Short communication

Depletion of Mac1-positive macrophages protects DBA/2 mice from encephalomyocarditis virus-induced myocarditis and diabetes

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DBA/2 mice treated with anti-Mac1 monoclonal antibody (MAb) failed to develop encephalomyocarditis virus (EMCV)-induced diabetes and myocarditis. Virus concentrations and the number of viral RNA-positive cells in the pancreas and heart were significantly reduced in mice treated with anti-Mac1 MAb. Mac1-positive macrophages seem to be involved in EMCV-induced disease and to affect the replication of EMCV in target organs.

Encephalomyocarditis virus (EMCV) produces insulin-dependent diabetes (Craighead & McLean, 1968), myocarditis, encephalomyelitis (Craighead, 1966) and orchitis (Hirasawa et al., 1991) in mice of susceptible strains. Although many explanations of the pathogenesis of EMCV have been proposed, the pathogenesis is still not clear. It is assumed that immunopathogenic mechanisms are implicated in EMCV-induced disease. Several earlier reports have suggested that T lymphocytes may be involved in the development of EMCV-induced disease (Babu et al., 1986; Sriram et al., 1989). More recently, it was reported that the administration of anti-CD4 monoclonal antibody (MAb) prevented the development of EMCV-induced pancreatitis and parotitis in A/J mice (Barger & Craighead, 1991). In contrast, it has been shown that macrophages played a critical role in the development of diabetes in SJL/J mice infected with the D variant of EMCV (EMC-D) (Baek & Yoon, 1990). In addition, in our previous study, we observed prominent infiltration of Mac1-positive macrophages in the pancreatic islets of EMCV-infected DBA/2 and BALB/c mice (Hirasawa et al., 1996). To confirm the immunopathogenic mechanisms underlying EMCV infection, we examined the effect of depletion of Mac1- and CD4-positive cells on the development of EMCV-induced diabetes and myocarditis. We now report that depletion of macrophages by treatment with anti-Mac1 MAb results in the prevention of EMCV-induced diabetes and myocarditis and the inhibition of viral growth in the target organs.

MAbs M1/70 and GK1.5 (rat IgG2b), which recognize murine Mac1 and CD4 antigens, respectively, were prepared with hybridomas obtained from the American Type Culture Collection. Hybridomas were grown in tissue cultures with a serum-free medium (SF-O; Sanko Pure Chemicals). The supernatant was harvested and purified by ammonium sulphate precipitation. We used purified rat IgG (Cappel Research Products) as an irrelevant control IgG.

Eight-week-old male DBA/2 mice were infected intraperitoneally (i.p.) with 100 p.f.u./head of EMC-D (gifts from Ji-Won Yoon, University of Calgary, Alberta, Canada). The animals were treated i.p. with 1 mg of MAb or rat IgG 1 and 2 days before and after EMCV infection and observed for up to 4 days post infection (p.i.). By flow cytometry analysis, we confirmed that approximately 90% of Mac1+ and CD4+ cells were depleted from spleen.

Blood glucose level (BGL), creatine phosphokinase activity (CPK) and virus concentrations in the pancreas and heart were measured at 4 days p.i. BGL and CPK were measured colorimetrically, using Glucose C-test kit and CPK-test Wako (Wako Pure Chemical Industries), respectively. An infected mouse was regarded as having diabetes or myocarditis if the BGL or CPK, respectively, was more than 3 SD above the mean values of the controls. The virus concentration in the tissue was determined by plaque assay on L-929 cells as described previously (Matsuzaki et al., 1989).

Anti-Mac1 MAb treatment completely prevented diabetes (0%) and myocarditis (0%) in EMCV-infected
Fig. 1. Effects of anti-Mac1 MAb and anti-CD4 MAb on the development of (a) diabetes and (b) myocarditis in EMCV-infected DBA/2 mice. DBA/2 mice were treated with 1 mg of MAb or rat IgG on days 1 and 2 before and after EMCV infection (100 p.f.u./head; i.p. MAb). The shaded areas represent the mean BGL (a) or CPK (b) values ± 3 SD for uninfected control mice. Each circle represents an individual animal. The percentages above the parts indicate the proportion of animals in each treatment group with diabetes or myocarditis.

Table 1. Effects of anti-Mac1 and anti-CD4 MAb on BGL, CPK and virus titre

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Number of animals</th>
<th>BGL (mg/dl)</th>
<th>CPK (IU/ml)</th>
<th>Virus titre (log_{10} p.f.u./g)*</th>
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<tr>
<td></td>
<td></td>
<td>Pancreas</td>
<td>Heart</td>
<td></td>
</tr>
<tr>
<td>EMCV</td>
<td>20</td>
<td>515 ± 142</td>
<td>566 ± 353</td>
<td>6.3 ± 0.3</td>
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<tr>
<td>Mac1 + EMCV</td>
<td>10</td>
<td>185 ± 20</td>
<td>138 ± 28</td>
<td>4.9 ± 1.4</td>
</tr>
<tr>
<td>CD4+ EMCV</td>
<td>10</td>
<td>481 ± 138</td>
<td>397 ± 107</td>
<td>6.5 ± 0.4</td>
</tr>
<tr>
<td>Rat IgG + EMCV</td>
<td>5</td>
<td>425 ± 155</td>
<td>655 ± 266</td>
<td>ND</td>
</tr>
<tr>
<td>Control</td>
<td>20</td>
<td>201 ± 32</td>
<td>126 ± 23</td>
<td>ND</td>
</tr>
</tbody>
</table>

* Virus titres in the pancreas and hearts of ten mice in each group were determined. ND, Not determined.

mice, whereas almost all of the untreated and rat IgG-treated mice developed diabetes (80–90%) and all developed myocarditis (100%). In contrast, anti-CD4 MAb-treated mice developed diabetes (90%) and myocarditis (100%) (Fig. 1).

Virus concentrations in the pancreas and heart were significantly reduced in mice treated with anti-Mac1 MAb (P < 0.05; Table 1). In contrast, there were no significant differences in virus concentrations in the pancreas and heart between anti-CD4 MAb-treated and untreated mice.

Small pieces of the pancreas and heart obtained from each mouse were frozen with dry ice/ethanol for immunohistochemical staining and in situ hybridization. For immunohistochemical examination, cryosections (5 μm) were stained with anti-Mac1 MAb by the avidin–biotin–peroxidase complex (ABC) method, using the Vectastain Elite ABC kit (Vector Laboratories). Prominent infiltration of Mac1-positive macrophages was observed in and around the islets of untreated mice at 4 days p.i. (Fig. 2a, b). On the other hand, few macrophages appeared in the islets of anti-Mac1 MAb-treated mice. In the heart, infiltration of Mac1-positive macrophages was slight at 4 days p.i. (data not shown).

In situ hybridization was performed according to Hirota et al. (1992) with modifications for cryosections. Briefly, a 1000 bp cDNA fragment of the specific region of EMC-D (VP3–VP1) was subcloned into pBluescript (SK–) transcription vector by standard techniques. After the plasmids were linearized with the appropriate
restriction endonuclease, digoxigenin (DIG)-labelled antisense and sense RNA probes were prepared with T7 and T3 RNA polymerases, using a DIG RNA labelling kit (Boehringer-Mannheim).

Cryosections were fixed with 3% paraformaldehyde for 60 min, 0.2 m-HCl for 8 min, 0.25% acetic anhydride/0.1 m-triethanolamine–HCl pH 8.0 for 15 min and 2×SSC for 10 min. These sections were then prehybridized with 50% deionized formamide/2×SSC for 60 min. After pretreatment, the sections were hybridized for 18 h at 50 °C in the following solution (probe concentration: 2 μg/ml): 50% deionized formamide, 100 μg/ml yeast t-RNA, 10% dextran sulphate, 1×Denhardt’s solution, 0.05 M-Tris–HCl pH 7.5, 5 mM-EDTA and 0.6 M-NaCl. After hybridization, the sections were rinsed with 5×SSC and washed with 50%
formamide/2 x SSC for 30 min at 50 °C and incubated with RNase A buffer (2 μg/ml RNase A, 10 mM-Tris–HCl pH 7.5, 0.5 mM-NaCl) for 30 min at 37 °C. The sections were then washed twice in 2 x SSC and 0.2 x SSC (30 min with each) at 37 °C.

For colorimetric detection after hybridization, the sections were treated with 1% blocking buffer (Boehringer-Mannheim) in DIG buffer 1 (0.1 M-Tris–HCl pH 7.5, 0.15 M-NaCl) for 30 min and then incubated with alkaline phosphatase-conjugated anti-DIG antibody (1:500) (Boehringer-Mannheim) overnight. The next day, sections were incubated with NBT/BCIP and counterstained with haematoxylin.

Viral RNA was observed in a large number of the pancreatic islet cells in untreated mice, whereas it was found only in a small number of islet cells in anti-Mac1 MAb-treated mice. In addition, the number of EMCV RNA-positive myocardial cells was greater in untreated mice than in anti-Mac1 MAb-treated mice (Fig. 2c–f).

On the other hand, the infiltration of Mac1-positive macrophages and the number of EMCV RNA-positive cells in the pancreatic islets and hearts of anti-CD4 MAb-treated mice were both similar to the findings in untreated mice (data not shown).

It is well known that the cellular receptors for some picornaviruses belong to the immunoglobulin superfamily, such as ICAM-1 for rhinoviruses (Tomassini et al., 1989), VLA-2 for echovirus (Bergelson et al., 1992) and poliovirus cell surface receptor (PVR) for poliovirus (Racaniello, 1990). Recently, it has been reported that VCAM-1 acts as a receptor for EMCV on vascular endothelial cells (Huber, 1994). As the expression of VCAM-1 is induced by several cytokines [tumour necrosis factor-α (TNF-α), interleukins-1 and -4 and interferon-γ] (Prober & Cotran, 1991; Thornhill et al., 1991), it is suggested that the cytokines derived from macrophages induce or upregulate EMCV receptors in EMCV-infected DBA/2 mice. In this regard, we reported that exogenously administered TNF-α enhanced EMCV-induced myocarditis in DBA/2 mice (Yamada et al., 1993). We recently found TNF-α mRNA expression in the islets of EMCV-infected DBA/2 mice by in situ hybridization (K. Hirasawa and others, unpublished results). A study of the production of cytokines derived from macrophages at the early stage of EMCV infection is now under investigation.

Baal & Yoon (1990) reported that activated macrophages were cytotoxic to β cells isolated from SJL mice infected with EMCV-D. In our experiment, prominent infiltration of Mac1-positive macrophages was observed in the pancreatic islets of DBA/2 mice infected with EMCV. Two of the 10 anti-Mac1 MAb-treated mice which exhibited almost the same levels of virus (1 x 10^6−8 and 1 x 10^6−8 PFU/g) as those of untreated mice (1 x 10^6−8), did not develop diabetes. These results suggested that macrophages affected the development of EMCV-induced diabetes by two different mechanisms, via influence on viral growth and via cytotoxicity to pancreatic β cells. The infiltration of Mac1-positive macrophages and other immunocytes was slight in the hearts at 4 days p.i. This finding suggests that the higher CPK activity in EMCV-infected mice at the early stage of EMCV infection was induced by direct destructive effects of virus on myocardial cells, which were prevented by anti-Mac1 MAb treatment.

In conclusion, anti-Mac1 MAb treatment inhibited EMCV replication in the target organs and protected EMCV-infected mice from diabetes and myocarditis. This finding indicates that macrophages influence virus replication in the pancreas and heart, thus affecting the pathogenicity of EMCV infection.

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References


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