STIMULATED MACROPHAGES AND RESISTANCE TO HERPES SIMPLEX VIRUS IN NEWBORN MICE

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SUBMITTED TO

DEPARTMENT OF BIOLOGY

CARROLL COLLEGE

APRIL 10, 1972

This thesis for honors has been approved for the Department of Biology.

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April 14, 1972
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ABSTRACT

Peritoneal macrophages from adult mice failed to protect 24-48 hr. newborn mice from intraperitoneal infection with Herpes Simplex Type I. Macrophages stimulated with either proteose peptone or thioglycollate medium showed no more increased protection than unstimulated macrophages.

Intraperitoneal inoculation of neonatal mice with Herpes virus is followed by encephalitis and death (1). Adult mice are resistant to infection by intraperitoneal route but remain susceptible to intraceberal infection (2). The macrophage appears to be the first line of defense in Herpes infections. (3). Johnson (4) found that the ability of the macrophage to phagocytize and contain virus particles is the initial factor in resistance. The second line of defense appears to be the immunocompetent lymphocyte (3) which may afford protection by elicitation of humoral antibody (6) or by cell mediated response (3).

A number of theories (5) have been suggested to account for age-dependent resistance in mice. The immunological immaturity of neonates may be due to the small numbers of cells, however mature, present in the animal. The lack of some necessary function, eg. antigen processing, may thwart early response. Other factors may be hormonal changes taking place later in development, the lack of antigenic stimulation in the fetal state, and residual maternal antibody acting to suppress antibody formation. Also, the decreased susceptibility to viral infections in older mice may be due to more efficient interferon mechanisms (1).

Peritoneal macrophages stimulated with irritants such as proteose peptone, thioglycollate medium, and glycogen (11) are found to differ from normal cells in a number of ways (12). A larger size and greater content of cytoplasmic organells is apparent. They also attach more readily to glass surfaces and spread rapidly, indicating greater phagocytic capacities (13). Blanden, MacKaness, and Collins (14) have shown that activated macrophages produced during Listeria infection can ingest Salmonella typhimurium in the absence of specific antibody, whereas those of normal mice cannot.

Strong evidence has been presented indicating that both macrophages (9) and lymphocytes (10) are necessary in many cases for primary antibody response. Argyris (6,7), Johnson (4), Hirsch et al. (8), and others have presented evidence that age dependent resistance in mice may be due, in part, to macrophage maturation. There appears to be a critical period in the development of the mouse during which time macrophages will allow the mouse to respond to antigenic stimuli and this critical period may vary from strain to strain (7).

The present study attempts to further elucidate the relation of stimulated macrophages to age-dependent resistance and to compare the effectiveness of two macrophage stimulants in this system. Macrophages transferred from adult BALB/C mice unexpectedly failed to protect 24-48 hr.newborn mice from intraperitoneal infection with herpes virus. This might be attributed to the administration of macrophages to newborn mice before the critical period when such cells may be beneficial to the recipient.

MATERIALS AND METHODS

Mice. Inbred BALB/C mice were obtained in 1971 from Dr. John Jutila of Montana State University in Bozeman, Montana.

Virus. Herpes Simplex Type 1 (15) was isolated from an oral lesion of a lab worker at the Montana State Department of Health Virology Laboratory, April, 1970.

Macrophages. Unstimulated macrophages were obtained from adult BALB/C males by washing the peritoneal cavity with three amounts of 10 ml phosphate-buffered saline. Macrophages were separated from this cell population by their ability to adhere to glass within one hour. Macrophages comprised more than 95% of these preparations.

Proteose-peptone stimulated macrophages (8) were prepared by inoculating male BALB/C mice intraperitoneally with 2.5 ml of proteose peptone from Difco # 0121 (2 gm in 98 ml PBS, pH 7.2), filtered and autoclaved (16). Macrophages were harvested 72 hr. after inoculation as described above and comprised approximately 95% of the cell population.

Thioglycollate-stimulated macrophages were provoked by the method of Argyris (7). Male BALB/C mice were inoculated with 3 ml thioglycollate medium from Difco (2.98 gm in 100 ml distilled water). Macrophages were harvested as above 72 hr. after inoculation and comprised approximately 90% of the cell population.

Peritoneal cells were placed in siliconized glass tubes (Clay-Adams Siliclad) and kept cold during the collection period. All steps were carried out under sterile conditions. Cells were spun at 1000 r.p.m. for 10 min. in a refrigerated International centrifuge. Cell counts were performed in a

Improved Neubauer hemocytometer. Differential counts were made with Wright's stain.

Virus titration. Ten-fold dilutions of herpes were made and .05 ml of each dilution was inoculated into two litters of newborn 24-48 hr. mice, and the times of death were recorded. The last dilution giving 100% death was used throughout the investigation. This dilution also equaled 100 TLD-50.

In vivo experimental procedures. Mice of ages 2 days to 12 days were inoculated i.p. with the above determined dose of herpes and the times of death were recorded. Newborn BALB/C mice 24-48 hr. old were inoculated with $5x10^6$ stimulated or unstimulated macrophages in a volume of 0.05 ml. To prevent leakage from the peritoneum, the cells were injected through the thigh muscles into the peritoneal cavity with a 26-guage needle. Twenty-four hours later the mice were inoculated i.p. with 0.05 ml of the determined dose of herpes. Littermate control mice received 1) herpes only, 2) saline plus herpes, 3) $5x10^6$ macrophages plus saline, 4) $10x10^6$ macrophages plus saline. Herpes Simplex was recovered from the brains of infected animals.

RESULTS

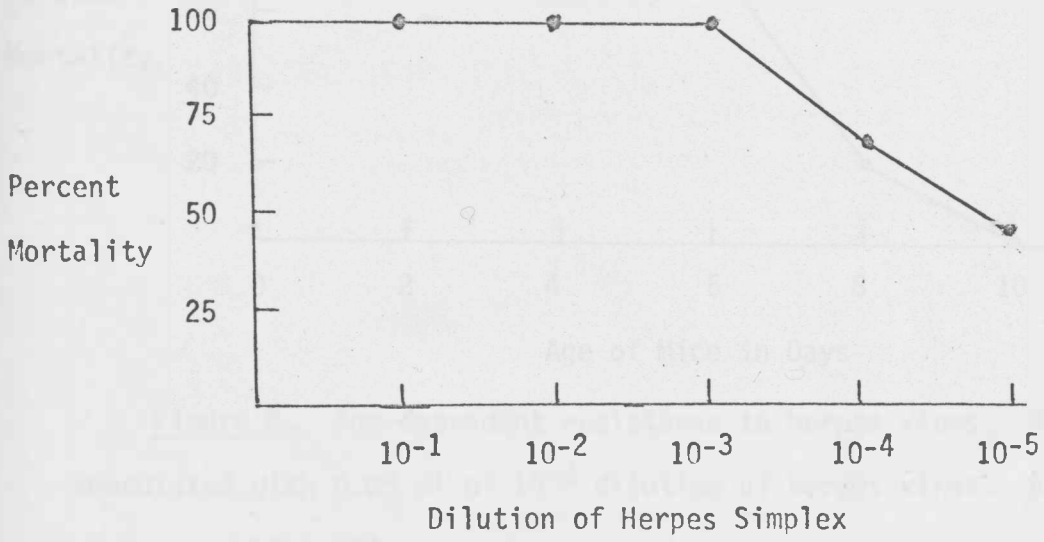

Figure 1. Determination of herpes dosage. Mice were inoculated with 0.05 ml of each of the above dilutions. Each point represents 12 mice.

The last dilution of herpes giving 100% mortality was 10^{-3} as shown in Figure 1. This was used throughout this investigation as the viral dosage which would be most sensitive to any host resistance.

The age-dependent resistance to herpes virus is illustrated in Figure 2. The development of resistance correlates with similar studies (4,8).

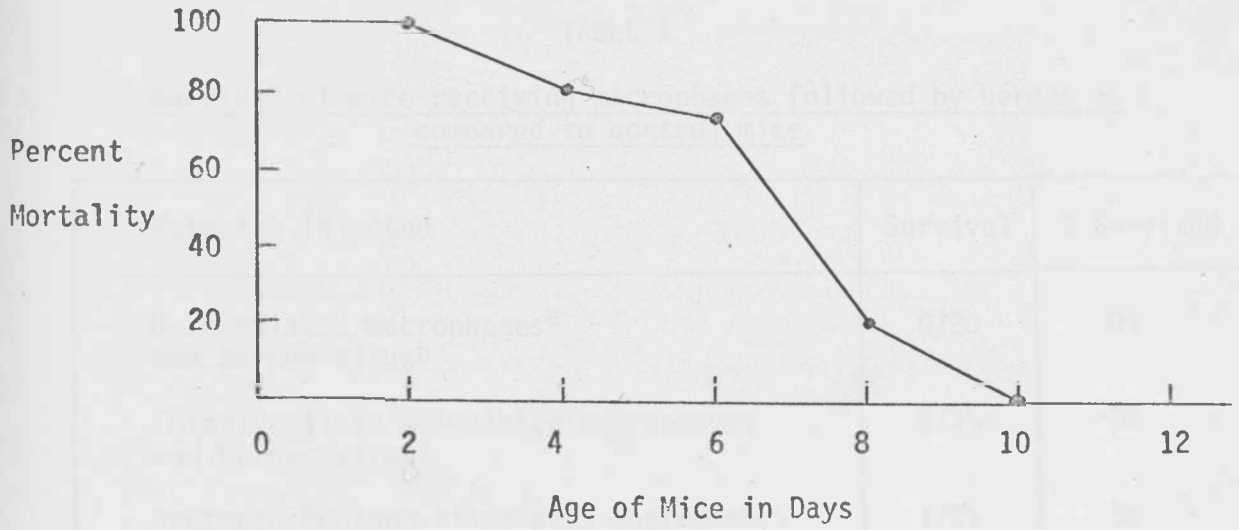

Figure 2. Age-dependent resistance to herpes virus. Mice were inoculated with 0.05 ml of 10^{-3} dilution of herpes virus. Each point represents 12 to 18 mice.

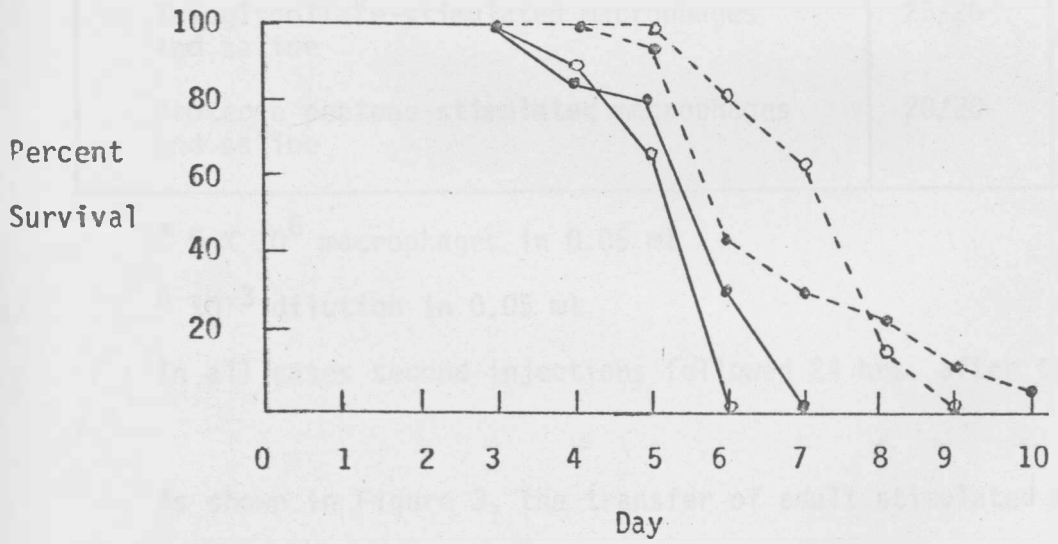

Figure 3. Survival rates of mice receiving macrophages followed by herpes inoculation. Mice were inoculated with 0.05 ml of 10^{-3} dilution of herpes on day 0. O—O, herpes virus control; O---O, transfer of unstimulated macrophages; ●—●, transfer of thioglycollate-stimulated macrophages; ●---●, transfer of proteose peptone-stimulated macrophages. Each group consists of 20 to 35 mice.

TABLE I

Survival of mice receiving macrophages followed by herpes as compared to control mice

Material Injected	Survival	% Survived
Unstimulated macrophages[a] and herpes virus[b]	0/20	0%
Thioglycollate-stimulated macrophages and herpes virus	0/35	0%
Proteose Peptone-stimulated macrophages and herpes virus	1/21	5%
Herpes virus and saline	0/20	0%
Unstimulated macrophages and saline	18/18	100%
Thioglycollate-stimulated macrophages and saline	25/26	96%
Proteose peptone-stimulated macrophages and saline	20/20	100%

[a] 5×10^6 macrophages in 0.05 ml

[b] 10^{-3} dilution in 0.05 ml

In all cases second injections followed 24 hrs. after the first.

As shown in Figure 3, the transfer of adult stimulated and unstimulated macrophages to newborn mice produced no significant difference in mortality as compared to the herpes control group. Both stimulated and unstimulated macrophages showed an increase in mean survival time (MST) over the herpes group. MST for the herpes group was 4.5 days; MST for the thioglycollate group was 5 days; MST for

the proteose peptone group was 6 days; MST for the unstimulated group was 6.5 days. The difference in MST between macrophage treated groups and the herpes control group was significant at the 95% confidence level of Student's t-distribution.

The macrophage preparations themselves and the trauma of inoculation had no singular effect on mortality as shown in Table I.

DISCUSSION

The study described in this paper was designed to determine the effectiveness of stimulated macrophages in protecting newborn 24-48 hr. mice against intraperitoneal infection with Herpes Simplex virus. The results showed that macrophages in all cases increased the mean survival time of the recipient mice, however, there was no significant difference in mortality from the control group. From these observations I conclude that macrophages alone do not confer resistance upon mice at the early age of 24-48 hr.

Hirsch et al. (8) found that stimulated macrophages transferred to five- to six-day-old CBA mice provided enhanced resistance to i.p. infection with herpes simplex. It appears that there is a critical period (7) in immunologic development, before which newborn mice are unable to benefit from macrophage treatment. Argyris (7) has suggested that this may be due to a functional immaturity of the immunocompetent cells, and without the action of the antibody eliciting lymphocyte, i.p. infections of herpes prove fatal (3).

The finding that adult macrophages produce more interferon than those from suckling mice after viral challenge (8) might also suggest a relationship between the ability to produce interferon and age-dependent viral resistance.

Stimulated macrophages showed no significant advantage over un stimulated macrophages in the system employed. Since these stimulated macrophages have been shown to be innumologically enhanced (8), the results must be attributed to the experimental system. A comparison of macrophage stimulants showed that proteose peptone-simulated macrophages gave a longer mean survival time than thioglycollate medium-simulated macrophages. However, more sensitive techniques are needed

to evaluate macrophage stimulants. The phenomenon of macrophage stimulation, the development of full cellular physiologic and immune functions through an obsecure mechanism (12), deserves further analysis.

The failure of stimulated macrophages to protect newborn mice remains unexplained. This situation leads to the conclusion only that further investigation into immunologic maturation may uncover more than has been found. The interplay of any number of factors may make up a delicate balance of common purpose in the maturing mouse.

ACKNOWLEDGEMENTS

For advice and skillful assistance I wish to thank Jessie Mola, Bob Clark, Dr. David Lackman, and Dr. James Manion.

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