Natural Killer Cells Are Not Required for Interferon-mediated Prophylaxis Against Vaccinia or Murine Cytomegalovirus Infections

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SUMMARY

Natural killer (NK) cell-depleted or control mice were treated prophylactically with polyinosinic:polycytidylic acid (polyI:polyC) or purified beta interferon (IFN) and then infected with either vaccinia virus or murine cytomegalovirus. NK cell depletion alone enhanced virus titres in the spleen and peritoneal cavity. However, poly I: polyC and IFN inhibited virus replication equally well in control and NK cell-depleted mice. This suggests that prophylactic IFN treatment mediates antiviral effects independently of NK cells.

Virus-induced interferon (IFN) stimulates the activation (Gidlund et al., 1978; Welsh, 1978) and proliferation (Biron et al., 1984) of natural killer (NK) cells which have been suggested to play a role in resistance to virus infection. Mice deficient in NK cell activity as a result of youth (Boos & Wheelock, 1971), immunosuppressive drugs (Osborn et al., 1968), genetic background (Bancroft et al., 1981), the beige mutation (Shellam et al., 1981), or treatment with antiserum to the ganglioside, asialo GM1 (Bukowski et al., 1983) are highly susceptible to murine cytomegalovirus (MCMV) infection. Adoptive transfer studies revealed that the protective cell population is asialo GM1⁺, NK 2.1⁺, Ly 5⁺, Thy-1⁻, Lyt-2⁻, Ia⁻, and is thus identifiable as NK cells (Bukowski et al., 1985). Treatment of mice with antiserum to asialo GM1 also lowers their resistance to vaccinia virus (VV) (Bukowski et al., 1983) suggesting a role for NK cells against this virus, as well.

Prophylactically administered IFN is capable of mediating an antiviral effect in many animal models (for review, see Stewart, 1979) but most studies have not distinguished between the possibilities that IFN mediates its antiviral effect through direct inhibition of virus replication or through stimulation of components of the host defence system, such as NK cells. In this study we show that NK cell depletion has no effect on the ability of prophylactically administered IFN to mediate antiviral effects against viruses known to be sensitive to NK cells.

Groups of four female C57BL/6j mice 6 to 12 weeks of age were either untreated or injected intraperitoneally (i.p.) with 20 μl of antiserum to asialo GM1 (Wako Chemical Corporation, Dallas, Tex., U.S.A.) and/or 100 μg of the IFN-inducing compound polyinosinic:polycytidylic acid (polyI:polyC; Sigma). Six h later they were challenged with 2 × 10⁴ p.f.u. of MCMV i.p. (Smith strain; Nedrud et al., 1979) and then sacrificed 3 days later for virus titrations and NK cell studies using a standard ⁵¹Cr release assay on YAC-1 target cells (Welsh, 1978). Treatment with polyI:polyC or infection with MCMV routinely resulted in a two- to threefold enhancement of spleen NK cell activity as compared with uninfected controls, as well as the induction of 512 to 2048 units of IFN/ml plasma. The results in Table 1 show that polyI:polyC had a modest three- to fivefold protective effect against MCMV replication, consistent with previously published results showing that polyI:polyC treatment decreased MCMV-induced mortality (Kern et al., 1977). Treatment with antiserum to asialo GM1 had a profound (> 60-fold) enhancing effect on MCMV replication, as expected (Bukowski et al., 1983). However,
Table 1. Prophylactic polyI:polyC inhibits MCMV replication in NK cell-depleted mice

<table>
<thead>
<tr>
<th>Treatment</th>
<th>None</th>
<th>Anti-asialo GM1</th>
<th>PolyI:polyC</th>
<th>Anti-asialo GM1 and polyI:polyC</th>
</tr>
</thead>
<tbody>
<tr>
<td>log_{10} P.f.u. MCMV/spleen</td>
<td>1.9 ± 0.2*</td>
<td>3.7 ± 0.1</td>
<td>&lt;1.3†</td>
<td>3.0 ± 0.1</td>
</tr>
<tr>
<td>Specific lysis (%) of YAC-1</td>
<td>22.0 ± 2.6</td>
<td>4.7 ± 2.5</td>
<td>33.0 ± 2.3</td>
<td>6.1 ± 1.7</td>
</tr>
<tr>
<td>targets by spleen cells‡</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Results are expressed as the arithmetic average of four samples titrated individually ± S.E.M.
† Two of the mice in this group had no detectable virus and thus were assigned a titre of <1.3 log_{10} p.f.u., the lowest level detectable in our assay. These numbers were averaged with the rest, and the result was designated 'less than (<)'.
‡ Effector to target ratio was 33:1; assay length was 6 h.

Table 2. Prophylactic polyI:polyC inhibits vaccinia virus replication in NK cell-depleted mice

<table>
<thead>
<tr>
<th>Treatment</th>
<th>None</th>
<th>Anti-asialo GM1</th>
<th>PolyI:polyC</th>
<th>PolyI:polyC and anti-asialo GM1</th>
</tr>
</thead>
<tbody>
<tr>
<td>log_{10} P.f.u. VV/spleen</td>
<td>4.6 ± 0.6*</td>
<td>6.2 ± 0.2</td>
<td>2.6 ± 0.2</td>
<td>2.8 ± 0.3</td>
</tr>
<tr>
<td>Specific lysis (%) of YAC-1</td>
<td>8.6 ± 0.8</td>
<td>1.9 ± 0.2</td>
<td>9.4 ± 0.6</td>
<td>2.5 ± 0.4</td>
</tr>
<tr>
<td>targets by spleen cells</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>log_{10} P.f.u. VV/ml peritoneal fluid</td>
<td>3.5 ± 0.7</td>
<td>5.5 ± 0.2</td>
<td>1.8 ± 0.3</td>
<td>1.7 ± 0.3</td>
</tr>
<tr>
<td>Specific lysis (%) of YAC-1</td>
<td>3.6 ± 1.2</td>
<td>3.4 ± 1.3</td>
<td>38.0 ± 0.9</td>
<td>17.0 ± 1.3</td>
</tr>
<tr>
<td>targets by peritoneal cells</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Results are reported as the arithmetic average of four samples titrated individually ± S.E.M.
† Effector to target ratio was 25:1 for spleen cells and 10:1 for peritoneal cells; assay length was 6 h.

NK cell depletion using antiserum to asialo GM1 failed to influence the antiviral effect of polyI:polyC. These data support the concept that prophylactic IFN can mediate anti-MCMV effects in the absence of detectable NK cell activity.

Since the antiviral effect of IFN against MCMV was only a modest one, we sought to employ another virus system whose sensitivity to IFN-mediated prophylaxis was greater. VV infection of mice is such a system (Baron et al., 1966), and 10^7 p.f.u. of VV (strain WR obtained from Dr Robert Singer, University of Massachusetts Medical School Worcester, Mass., U.S.A.) were used in the above protocol outlined for MCMV. Virus titres and NK cell activity were also assessed in the peritoneal wash-out fluid, since the injection site is the site of initial virus replication. Table 2 shows that antiserum to asialo GM1 enhanced VV titres in both the spleen and peritoneal fluid, as previously shown (Bukowski et al., 1983), while reducing NK cell activity to very low levels. PolyI:polyC and 10^5 units of purified beta interferon (IFN-β; Lee BioMolecular Research Laboratories, San Diego, Ca., U.S.A.) each elicited a dramatic protective effect (up to 6000-fold) against VV (Table 3), also shown previously (Baron et al., 1966). However, NK cell depletion had no effect on the ability of polyI:polyC (Table 2) or IFN-β (Table 3) to mediate antiviral effects against VV.
Table 3. Prophylactic IFN inhibits vaccinia virus replication in NK cell-depleted mice

<table>
<thead>
<tr>
<th>Treatment</th>
<th>log₁₀ P.f.u. VV/spleen</th>
<th>Specific lysis (% of YAC-1 by spleen cells†)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>4.8 ± 0.1*</td>
<td>37.0</td>
</tr>
<tr>
<td>Anti-asialo GM1 IFN</td>
<td>5.7 ± 0.2</td>
<td>4.3</td>
</tr>
<tr>
<td>IFN</td>
<td>2.2 ± 0.1</td>
<td>34.0</td>
</tr>
<tr>
<td>IFN and anti-asialo GM1</td>
<td>2.3 ± 0.2</td>
<td>6.4</td>
</tr>
</tbody>
</table>

* Results are expressed as the arithmetic average of four samples titrated individually ± S.E.M.
† Effector to target ratio was 100:1, assay length was 5 h. Spleen cells from four mice were pooled for each datum.

A wide body of evidence indicates that NK cells play a major role in resistance to MCMV (for review, see Welsh, 1986), and the fact that VV titres are higher in NK cell-depleted mice suggests that these cells also play a role in VV infection (Bukowski et al., 1983). These results showing that NK cells are not required for the prophylactic effect of IFN in these systems may seem surprising since IFN is capable of activating NK cells (Welsh, 1978; Gidlund et al., 1978) and inducing them to proliferate (Biron et al., 1984). However, activation of NK cells by prophylactic IFN may be superfluous to that which already occurs during a virus infection. The IFN produced locally at sites of virus infection may be more than enough to activate NK cells locally, and NK cells activated systemically by prophylactic treatment may not have a greater capability of accumulating at local sites of virus infection. NK cells respond to chemotactic agents produced locally at sites of infection (Natum & Welsh, 1987) and a large non-localized release of IFN may impair the movement of NK cells towards areas of virus-infected cells.

These results suggest that IFN acts by different mechanisms, such as direct inhibition of virus replication, or by activating another arm of the host defence system. In vitro studies revealed that VV is extremely resistant to the direct antiviral effects of IFN, and that systemic treatment with purified IFN must prevent VV-induced skin lesions by a mechanism other than that of direct antiviral effects, but the mechanism has not been characterized (Schellekens et al., 1979). Though direct evidence is lacking, macrophages are good candidates for this alternative mechanism, since they are activated by IFN (Schultz et al., 1977) and are suspected of inhibiting the spread of VV by failing to support active growth of the virus (Natum & Holowczak, 1985).

To summarize, NK cells do not appear to be required for the antiviral effect of prophylactically administered IFN in two viral systems thought to be sensitive to the antiviral effect of NK cells. The mechanisms responsible for the IFN-mediated effect remain to be elucidated.

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REFERENCES


Short communication


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