Key words: HSV-1/B cell-suppressed mice/antibodies/CMI

Pathogenesis of Herpes Simplex Virus in B Cell-suppressed Mice: the Relative Roles of Cell-mediated and Humoral Immunity

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SUMMARY

B cell responses of Balb/c mice were suppressed using sheep anti-mouse IgM serum. At 4 weeks, both B cell-suppressed and normal littermates were infected in the ear pinna with herpes simplex virus type 1 (HSV-1). The B cell-suppressed mice failed to produce neutralizing herpes antibodies in their sera but had a normal cell-mediated immunity (CMI) response as measured by a delayed hypersensitivity skin test. Although the infection was eliminated from the ear in both B cell-suppressed and normal mice by day 10 after infection, there was an indication that B cell-suppressed mice had a more florid primary infection of the peripheral and central nervous system and also a higher incidence of a latent infection. These results support the hypothesis that antibody is important in restricting the spread of virus to the central nervous system, whereas CMI is important in clearing the primary infection in the ear pinna.

Recovery from herpesvirus infection involves elements of both cell-mediated (Rager-Zisman & Allison, 1976; Oakes, 1975) and humoral immune mechanisms (Oakes & Lausch, 1981). However, the relative role of either form of immunity is still somewhat controversial. It is generally believed that cell-mediated immunity (CMI) plays an important role in the elimination of virus-infected cells, whereas antibody-mediated responses are thought to be directed both at the free virus and on the virus-infected cells. Antibodies either alone or in conjunction with complement components can neutralize the infectious virus. Antibody-mediated complement-dependent responses (Oldstone & Lampert, 1979) or antibody-dependent cellular cytotoxicity (Shore et al., 1976) can result in lysis of virus-infected cells. These mechanisms are believed to play an important role in the recovery from virus infection. However, the relative importance of each mechanism appears to vary in different virus–cell combinations both in in vitro and in vivo situations.

Rapid clearance of infectious virus from the ear pinna has been shown to occur in mice following the adoptive transfer of immune cells (Nash et al., 1980a). The cells involved in this reaction map to the H2K(D)/IA regions, and presumably involve cytotoxic T cells and either T helper or delayed hypersensitivity T cells (T₉ cells) (Nash et al., 1981). The contribution of anti-herpesvirus antibody has not yet been considered in this model. To examine further the role of B cells in the primary herpesvirus infection, the B cell responses of mice were suppressed by the administration of anti-IgM serum; such treatment is known to suppress the appearance of IgM and certain classes of IgG antibodies, to inhibit proliferative responses to lipopolysaccharides and to deplete B cell-dependent areas of the lymphoid system (Gordon, 1979; Friend et al., 1981).

Balb/c mice were injected intraperitoneally three times weekly with sheep anti-mouse IgM serum (a gift from Dr J. Bradwell and Mr R. Drew, Department of Immunology, University of Birmingham, U.K.) until they were 4 weeks of age (Gordon, 1979). At this stage, both treated and untreated age-matched groups of mice were infected subcutaneously in the left pinna (10⁴ p.f.u./mouse) with SC16, a herpes simplex type 1 virus (Hill et al., 1975). Anti-IgM-treated mice were thereafter injected intraperitoneally twice weekly with anti-mouse IgM serum until completion of the experiment. At various times after infection, groups of
three mice were killed; the left ear pinna, left sensory cervical dorsal root ganglia (C2, C3 and C4) and a portion of spinal cord adjacent to C2, C3 and C4 (approx. 3 mm in length) were removed and assayed for infectious virus using BHK21 cells (Russell, 1962). The serum from each mouse was also collected separately for antibody and immunoglobulin (IgM and IgG) estimations along with spleen and lymph nodes for histological examination. In addition, 24 h after infection and on successive days thereafter, the primary ear thickness response was measured using a screw gauge micrometer (Nash et al., 1980b). In Fig. 1 the results of the primary ear thickness response are shown. The anti-IgM treatment significantly reduced the inflammatory response from the 4th day onwards; a similar observation was made in a second experiment (data not shown). This indicates that in the normal situation B cells or their products contribute to the primary ear thickness/inflammatory response. In anti-IgM-treated mice the cell-mediated immune responses were not compromised, as measured by a delayed hypersensitivity (DH) skin test. For elicitation of DH, groups of four mice were challenged 20 days after infection in the right pinna with 10⁶ p.f.u. of heat-killed SC16 virus (Nash et al., 1980a) and the DH response measured after 24 and 48 h. As shown in Fig. 1 no difference was seen between untreated and anti-IgM-treated mice.

Histological examination of sections of spleen and lymph nodes from anti-IgM-treated mice showed a marked depletion of the B cell-dependent areas. The B cell-suppressed mice also failed to produce IgM in their sera and had greatly reduced amounts of IgG as revealed by a double-immunodiffusion test. Furthermore, in the anti-IgM-treated group no anti-herpesvirus antibodies were detected by the 50% plaque reduction technique (Table 1). In normal HSV-infected mice neutralizing antibodies appear in serum between 5 and 8 days after infection (Burnet & Lush, 1939; Nash et al., 1980b; Morahan et al., 1981). This observation is important because anti-IgM-treated mice eliminated infectious herpesvirus from the pinna as efficiently as untreated neutralizing antibody-positive mice. In fact, the infectious virus titres in B cell-suppressed mice 5 to 6 days after infection were up to 1 log₁₀ p.f.u. lower than untreated controls (Table 1). This rather suggests that B cells or their products actually interfere with the clearance of virus presumably mediated by local CMI responses. However, despite the lower titres in the ear pinna, the B cell-suppressed mice had an increase in the amount of infectious virus detected in the dorsal root ganglia and spinal cord (Table 1, experiment 1). In another experiment, infectious virus was detected in the
Table 1. *Infectious herpes simplex virus titres in various tissues of normal and B cell-depleted mice at different days after infection*

<table>
<thead>
<tr>
<th>Expt.</th>
<th>Time after infection (days)</th>
<th>Infectious virus titre (log_{10} p.f.u./organ)†</th>
<th>Reciprocal of serum-neutralizing antibody titre</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Ear B − ve Ganglia (C₂, C₃, C₄) Spinal cord</td>
<td>Normal B − ve</td>
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<td>Normal B − ve</td>
<td>Normal B − ve</td>
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<td>(3-6) 3-7, 4-0, 4-0</td>
<td>(9) −, −, 1-3</td>
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<td>(3-6) 3-0, 3-4, 4-4</td>
<td>(9) 2-0, 3-3, 3-4</td>
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<td>1</td>
<td>3</td>
<td>(3-6) 3-9, 3-9</td>
<td>(9) −, −</td>
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<td>6</td>
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<td>10</td>
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<td>(9) 2-8, 2-7, −, 2-6</td>
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* Mice were infected with 10⁴ p.f.u. SC16 virus in ear pinna.
† Infectivity values are shown for individual mice. The first value given for each organ (ear, ganglia and spinal cord) is from the same animal (ditto for second, third etc. values). −, No infectious virus detected. Figures in parentheses indicate mean value.
‡ Sera from three to five mice/group were tested individually and no antibodies were detected.
§ ND, Not determined.
† Infectious virus titre less than 5 p.f.u./organ.
ganglia and spinal cord at days 5 and 6 after infection; again the number of positives detected in the B cell-suppressed mice (4/9) was higher than untreated controls (1/9). These results indicate an increased probability of infection of the peripheral and central nervous system in anti-IgM-treated mice.

These observations were further strengthened when infected B cell-suppressed mice were tested for latent virus. Using the in vitro reactivation technique (Field et al., 1979), the left cervical dorsal root ganglia (C2, C3, and C4) were removed 40 days after left ear infection and cultured in vitro for 6 days; infectious virus was then assayed using BHK21 cells. In the anti-IgM-treated group, ganglia from 7 out of 8 mice were positive (87%), whereas among the untreated group only 3 out of 8 mice were positive (37%). Although these are preliminary findings and clearly more experiments will be required to provide a definitive answer, taken together all these observations suggest that the B cell suppression leads to a more florid infection of the ganglia and spinal cord, and also leads to a higher incidence of latency. Furthermore, the above observations also support our earlier findings in athymic nude mice that neutralizing antibodies are important in restricting the spread of herpesvirus to the nervous system whereas CMI is important in clearing virus from the primary infection site (Kapoor et al., 1982).

This model might be considered analogous to hypogammaglobulinaemia in man. Such individuals recover normally from a primary herpetic infection, implying that CMI and not humoral immunity is central to recovery (Merigan & Stevens, 1971). However, our observations on mice lead one to speculate whether herpesvirus-infected hypogammaglobulinaemic patients may have an increased incidence of latent infection and may conceivably be more prone to develop demyelinating neurological disorders (Kristensson et al., 1979).

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REFERENCES


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