Alteration of Rabbit Alveolar and Peritoneal Macrophage Function by Herpes Simplex Virus

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Exposure of rabbit alveolar and peritoneal macrophages to infectious herpes simplex virus resulted in inhibition of antibody-dependent cellular cytotoxicity and either enhancement or inhibition of phagocytosis. At all times after exposure, virus was inhibitory to peritoneal macrophage phagocytosis. Alveolar macrophages showed enhanced phagocytic activity in the early stages of virus exposure and inhibition at later time points.

There is an increasing body of evidence to indicate that interaction between macrophages (MØ) and herpes simplex virus (HSV) affects the course of herpetic disease. Studies using agents that modulate MØ function have linked MØ to the limitation of the spread of herpetic infection in mice (8, 11, 15–17, 25, 33). The ability of HSV to replicate in MØ of young mice (5, 7, 14) and human neonates (13) has been correlated with increased susceptibility to herpetic infection.

Our laboratory has been investigating several aspects of the interaction between HSV and MØ of adult New Zealand white rabbits. In a recent study of MØ permissiveness to HSV infection, we found different levels of interaction with HSV, which appeared to relate to the state of MØ differentiation (21). The present report compares the effects of in vitro exposure to HSV on alveolar MØ (AM) and peritoneal exudate MØ (PEM) function.

AM were obtained from adult New Zealand white rabbits by lavage of excised lungs (18). PEM were obtained after elicitation by intraperitoneal inoculation of sterile paraffin oil (21). The RE strain of HSV type 1 (6) was prepared in Vero cells as previously described (21, 29). Inactivation of virus was accomplished by incubation at 56°C for 1 h.

For assay of phagocytosis, MØ were allowed to adhere to 16-mm microplate wells (ca. $4 \times 10^5$ cells per well). Cells were exposed to 0.1 ml of various multiplicities of virus for various intervals, washed to remove extracellular virus, and assayed for ingestion of $^{51}$Cr-labeled, opsonized sheep erythrocytes (12).

For assay of antibody-dependent cellular cytotoxicity (ADCC), MØ were incubated in suspension (ca. $5 \times 10^6$ cells) for various intervals in the presence of 0.2 ml of various multiplicities of virus. After being washed to remove extracellular virus, MØ were assayed for their ability to mediate ADCC of HSV-infected target cells. ADCC was determined as previously described (28, 30), with the following modifications. Cells of the BHK-21 line were used as targets, seeded in 96-well microplates ($5 \times 10^3$ cells per well), and labeled with $^{51}$Cr 18 h before infection with HSV. Radiolabeled target cells were then infected in a monolayer 4 h before the addition of MØ effector cells.

The effect of HSV on phagocytosis is shown in Fig. 1. MØ from three animals were exposed to virus for 2 to 10 h before assay of phagocytosis. Virus appeared to have an inhibitory effect on PEM phagocytosis, the magnitude of which increased with time. In contrast, AM phagocytosis was enhanced after a brief exposure to HSV. With exposures of longer than 5 h, the effect was again inhibitory. Figure 2 shows effects of virus exposure on MØ-mediated ADCC. The ability of both AM and PEM to function as effector cells was adversely affected by virus, the inhibitory effect on PEM being more pronounced than that on AM.

The dose dependency of the virus effect on both functional parameters is shown in Table 1. Representative data from at least five animals tested show that inhibition or enhancement of MØ function was increased with increased virus doses. Heat-inactivated virus was unable to inhibit either ADCC or phagocytosis, although inactivated HSV was able to enhance AM phagocytosis (Table 1).

The data indicate that the effect of HSV on MØ function in vitro depends upon the MØ source used, the functional parameter examined, and the length of virus exposure. Enhancement of AM phagocytosis after exposure to

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increased enzyme content allow for a rapid response to a sudden bombardment by a load of inhaled particles (1). It is, therefore, possible that AM respond initially to HSV simply as a particulate. This hypothesis is strengthened by our observation that noninfectious virus induced enhancement of phagocytosis, which persisted for up to 10 h (data not shown).

HSV-induced inhibition of PEM function might be explained in light of our previous study showing that rabbit PEM can support virus replication (21), since HSV induces extensive cytopathic changes in infected cells (2). Inhibition of PEM function, however, exceeded the level expected if virus replication were the only mechanism of dysfunction. Both phagocytosis and ADCC could be almost totally inhibited by exposure to virus, whereas only about 40% of PEM become infected with HSV (21).

The magnitude of PEM dysfunction could be the result of the elimination of a subpopulation of cells with specific functional capabilities. Differences in several functions, including phagocytic activity, have been reported among subpopulations of rabbit PEM (22, 32). Although permissiveness of PEM for HSV appears to be limited to less than half of the total population (21), infection could involve subpopulations of cells highly active in phagocytosis and ADCC. It is also possible that the degree of functional

HSV has not previously been reported and was unexpected in light of previous studies in a variety of animal and virus systems (3, 9, 20, 23, 24, 26). The mechanism of HSV-induced enhancement is unknown. Infection with a variety of microorganisms leads to enhanced functional activity (i.e., activation) of MØ recovered from the infected host (19). Activation of MØ is the result of the action of lymphokines secreted by immunologically stimulated T lymphocytes. The animals used in our study had no previous exposure to HSV, as determined by serological assay. It is therefore improbable that any contaminating T cells in our preparations might have produced MØ-activating lymphokines during the brief virus exposure periods after which enhancement was seen. Additionally, PEM populations, which might be more likely than AM to contain T cells, showed no enhancement of phagocytosis.

The enhancement phenomenon that we observed might be the result of the particular adaptation of AM to their environment in the lung. It appears that AM characteristics such as

![Graph](image1)

**FIG. 1.** Time course of the virus effect on phagocytosis of $^{51}$Cr-labeled opsonized erythrocytes by MØ. Cells were exposed to 20 PFU of HSV per cell. Values were determined by dividing the mean counts per minute obtained in triplicate wells of virus-treated MØ by the mean counts per minute obtained by triplicate wells of control, untreated MØ. Each point represents the mean ± standard error of three experiments. Symbols: ●, PEM; ○, AM; ---, control values.

![Graph](image2)

**FIG. 2.** Time course of the virus effect on MØ-mediated ADCC. Cells were exposed to 20 PFU of HSV per cell. Values were determined by dividing the mean percent specific $^{51}$Cr release obtained in quadruplicate wells of virus-treated MØ by the mean specific $^{51}$Cr release obtained in quadruplicate wells of control, untreated MØ. Each point represents the mean ± standard error of three experiments. Symbols: ●, PEM; ○, AM; ---, control values.
TABLE 1. Effects of HSV on phagocytosis and ADCC by alveolar and peritoneal MØ

<table>
<thead>
<tr>
<th>Virus multiplicity (PFU per cell)*</th>
<th>0 (control)</th>
<th>1</th>
<th>10</th>
<th>20</th>
<th>30°F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phagocytosis AM PEM</td>
<td>100 100</td>
<td>88 70</td>
<td>114 50</td>
<td>133 50</td>
<td>140 96</td>
</tr>
<tr>
<td>ADCC AM PEM</td>
<td>100 100</td>
<td>89 55</td>
<td>77 15</td>
<td>58 5</td>
<td>96 93</td>
</tr>
</tbody>
</table>

* MØ were exposed to virus at the indicated multiplicities for 5 h.

Values were determined by dividing the average of results obtained in triplicate experimental wells by the average of results obtained in triplicate control wells and multiplying by 100.

Impairment of PEM was the result of virus-mediated toxicity which occurred independently of viral replication. Prior studies revealed that after exposure to high multiplicities of infectious virus, more than 95% of the PEM could be killed (21). The cytoidal effect of HSV might also account for the impairment of alveolar MØ function after prolonged virus exposure, as up to 80% of AM can ultimately be killed by virus (21).

Since HSV induces receptors for the Fc portion of immunoglobulin G on infected cell surfaces (31), it is also possible that the alteration in the expression of Fc receptors might be responsible for the alteration of Fc receptor-dependent functions, such as the ones tested here (4, 27). Although we did not investigate the effect of HSV on the number of Fc receptors per cell, exposure to HSV does not appear to alter the percentage of Fc receptor-bearing MØ (unpublished data).

Our findings underscore the importance of characterizing the system utilized in defining virus-MØ interactions. The characteristics of MØ are influenced by their in vivo environment and, as has been suggested previously (10), by the experimental manipulations to which they are subjected. Although the outcome of the interaction between HSV and HØ may be significant to the course of herpetic disease, that outcome may depend entirely on the MØ population encountering the virus.

ACKNOWLEDGMENTS

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