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Mechanism of Recovery from Systemic Herpes Simplex Virus Infection. II. Effectiveness of Antibody Reconstitution of Nude and Neonatally Thymectomized Mice (41005)

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Abstract. A series of investigations was designed to study the role of cellular immunity, humoral antibody, and interferon in recovery from primary systemic herpes simplex virus type I (HVH) infection in mice. Nude and neonatally thymectomized mice, which have a specific defect in T-cell function, were dramatically more susceptible to primary systemic HVH infection. These immunosuppressed mice did not form neutralizing antibody to HVH, and had pathological lesions involving primarily the liver and brain. Passive transfer of physiologic amounts of neutralizing antibody after infection largely protected these T-cell-deficient HVH-infected mice. The results thus suggest that the formation of anti-HVH antibody in the nonimmunosuppressed mouse is critically important in promoting recovery from systemic HVH infection. The findings do not favor a dominant defensive role for immune cells, but they do not exclude a defensive role for these cells in this experimental primary HVH infection.

Herpes group viruses, including herpes simplex virus type I (HVH), have caused severe and fatal infections in immunosuppressed cancer patients and organ transplant patients (1, 2). Better understanding of the normal mechanism of recovery from a primary HVH infection should allow more rational attempts to prevent or treat severe HVH infections in these patients. In recent years increased emphasis has been placed on the importance of cellular immunity and the relative lack of importance of humoral antibody in the recovery of an animal from a primary viral infection (3, 4). In particular, it has been generally believed for a number of years that the formation of specific antiviral antibody is not important in promoting recovery from a primary or recurrent HVH infection, and that the major host defense mechanism against this virus is some aspect of cell-mediated immunity which is independent of antibody (3–9). The concept that cell-mediated immunity, rather than humoral immunity, is the major host defense has been based in part on the increased severity of HVH infection after impairment of T-lymphocyte function. (5, 7–9).

More recently, however, a number of observations have suggested that perhaps antibody could play a role in recovery from HVH infection in a normal host. First, we and others have demonstrated that the formation of antibody to HVH in mice is thymus dependent (6, 10, 11). Thus, any method that suppressed thymus-dependent cell-mediated immunity would also suppress the formation of thymus-dependent antibody. In addition, we and others have recently demonstrated that administration of anti-HVH antibody protected neonatal mice from fatal HVH infection when administered several hours after virus (12, 13). Previous studies have also shown that antiviral antibody can protect nonimmunosuppressed mice against fatal HVH infection in a variety of experimental situations (14–19).

Therefore, a series of investigations was designed to study the role of cellular immunity, humoral antibody, and interferon in recovery from primary systemic HVH infection in mice. In a previous paper we reported that mice immunosuppressed by three different methods (X-irradiation or the administration of Cytoxan or an-

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tithymocyte serum), were markedly more susceptible to primary systemic HVH infection (20). These immunosuppressed mice also did not form neutralizing antibody to HVH. Passive transfer of physiologic amounts of antibody as late as Day 6 after infection exerted a protective effect in these immunosuppressed HVH-infected mice. Immune spleen cells were less effective when transferred after infection, and mice which received these cells appeared to make sufficient antibody to explain the protective effect of the transferred cells.

The results in this previous paper thus suggest that antiviral antibody has a critical role in promoting recovery from primary HVH infection in this experimental model (20).

It seemed important to attempt to confirm and extend these observations in nude and neonatally thymectomized mice, which have been shown to have a specific and profound defect in T-cell function (21, 22). In the present paper, we report the results of our studies in nude and neonatally thymectomized mice infected with HVH.

Materials and methods. Herpes simplex virus type I. Stocks of a type I HVH (strain VR3) originally obtained from Dr. A. J. Nahmias, Emory University, Atlanta, Georgia, were grown in primary rabbit kidney cell monolayers in 32-oz bottles.

Virus assay. Individual mice were bled by an orbital technique and serial three-fold dilutions of each serum in Eagle's medium with 2% fetal calf serum (FCS) were assayed for HVH content by a microplaque titration on BHK-21 cells. At various intervals after virus infection, mice were bled to death and 20% (w/v) suspensions of brain, heart, lung, liver, spleen, and kidney were prepared in Eagle's medium with 2% FCS. Samples of each of these suspensions were assayed for HVH content on BHK-21 cells.

Mice. Neonatally thymectomized CD1 Swiss male mice and normal nonthymectomized CD1 Swiss male mice were obtained from Charles River Laboratory. Outbred athymic nude mice and fully immunocompetent littermate control mice were obtained from the NIH colonies.

Antibody assays. Individual mouse sera were assayed for anti-HVH antibody by a plaque reduction method. Samples containing 50 plaque-forming units (pfu) of HVH/0.2 ml were incubated with an equal volume of three-fold dilutions of serum at 37° for 2 hr; each mixture was then plaque-assayed on BHK-21 cells. A control sample of HVH incubated with normal mouse serum was included in each experiment.

Hyperimmune anti-HVH antibody. Female rabbits were injected subcutaneously with 10^4 pfu of HVH on four occasions, with at least 1 week between any two injections. A week after the fourth HVH injection they were bled by intracardiac puncture. The blood was allowed to clot overnight at 0° and sera were then separated by centrifugation at 1500 rpm for 25 min.

Statistics. Significance of differences in mortality was determined using the χ^2 test.

Experimental design. Mice were injected ip with 10^3 pfu of HVH in 0.2 ml of Eagle's medium with 2% FCS. On various days after injection of virus, titers of HVH in the serum and other organs of individual mice were determined, as previously described; three mice from each group were also bled and the titer of serum antiviral antibody in individual mice was determined. After these initial studies, replacement with immune serum was performed on various days after injection of virus. All mice were held for at least 35 days before an experiment was terminated. Unless otherwise stated, each experimental group consisted of 20 mice.

Results. Effect of neonatal thymectomy on mortality from primary HVH infection (Table I). Neonatally thymectomized CD1 Swiss male mice had a mortality of 60%, while nonthymectomized control mice had a mortality of 15% in combining three experiments ($P<0.01$). Neonatal thymectomy thus dramatically increases the severity of primary HVH infection in CD1 Swiss mice.

In addition, we and others have previously reported that athymic nude mice are dramatically more susceptible to mortality from HVH infection than thymus-bearing littermate control mice (6, 10). The pathogenesis of HVH infection in these T-cell-deficient mouse models was studied. In particular, the effect of these methods of

TABLE I. EFFECT OF NEONATAL THYMECTOMY ON HVH INFECTION[a]

Exp. No.	Percentage mortality	
	Control	Thymectomy
1	25	70
2	20	60
3	0	50
Average	15	60[b]

[a] All mice received 10^3 pfu of HVH ip; there were 20 mice in each group.
[b] $P < 0.01$.

immunosuppression on serum neutralizing antibody formation, and the effectiveness of passive antiviral antibody administered after HVH infection were examined.

Effect of neonatal thymectomy on virus content of serum and organs. Three mice from each experimental group were sacrificed on Days 4, 6, and 8 after HVH infection, and virus content in each individual organ was assayed. Mice from each group were also bled daily in an attempt to detect viremia.

In spite of numerous attempts to demonstrate viremia, HVH was never demonstrated in serum or blood of any control or neonatally thymectomized mouse. Specifically, mice from each group were bled on Days 1 to 10 after infection in the negative attempts to detect viremia. On four separate occasions whole blood or the buffy coat fraction of blood were subjected to three cycles of freezing and thawing in an attempt to isolate intracellular HVH. These isolation attempts were also unsuccessful. Virus also was not demonstrated in any of the organs of control mice which were harvested on Days 4, 6, and 8. Neonatally thymectomized mice had virus in their livers and brains, but not other organs. These mice had 10^3 pfu of HVH per gram of tissue in their brains by Day 6 after infection, and this rose to 10^5-10^6 pfu of HVH per gram of tissue by Day 8 after infection. HVH was inconsistently demonstrated on Day 6 after infection in the livers of neonatally thymectomized mice, but by Day 8 after infection 10^3-10^4 pfu of HVH per gram of tissue was consistently demonstrated in the livers of these mice. Detailed attempts to isolate virus from the organs of nude mice were not performed.

Thus, neonatally thymectomized mice consistently had markedly higher titers of HVH in their brains by Day 6 after infection and in their livers by Day 8 after infection than did control mice. These results suggest that neonatal thymectomy interfered with some host defense mechanisms which became effective before the sixth day of HVH infection.

Effect of neonatal thymectomy on serum interferon levels. Serum interferon levels were determined as previously described (23). No interferon could be detected on Days 1 through 10 after infection in the serum of neonatally thymectomized or control mice. Interferon levels in organs were not studied.

Pathology. On Days 3, 5, 7, and 9 after injection of HVH, three mice from the neonatally thymectomized and control groups were sacrificed; the brain, heart, lungs, liver, spleen, and kidneys of each mouse were removed, fixed in B-5 solution (formal sublimate), sectioned, and stained with hematoxylin and eosin.

Control mice on Day 5 after infection had a few areas of necrosis in their livers, with no pathology evident in any other organ. By Day 7 after infection most control mice were entirely normal on pathological examination. Neonatally thymectomized mice were found on Days 7 and 9 after virus infection to have many areas of focal necrosis in the liver, with some areas of more extensive hepatic necrosis and polymorphonuclear cell infiltration. They also had areas of focal necrosis in the brain, while the other organs examined appeared normal.

Effect of neonatal thymectomy on serum neutralizing antibody after HVH infection. Neonatally thymectomized CD1 Swiss male mice failed to make detectable serum neutralizing antibody, while control CD1 Swiss mice had significant levels of neutralizing antibody by Day 6 after HVH infection (Fig. 1). As previously reported, nude mice also failed to make detectable serum neutralizing antibody, while their littermate controls produced antibody beginning at least by Day 7 after HVH infection (10).

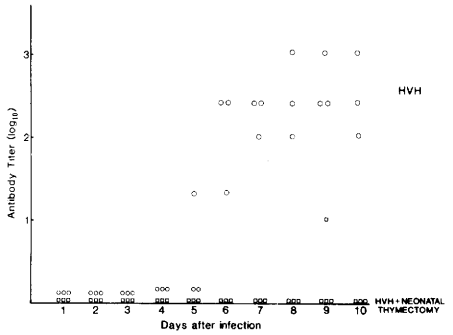

FIG. 1. Effect of neonatal thymectomy of formation of serum neutralizing antibody to HVH.

Thus, both of these methods of immunosuppression suppressed the formation of serum neutralizing antibody to HVH. Control nonimmunosuppressed mice consistently had significant titers of serum neutralizing antibody by Day 6 or 7 after HVH infection.

Effect of antibody replacement on HVH infection in neonatally thymectomized mice (Fig. 2). We have previously reported that mice injected with 0.4 ml of our undiluted HVH immune serum had serum antibody levels around 300 units immediately after antibody transfer (20). Lower levels of antibody were still present 5 and 8 days after passive antibody administration. The levels present immediately after transfer are similar to the serum antibody titers of nonimmunosuppressed mice 6 or 7 days after primary HVH infection (Fig. 1).

In this experiment there were 20 neonatally thymectomized mice which received HVH in the control group and 15 neonatally thymectomized mice which received HVH plus antibody on Day 1. A single ip dose of immune serum on Day 1 after HVH infection reduced the mortality from 90 to 0% in these mice ($P < 0.01$). Similar results were obtained in a second experiment. When the immune serum was diluted 1:10, a loss of the protective effect of this immune serum was noted (results not shown).

Effect of antibody replacement on HVH infection in nude mice. The results in Table II demonstrate that antibody passively transferred on Day 1 or 2 after HVH infection reduced the mortality in nude mice infected with HVH ($P > 0.01$). Mortality in these nude mice infected with HVH and treated with immune serum was not significantly different from the immunologically normal and resistant littermate controls infected with HVH.

Thus, these results strongly suggest that physiologic levels of neurtralizing antibody can largely replace the defect in resistence to HVH found in these nude and neonatally thymectomized mice.

Discussion. In the present paper, the pathogenesis of HVH infection in nude mice and neonatally thymectomized mice was studied. In these mice with a specific

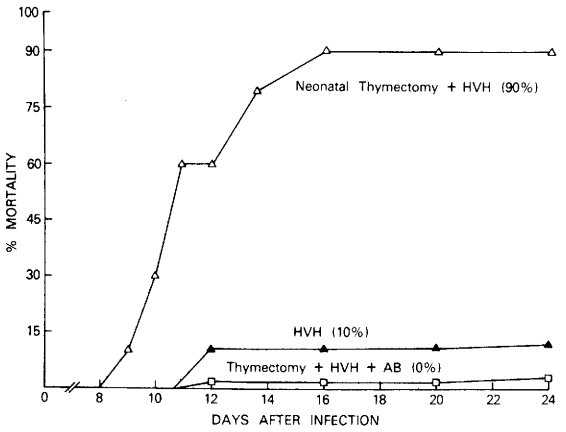

FIG. 2. Effect of antibody (AB) replacement on HVH infection in X-irradiated mice.

TABLE II. EFFECT OF ANTIBODY REPLACEMENT ON HVH INFECTION IN NUDE MICE (EXPRESSED AS PERCENTAGE MORTALITY)[a]

	Nude mice[b]		
Littermate control	Nude mice	AB on Day +1	AB on Day +2
14% (2/14)	82% (14/17)	15% (2/13)[c]	17% (2/12)[c]

[a] All mice were injected ip with 10^3 pfu of HVH.
[b] Mice were injected with 0.4 ml of immune serum (AB) ip on day indicated; in the parentheses are the number of mice which died/number of mice infected with HVH.
[c] $P < 0.01$ compared with nude mice controls.

and profound defect in T-cell function, HVH infection was dramatically increased in severity. Formation of serum neutralizing antibody was markedly suppressed in these nude or neonatally thymectomized mice. Most importantly, in these immunosuppressed mouse models protection was obtained by the passive transfer of apparently physiologic amounts of antibody after HVH infection. In a previous paper we reported that mice immunosuppressed by three different methods (X-irradiation or the administration of Cytoxan or antithymocyte serum), were markedly more susceptible to primary systemic HVH infection. (20). These immunosuppressed mice also did not form neutralizing antibody to HVH. Passive transfer of physiologic amounts of antibody as late as Day 6 after infection exerted a protective effect in these immunosuppressed HVH-infected mice. Immune spleen cells were less effective when transferred after infection, and mice which received these cells appeared to make sufficient antibody to explain the protective effect of the transferred cells (20). Taken together, these results help to reopen the possibility that antibody may play one of the essential roles in recovery from primary HVH infection in the mouse. They also demonstrate that antibody can be effective in the treatment of HVH infection in the severely immunosuppressed mouse.

It has been generally believed for a number of years that the formation of specific antiviral antibody is not important in promoting recovery from a primary or recurrent HVH infection, and that the major host defense against this virus is some aspect of cell-mediated immunity (3–9). This concept is based largely on several types of evidence: (a) the belief that patients who have an increased susceptibility to HVH infection have defects primarily of cell-medicated immunity (3, 4); (b) the observation that experimental animals with impaired T-cell function, in whom a cell-mediated immune response should be suppressed, have an increased severity of HVH infection (5, 9); (c) the ability of HVH to spread directly from cell to cell and thus avoid direct inactivation by extracellular antibody has been felt to support an important role for cell-mediated immunity, rather than antibody, in recovery from HVH infection (8).

There is reason to question the validity of these three types of evidence. First of all, we and others have recently demonstrated that antibody formation to HVH in mice is thymus dependent (6, 10, 11). It is therefore not surprising that experimental animals with impaired T-cell function (nude and neonatally thymectomized mice and ATS-treated mice) have been found to have severely decreased ability to produce antibody to HVH in our studies here and in previous reports (6, 10, 11). In experimental animals, and probably in patients, any disease or therapeutic intervention which impairs T-cell function will impair antibody formation to HVH, as well as cell-mediated immunity to HVH (6, 10, 11).

The argument that the cell-to-cell spread of HVH allows this virus to avoid direct inactivation by antibody has been seriously weakened by several recent reports. One such report has demonstrated that specific antiviral antibody is capable of curing HVH plaques in an *in vitro* system (24). In addition, the phenomenon of antibody-dependent, cell-mediated cytotoxicity has recently been extended to include target cells

acutely infected with HVH in a variety of *in vitro* systems (6). This provides another mechanism which could enable anti-HVH antibody to directly promote recovery of an infected end-organ, such as liver or brain, *in vitro*.

Two recent studies have appeared to support a primary role for cell-mediated immunity in promoting recovery from a primary HVH infection, while suggesting that the formation of antiviral antibody played a relatively unimportant role in this process (7, 8). In these studies passively transferred antibody, unlike our results, did not provide protection against HVH infection in mice immunosuppressed by X-irradiation or by the administration of Cytoxan or ATS (7, 8). In one of these papers it appears quite possible that mice injected ip with HVH were not protected because the titer of antiviral antibody transferred was too low (8). In this experiment a single dose of 0.3 ml of mouse immune serum with a titer of 1:32 was transferred to immunosuppressed mice. It is likely that this antibody would undergo at least a 5- to 10-fold dilution in the recipient mice. Titers of antibody at this level would be unlikely to be protective, based on our results. In the second study no protective effect of passively transferred antibody was noted after sc innoculation of X-irradiated or ATS-treated mice with HVH (7). Following sc innoculation of HVH in this study, the virus appeared to multiply in the sciatic nerve and in this way spread to the spinal cord and brain (7). It is possible that the pathogenesis of this experimental viral infection is different from the pathogenesis of the HVH infection following ip innoculation in our immunosuppressed mouse models.

There is abundant evidence that the methods of immunosuppression used in this and in our previous studies effectively suppress cellular immunity under a variety of experimental conditions (20–22). We have previously confirmed, under the conditions of the present experiments, the protective effect of neonatal thymectomy against acute LCM disease (23), which is believed to be a cell-mediated immunopathological disease (25–27). The evidence thus suggests that these methods of immunosuppression probably had a very significant inhibitory effect on cellular immunity to HVH in our experiments. Whatever degree of suppression of cellular immunity to HVH virus was obtained, it alone did not appear to be enough to explain the potentiation of this experimental infection, since the effect of immunosuppression was almost entirely reversed by administration of physiologic levels of antibody.

In the present study with primary systemic HVH infection we have been unable to demonstrate viremia in mice immunosuppressed by the techniques used. We have also been unable to demonstrate any HVH associated with red cells or white cells in the blood. This inability to demonstrate a viremia in immunosuppressed mice with fatal HVH infection has been reported by others (7,8). We are, therefore, unable to determine whether antiviral antibody exerted a protective effect by limiting virus dissemination or by directly promoting recovery of the infected target organs.

Several very promising antiviral agents have recently been shown to be effective in the prevention or therapy of herpes simplex virus, cytomegalovirus and herpes zoster infections in immunosuppressed or nonimmunosuppressed patients (28–30). In experimental situations these antiviral agents have appeared to have an additive or synergistic effect with specific antiviral antibody (31). It would thus seem reasonable to consider the use of high titered antiviral antibody in severe local and systemic HVH infection in immunosuppressed patients lacking antibody, either alone or in combination with promising antiviral drugs.

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