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Roles of Different T-Cell Subsets in Control of Herpes Simplex Virus Infection Determined by Using T-Cell-Deficient Mouse Models

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Herpes simplex virus infection of the scarified dermis results in infection of the nervous system and, subsequently, a cutaneous lesion in the innervated dermatome. We compared the pathogenesis of such zosteriform lesions in mice lacking or severely depleted of $CD4^+$ or $CD8^+$ T cells because of targeted gene disruption. Mice without $CD4^+$ cells showed markedly increased susceptibility, whereas β2 microglobulin knockout mice lacking $CD8^+$ T cells were as resistant to challenge as were immunocompetent mice with the same genetic background. Our results demonstrate that $CD4^+$ T cells are of primary importance in the control of herpes simplex virus infections of the skin and nervous system.

Herpes simplex virus (HSV) types 1 and 2 (HSV-1 and HSV-2, respectively) cause a wide spectrum of human infections. After an acute episode, the virus invariably ascends to the neural ganglia, where it remains latent indefinitely (10). During reactivation, the virus undergoes rounds of replication and migrates via the sensory nerve to the original site of acute infection, where it may cause recurrent lesions. The mouse zosteriform model resembles this pathogenesis except that the skin lesions result from spread from ganglia following primary cutaneous infection (12). We and others have used the mouse zosteriform model to assess vaccine effectiveness and to determine immunological mechanisms involved in antiviral immunity (4–6, 13). Such studies indicate that virus clearance from the cutaneous lesions depends largely on antibody and $CD4^+$ T-cell function (4, 6, 7, 12). The dominant role of the latter cell type became evident in comparisons of the effectiveness of immune populations of $CD4^+$ and $CD8^+$ cells at preventing zosteriform lesions in nude mice or mice with severe combined immunodeficiency given adoptive transfers of individual cell types (4). In fact, the cytokine profiles induced by viral antigen stimulation of the transferred population indicated that $CD4^+$ T cells of the type 1 cytokine-producing phenotype are involved in viral clearance. In a model measuring the rapidity of viral clearance from a subcutaneous site, both $CD4^+$ and $CD8^+$ T cells were shown to function and both subsets appeared to operate by the release of gamma interferon (14).

To gain further insight into the cellular mechanisms which contribute to viral clearance and hence recovery from HSV infection, the susceptibility of mice lacking $CD4^+$ (8) or $CD8^+$ (2) T cells because of gene knockout were compared for susceptibility to HSV-induced zosterification.

Animals were infected on the shaved, scarified left flank with either a high dose (for both HSV-1.17 and HSV-2-MS, it was 10^8 50% tissue culture-infective doses [$TCID_{50}$]) or a low dose (for HSV-1, 10^4 $TCID_{50}$; for HSV-2, 5.2×10^3 $TCID_{50}$) of virus as described elsewhere (4, 5). Animals were observed daily for signs of lesion development and severity. Severity was judged subjectively in accordance with the following criteria: 1+, vesicle formation; 2+, local erosion and ulceration of the

TABLE 1. Susceptibility of mice genetically deficient in $CD4^+$ T cells to HSV-1 and HSV-2 infections[a]

Day post-challenge	CD4 −/−				CD4 +/−				C57.BL/6			
	HSV-1.17		HSV-2-MS		HSV-1.17		HSV-2-MS		HSV-1.17		HSV-2-MS	
	LD	HD	LD	HD	LD	HD	LD	HD	LD	HD	LD	HD
5	—	10 (1.0)	20 (1.0)	60 (1.0)	—	—	—	—	—	—	—	—
7	40 (2.0)	80 (2.5)	60 (3.7)	100 (3.8)	—	40 (2.0)	—	40 (2.0)	—	40 (2.0)	—	40 (2.0)
9	90 (3.1)	100 (3.7)	100 (4.0)	NS	—	100 (3.5)	—	80 (3.3)	—	80 (3.0)	—	80 (3.5)
11	100 (4.0)	NS	NS	NS	—	100 (4.0)	—	100 (4.0)	—	100 (4.0)	—	100 (4.0)
14	NS	NS	NS	NS	—	NS	—	NS	—	NS	—	NS

[a] —, no lesion development; NS, no survivors. Anesthetized homozygous heterozygous, and C57.BL/6 mice were shaved, and the skin of the midflank was scarified. The animals were infected with low (LD) and high (HD) doses of HSV-1.17 or HSV-2-MS. For HSV-1.17, the low dose was 10^4 $TCID_{50}$ and the high dose was 10^8 $TCID_{50}$. For HSV-2-MS, the low dose was 5.2×10^3 $TCID_{50}$ and the high dose was 10^8 $TCID_{50}$. Shown are the percentages of animals that developed zoster lesions and the mean clinical severity (in parentheses). The data represent one of the three experiments conducted with 10 mice per group. CD4 −/− and CD4 +/− mice were kindly provided by Dan R. Littman, Howard Hughes Medical Institute, San Francisco, Calif. The mice were bred by Virginia Godfrey, Oak Ridge National Research Laboratory, Oak Ridge, Tenn. The offspring from heterozygous matings that were CD4 −/− were excluded.

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TABLE 2. Susceptibility of β2 microglobin-deficient mice to HSV-1 and HSV-2 infections[a]

Day post-challenge	β2M −/−				C57.BL/6			
	HSV-1.17		HSV-2-MS		HSV-1.17		HSV-2-MS	
	LD	HD	LD	HD	LD	HD	LD	HD
5	—	—	—	—	—	—	—	—
7	—	30 (2.0)	—	60 (1.7)	—	40 (2.0)	—	50 (2.0)
9	—	90 (3.0)	—	100 (3.4)	—	80 (3.0)	—	90 (3.0)
11	—	100 (4.0)	—	100 (4.0)	—	100 (4.0)	—	100 (4.0)
14	—	NS	—	NS	—	NS	—	NS

[a] —, no lesion development; NS, no survivors; LD, low dose; HD, high dose. Homozygous and C57.BL/6 mice were anesthetized and skin of the midflank was depiliated. A low or high-dose of HSV-1 or HSV-2 (as mentioned in the Table 1 footnote) was used to infect the mice. Shown are the percentages of mice that developed lesions; the values in parentheses are mean clinical scores. The data represent one of three experiments done with 10 mice per group. β2 microglobulin −/− mice were obtained from R. Jaenisch, Howard Hughes Medical Institute. The mice which had been backcrossed five times were further backcrossed for five more generations with C57.BL/6 mice by Virginia Godfrey, Oak Ridge National Research Laboratory.

local lesion; 3+, mild-to-moderate ulceration; 4+, severe ulceration, hind limb paralysis, and encephalitis.

Mice vary markedly in susceptibility to HSV infection. Thus, the low dose of both HSV-1 and HSV-2 chosen for study was lethal to susceptible mouse strains, such as BALB/c. C57BL6 mice, however, were totally resistant to the low-dose challenge. However, C57BL6 mice were susceptible and developed zosteriform lesions and usually a lethal encephalomyelitis following infection with the high challenge dose of either HSV-1 or HSV-2 (Table 1). Mice virtually lacking $CD8^+$ T cells because of targeted deletion of the β2 microglobulin gene and backcrossed more than 10 times with strain C57BL6 retained the resistance pattern of C57BL6 mice, succumbing to a high-dose challenge but resisting a low-dose challenge (Table 2). On the other hand, mice lacking $CD4^+$ T cells were highly susceptible to the low-dose challenge of both HSV-1 and HSV-2 (Table 1). In the experiments with CD4 −/− mice, heterozygous mice with the identical genetic background were also investigated. As is evident in Table 1, the heterozygous mice retained the high-resistance pattern of immunocompetent C57BL6 mice.

Taken together, the results confirm that immunity to cutaneous infection with HSV is essentially a property of $CD4^+$ T cells. Moreover, if adequate clearance of the infection does not occur, the virus enters the nervous system and the animal usually succumbs to encephalitis. Indeed, we have observed only very few animals which developed zosteriform lesions and survived. Apparently, once the virus is within the nervous system and disseminates to the skin it usually also spreads to the brain and causes death. This result always occurs with HSV-2. Our data indicate that effective immunity to HSV can be attained without $CD8^+$ T-cell function. Although β2 microglobulin −/− mice do have very few $CD8^+$ T cells (3), the ability to generate HSV-specific $CD8^+$ cytotoxic T-lymphocyte responses is virtually absent (9). Accordingly, we suggest that the $CD8^+$-mediated cytotoxic T-lymphocyte response, essential for immunity to several viruses, can be dispensed with for HSV. Indeed, in humans, the anti-HSV cytotoxic T-lymphocyte response is mediated largely by $CD4^+$ T cells (11) and it seems that HSV expresses strategies that minimize or prevent cytotoxic T-lymphocyte recognition (1, 15).

In conclusion, the results of the present study with knockout mice confirm the primary importance of $CD4^+$ T cells for immunity to HSV. The present study sheds no light on how $CD4^+$ T cells subserve this function, although the work of others indicates that the elaboration of type 1 cytokines, particularly gamma interferon, may be the crucial function involved.

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