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Effects of Immunostimulants on Resistance of Newborn Mice to Herpes Simplex Type 2 Infection (39327)

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Herpes simplex virus (HSV) infection of human neonates is associated with a high mortality rate, and survivors frequently have severe neurological or ocular sequelae (1, 2). Although antiviral chemotherapy with iododeoxyuridine, cytosine arabinoside, or adenine arabinoside has been used for the treatment of neonatal herpes, the efficacy of these drugs is currently undetermined (1, 2). Other agents, which might inhibit virus replication or bolster the newborn's immune system specifically or nonspecifically, require investigation.

Similar to the human newborn, who is more susceptible to viral dissemination than older individuals, the newborn mouse has been noted to die when inoculated with doses of virus which would not kill older mice (3–5). Survival of newborn mice can be significantly increased by the transfer of adult mouse peritoneal macrophages prior to viral challenge (4). Since transfer of macrophages is not readily feasible in human newborns it was of interest to determine if agents which might stimulate macrophages or other cells involved in immunity would lead to increased survival of newborn mice infected with HSV. Blaese has reported enhanced survival of newborn rats challenged with *Listeria monocytogenes* after administration of a number of such agents (6). A strain of HSV-2 was used in these studies since about three-quarters of HSV strains recovered from neonates have been typed in our laboratory as HSV-2, which is also the virus most often associated with genital infections in adults (1, 2).

Materials and methods. Mice. Outbred adult and newborn Swiss mice were used. For the latter, pregnant mice were obtained (Southern Animal Farms, Prattville, Ala.) and the dates of delivery of the litters were recorded.

Virus. A strain of HSV-2 isolated from a newborn with disseminated herpetic infection was used. A virus pool prepared from infected primary rabbit kidney (RK) cells had a titer of 4×10^6 plaque-forming units (PFU) per ml.

Immunostimulants. The following putative immunostimulants were employed: BCG, Tice strain, $2–8 \times 10^8$ viable organisms per ml (obtained from Dr. Roy Crispen, University of Illinois Medical Center, Chicago, Illinois); Levamisole, 2.5–25 mg/kg (Janssen R & D, Inc., New Brunswick, N.J.); Staphage lysate, undiluted (Delmont Laboratories, Swarthmore, Pa.); Typhoid vaccine 1–8 units/ml (Wyeth Laboratories, Marietta, Pa.); Brucella vaccine, 10^9 organisms per ml (obtained from Dr. Michael Blaese, NIH, Bethesda, Md.). BCG and levamisole were reconstituted or diluted with normal saline.

Antisera to HSV-2. Antiserum to HSV-2 was prepared in rabbits, as previously described (7), and had a neutralizing titer of 1:1024.

Inoculation of mice. One- to two-day-old litters of newborn mice were inoculated via the intraperitoneal (ip) route with 0.05 ml of one of the immunostimulants to be evaluated. In some experiments BCG was administered intradermally (id). Two, four, and six days later the animals were challenged ip with 10 LD_{50} of HSV-2 in 0.05 ml. Controls consisted of age-matched animals receiving only an immunostimulant, and age-matched animals receiving saline and then challenged with virus. In other experiments, the effect of HSV antiserum administered 24 hr following viral challenge was determined. Mice were observed daily for 3 weeks and the times of death recorded. *P* values were calculated by use of the chi-square test or the exact test.

Results. Determination of LD_{50}. Newborn and 6-week-old mice were inoculated with

serial dilutions of the virus. The results (Fig. 1) are similar to previous findings obtained by others (4, 5) with HSV-1, in that newborn mice succumb when inoculated ip with doses of HSV-2 that do not kill adult mice. The LD_{50}s for newborn and adult mice, calculated by the Reed-Muench method, were 67 PFU and greater than 2×10^4 PFU, respectively.

Survival of newborn mice receiving immunostimulants. In preliminary experiments no protective effect could be demonstrated when undiluted BCG was administered either 2 or 4 days prior to viral challenge with 10 LD_{50} of HSV-2; 3 of 31 mice given BCG 2 days prior to challenge survived; 2 of 24 mice given BCG 4 days prior to challenge survived. When undiluted BCG was given 6 days prior to viral challenge, highly significant protection was observed ($P < 0.0005$) as shown in Fig. 2. No protective effect could be demonstrated when any of the other putative immunostimulants were administered 2, 4, or 6 days prior to viral challenge at the doses indicated. Deaths in control animals receiving any of the immunostimulants alone were not observed.

Effect of dose and route of administration of BCG. Litters of newborn mice were inoculated with 0.05 ml BCG, undiluted or diluted 1:5, by the ip or id route 6 days prior to viral challenge. Mice receiving undiluted BCG ip or id demonstrated significantly increased survival as did mice receiving BCG diluted 1:5 ip (Table I). Animals receiving BCG diluted 1:5 id were not protected.

Effect of HSV-2 antiserum. When 0.05 ml of high titered anti-HSV-2 rabbit serum was administered ip to newborn mice 24 hr after virus inoculation, a significant increase ($P < 0.01$) in survival rate as compared to controls was noted and the animals that succumbed died later than control animals (Fig. 3). This partial protective effect of immune serum is similar to that described previously (8). No significant difference in survival was

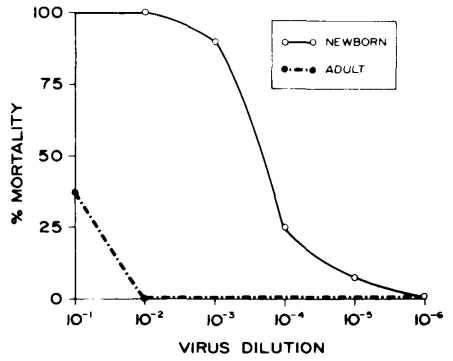

FIG. 1. Mortality of newborn and adult mice inoculated with 0.1-ml aliquots of dilutions of HSV-2 by the ip route. Each group consisted of 4 adult mice or 7–11 newborn mice.

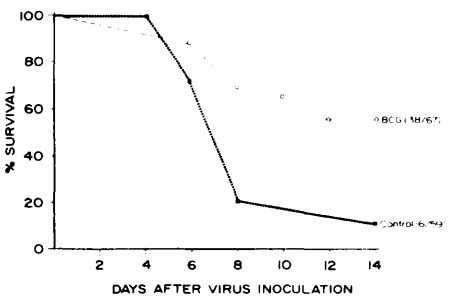

FIG. 2. Survival of mice receiving BCG or saline 6 days prior to ip inoculation with 10 LD_{50} of HSV-2.

TABLE I. SURVIVAL OF NEWBORN MICE RECEIVING VARYING AMOUNTS OF BCG INTRAPERITONEALLY OR INTRADERMALLY 6 DAYS PRIOR TO CHALLENGE WITH 10LD_{50} OF HSV-2.

Treatment	Number of mice	
	Alive	Dead
None	0	9
Undil. BCG ip[a]	3	3
1:5 BCG ip[b]	11	9
Undil. BCG[c] id	5	2
1:5 BCG id	0	5

[a] $P < 0.05$ by exact test.
[b] $P < 0.005$ by exact test.
[c] $P < 0.0005$ by exact test.

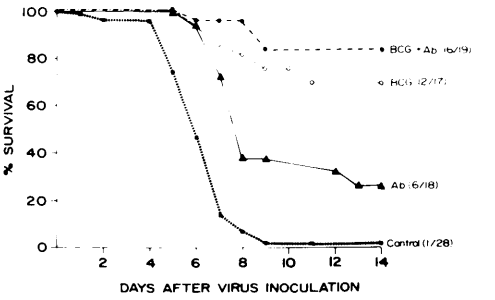

FIG. 3. Survival of mice receiving BCG 6 days prior to inoculation with 10 LD_{50} of HSV-2 or HSV-2 antibody 24 hr after virus inoculation, or both.

noted in mice receiving BCG 6 days prior to virus inoculation, then antibody 24 hr later, as compared to mice receiving BCG alone and then challenged with virus ($P > 0.05$).

Discussion. Administration of adequate doses of BCG ip or id to newborn mice 6 days prior to ip challenge with HSV-2 resulted in significantly increased survival. A protective effect could not be demonstrated when BCG was given 4 days or less before challenge with HSV-2, suggesting that stimulation of effective immune mechanisms requires several days. In this regard, Young and Skinsnes reported that it takes 5 days for increased numbers of lymphocytes and macrophages to appear in the peritoneal cavities of newborn mice inoculated with *Mycobacterium lepraemurium* ip (9).

A protective effect was not obtained with any of the other agents used. It is possible that higher doses or multiple administrations might have been effective. It is interesting to note that of the agents used BCG is the only one to contain live organisms.

Several mechanisms might be involved in the observed increased resistance of newborn mice to HSV after administration of BCG. Earlier studies emphasized the role of macrophages in immunity to HSV (4), and more recently, activated macrophages have been shown *in vitro* to decrease HSV cell-to-cell spread (10). It is possible that BCG stimulates macrophages which are then able to limit infection with HSV. The activation of macrophages might involve mediators produced by T lymphocytes stimulated by BCG. Alternately, BCG might act by stimulating production of other lymphokines or of lymphocyte-dependent cytotoxicity.

There have been several reports on induction of immunity to HSV infections after BCG administration, but the present report is the first in which protection of newborn animals is demonstrated. Baker *et al.* (11) have reported a synergistic effect of HSV immune serum and BCG on the survival of adult mice challenged with HSV-2 intravaginally. No protective effect could be demonstrated when BCG was administered alone prior to viral challenge, a finding we have corroborated in unpublished experiments. This group of workers had also reported earlier on the partial protection conveyed by BCG on the mortality of adult rabbits infected with HSV-2 (12).

Favorable results have been claimed on the use of BCG for the treatment of recurrent genital herpetic infections (13), but the efficacy of this regimen requires further evaluation. Whether BCG might be helpful for the treatment or prevention of human neonatal HSV infections also remains to be ascertained. The studies reported here suggest, however, that newborns with already disseminated infection would be unlikely to benefit from BCG administration, since BCG was effective only if given 6 days prior to viral challenge. However, since the incubation period of HSV is around 6 days and it may take several days for disseminated disease to occur even after skin lesions are noted, BCG might affect the course of babies delivered vaginally from mothers with genital HSV infection at the time of delivery, or of newborns with herpetic skin lesions without signs of dissemination. The combination of BCG with other modalities of therapy, such as passively administered antibodies or antiviral agents, also remains to be determined.

A small, but significant, increase in survival rate was noted when high-titered anti-HSV-2 rabbit serum was administered to newborn mice 24 hr after virus inoculation (Fig. 3). Recently it has been shown that administration of similarly prepared antisera to newborn mice 1 hr after intranasal inoculation with HSV-2 results in increased survival (14). Since high-titered anti-HSV sera could conceivably be employed clinically, further studies on the use and activity of high-titered anti-HSV sera are justified.

Summary. Since age-dependent diminished macrophage function has been related to the increased susceptibility of newborn mice to herpes simplex virus (HSV) infection, the effect of several agents which might activate macrophages or other cells involved in immunity was investigated. BCG, typhoid vaccine, brucella vaccine, levamisole, or staphage lysate were administered to newborn mice prior to challenge with HSV-2. Of these agents, only BCG, administered ip or id 6 days prior to challenge, was found to increase the survival rate of newborn mice. The possible use of

BCG, alone or in combination with other modalities, for the prevention or treatment of neonatal HSV infections is discussed.

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