (10^4 PFU/ml). At this concentration of virus 30 of 33 pregnant mice (90.9%) and 19 of 30 nonpregnant mice (63.3%) died ($p < .05$), confirming previous observations that preg-

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nant mice are more susceptible to HSV-2 following IVAG challenge.

Figure 2 compares mortality rates in pregnant and nonpregnant mice infected with HSV-2 by the IP route. At the concentrations tested there was no difference in rates of death or overall mortality between the two groups. In these animals death appeared to be due to encephalitis.

Figure 3 compares the mortality rates in pregnant and nonpregnant mice infected with HSV-2 by the IV route. At a high challenge dose (10^5 PFU/ml) pregnant mice had a greater early mortality than nonpregnant mice ($p < .001$ at day 4) but the overall mortality was not different (100% vs 90%). At lower concentrations of virus there were no significant differences between the two groups. Deaths between 2 and 8 days appeared to result from massive hepatic necrosis while later deaths were due to encephalitis.

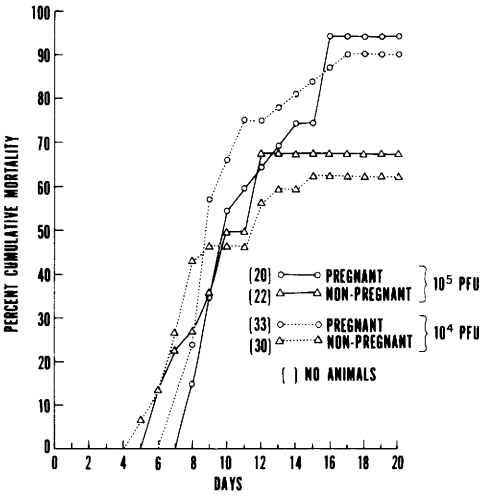

FIG. 1. Intravaginal infection of pregnant and nonpregnant mice with HSV-2.

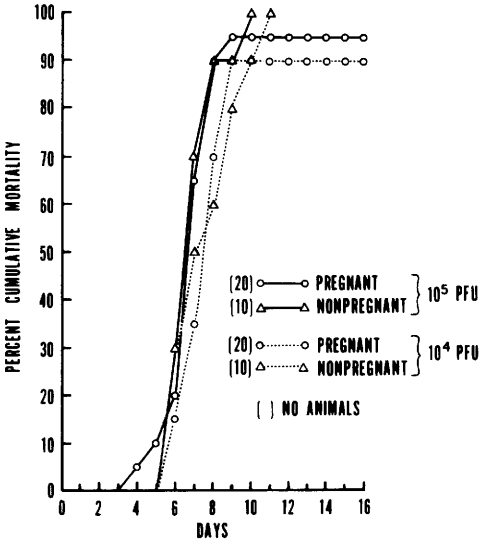

FIG. 2. Intraperitoneal infection of pregnant and nonpregnant mice with HSV-2.

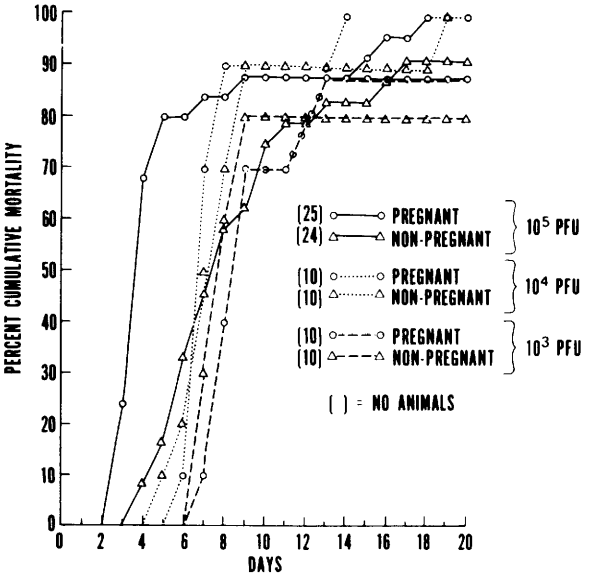

FIG. 3. Intravenous infection of pregnant and nonpregnant mice with HSV-2.

Figure 4 compares the mortality rates between pregnant and nonpregnant mice infected with HSV-2 by the INAS route. At all concentrations of virus tested there were no differences between the two groups. Early deaths were due to pneumonia although virus was also detected in the livers and brains, and late deaths were due to encephalitis.

Effect of pregnancy on influenza infection. Since pregnancy did not enhance HSV-2 infection when given by the INAS route, groups of pregnant and nonpregnant mice were infected by the INAS route with the PR-8 strain of influenza A. Figure 5 shows the mortality rates following infection with 6 × LD_{50} dose of virus. Twenty-nine of 32 (91%) of nonpregnant mice died whereas 20 of 31 (74%) of pregnant animals died ($p < .05$) suggesting that pregnant mice are actually less susceptible to influenza infection than nonpregnant mice.

Discussion. These data confirm that pregnant mice are more susceptible than nonpregnant mice to IVAG infection with HSV-2. If the route of infection in humans is generally IVAG (i.e. venereal), then the data support the clinical observation that primary HSV-2 infection during pregnancy is associated with severe disseminated disease (6).

The mechanism of this enhanced susceptibility to IVAG infection with HSV-2 remain unknown. Numerous investigators have established the importance of immune mechanisms, especially cell mediated immunity (CMI) in recovery from herpesvirus infections (11, 12). Impairment of CMI with cyclophosphamide (13) or neonatal thymectomy (14), for example, results in increased mortality from herpesvirus infections. The effect of pregnancy itself on the immune system has received much attention from the standpoint that the fetus is not rejected by the maternal immune system. Alterations in CMI which have been documented in pregnant women include a diminished response of lymphocytes to mitogens (15, 16) decreased tuberculin skin test reactivity (17) and prolonged skin graft survival (18). While it is tempting to ascribe enhanced susceptibility to infection to such immunologic alterations, the significance of such *in vitro* observations remain conjectural (19). The importance of the route of infection was highlighted by Morahan and co-workers who showed that mice surviving IVAG infection with HSV-2 were resistant to subsequent vaginal infection but were not resistant to subsequent IV infection with this virus (20). Vaginal infection was associated with significant CMI responses but no systemic neutralizing antibodies to HSV-

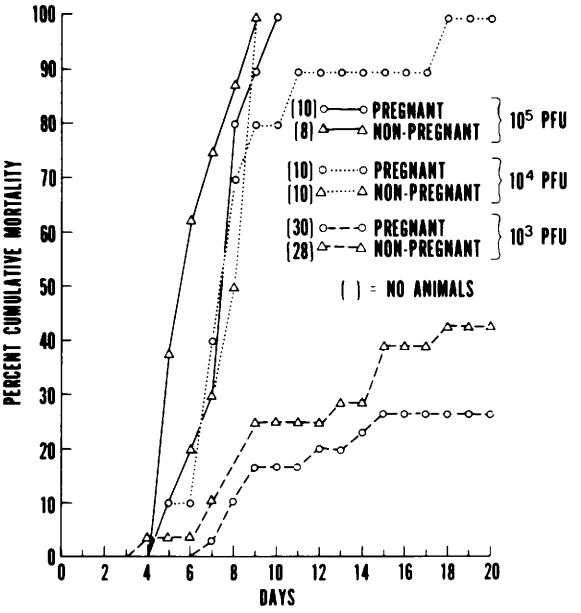

FIG. 4. Intranasal infection of pregnant and nonpregnant mice with HSV-2.

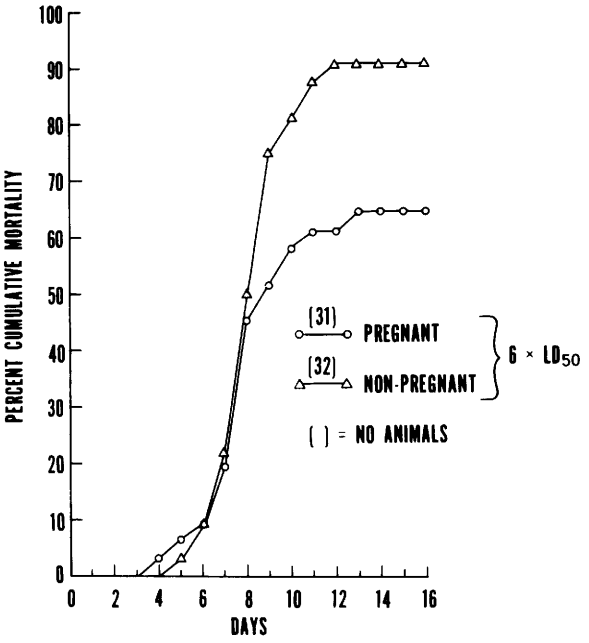

FIG. 5. Intranasal infection of pregnant and nonpregnant mice with influenza A virus.

2. In contrast, IV infection with HSV-2 resulted in both CMI and humoral antibodies.

Hormonal alterations in pregnancy have also been studied with regard to their effects on resistance to virus infection, and progesterone was shown to enhance IVAG infection with HSV-2 (21) but had no effect on encephalomyocarditis virus infections which was given intraperitoneally (22, 23).

Our data are in agreement with previous observations showing that pregnant mice are more susceptible to IVAG infection with HSV-2, the natural route of infection with this virus. If pregnancy resulted in reduced resistance to virus infection as a result of generalized suppression of immunity, then it would be expected that infection with HSV-2 would be enhanced regardless of the route of infection. That this is not the case, strongly suggests that the effect of pregnancy is a local one, at the level of the portal of entry. Pregnancy is associated with vascular changes and alterations in permeability of the cervical–vaginal epithelium which may enhance virus penetration. Cervical mucus is known to contain immunoglobulin A (24) which is felt to be important in protecting mucocutaneous tissues from herpesvirus (25). The demonstration that pregnant mice are not more susceptible to influenza virus, a respiratory pathogen, is further evidence against a generalized state of altered susceptibility, and does not support the scanty clinical evidence for increased susceptibility of pregnant women to epidemic influenza (4, 5). Moreover, this observation favors the hypothesis that pregnancy enhances genital virus infection by a direct effect within the vagina.

Summary. Pregnant and nonpregnant mice were infected with HSV-2 by the IVAG, IP, IV and INAS routes. An enhancement of infection in pregnant mice was demonstrated only by the IVAG route. In addition, infection with a respiratory virus (influenza A) demonstrated that pregnant mice were not more susceptible and were perhaps more resistant than nonpregnant mice to this infection.

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