Effects of Interleukin-2 on Active Hepatitis in Athymic Nude Mice due to Low-Virulence Mouse Hepatitis Virus

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ABSTRACT. The effects of a recombinant interleukin-2 (rIL-2) on active hepatitis due to low virulence mouse hepatitis virus, MHV-2-CC, in athymic nude mice, were studied. Athymic nude mice were inoculated intraperitoneally with 6 × 10⁵ PFU of the virus and given daily with 0.1, 1 and 10 μg of rIL-2. The nude mice treated with 1 μg of rIL-2 showed virus titers 1 log lower than those of untreated mice, through Days 7 to 21 of postinoculation. Liver lesions in mice treated with 1 or 10 μg of rIL-2 were characterized by multiple round and circumscribed necrotizing foci without inflammatory cells, whereas inflammatory reactions were noticeable in necrotizing lesions of non-treated controls. At the periphery of the lesions of rIL-2 treated mice there were many degenerated hepatocytes being positive for viral antigen surrounded by mononuclear cells, some of which were positive with asialo-GM1.—KEY WORDS: hepatitis, MHV, nude mouse, rIL-2.

Interleukin-2 (IL-2) is known to have various immunoregulatory activities including T-lymphocyte stimulation and it has been expected to have therapeutic effects on infectious disease in man and animals. Recently, the human IL-2 gene was cloned and expressed in Escherichia coli resulting in production of a recombinant IL-2 (rIL-2), which was biologically as active as the native IL-2 [13]. The rIL-2 was shown to be effective on either malignant tumors [21] or infectious diseases [2, 12, 23] in laboratory animals.

On the other hand, the rIL-2 and IL-2 were revealed to restore some immune functions in congenitally athymic nude mice [18, 22], augmenting the natural killer (NK) cells as well as cytotoxic T-lymphocytes [6]. The IL-2 and rIL-2 were shown to have also a restoring effect on cellular immunity which might be depressed in herpetic diseases of animals [12, 14].

The present study was designed to see the effects of an rIL-2 on subacute or chronic active hepatitis in athymic nude mice [3, 4] due to a low-virulence mouse hepatitis virus, MHV-2-CC [8].

MATERIALS AND METHODS

Mice: Athymic nude mice 8 to 12 weeks of age having background of BALB/c, were bred at this laboratory in a barrier system. Mice were kept in plastic cages with a filter cap and given autoclaved commercial pellets CE-1 (Japan CLEA, Tokyo) and water.

Virus: Mice were inoculated intraperitoneally (i.p.) with 6 × 10⁵ PFU of MHV-2-CC [8]. Virus titers were determined with DBT cells as described earlier [7].

rIL-2: rIL-2 was provided from the Central Research Division, Takeda Chemical Industries (Osaka, Japan). A 1mg/ml solution of rIL-2 in phosphate buffered saline, pH 7.2, containing 5% of normal mouse serum, was prepared and stored at −20°C. One to 21 days after MHV inoculation mice were received i.p. with daily dose of 0.1, 1
Table 1. Effects of rIL-2 on mortality and distribution of viral antigen in athymic mice

<table>
<thead>
<tr>
<th>rIL-2 (µg/day)</th>
<th>Died/ Tested³</th>
<th>Virus titer (PFU/0.2 g)</th>
<th>Viral antigen</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
<td>1/12</td>
<td>1.0×10⁵</td>
<td>++ (Diffuse &amp; Localized)</td>
</tr>
<tr>
<td>1</td>
<td>0/12</td>
<td>4.2×10⁴</td>
<td>+ (Localized)</td>
</tr>
<tr>
<td>10</td>
<td>3/15</td>
<td>8.0×10⁵</td>
<td>++ (Localized)</td>
</tr>
<tr>
<td>Non-treated</td>
<td>0/ 5</td>
<td>6.4×10⁵</td>
<td>++ (Diffuse)</td>
</tr>
</tbody>
</table>

a) on Day 14 p.i. with MHV-2-CC (6×10⁵ PFU).

or 10 µg of rIL-2.

**Immunofluorescence:** Liver tissues were sampled on days 7, 14 and 21, fixed in 95% ethanol, dehydrated at 4°C and embedded in paraffin at 58°C [15]. For MHV antigen, sections were treated first with anti-MHV-2 rabbit serum [9] and then with fluorescein-isothiocyanate (FITC) conjugated goat anti-serum to rabbit IgG (Cappel, CA, USA). For the surface antigen of lymphocytes, sections were treated with rabbit or mouse monoclonal antibodies to asialo-GMI (Wako Pure Chemical, Osaka), Thy 1, 2 and Lyt 2, 2 (Dainihon Seiyaku Laboratory, Osaka) and then with FITC-conjugated goat IgG to rabbit IgG or mouse IgG (light & heavy chain, Cappel, CA, USA).

**Histopathology:** The livers of mice sacrificed on Days 7, 14 and 21 were fixed in neutral buffered formalin and embedded in paraffin. Sections were stained with hematoxylin and eosin (HE) or Masson’s trichrome. For electron microscopy small blocks of the liver from infected mice were fixed in 5% glutaraldehyde and post fixed in 1% osmium tetroxide. After dehydrating by an ascending ethanol series, samples were immersed in propylene oxide and embedded in an epoxy resin, Epok 812 (Oken, Tokyo). Ultrathin sections were stained with uranyl acetate and lead nitrate and examined by an electron microscope JEM 100CXII with accelerated voltage at 80kv.

**RESULTS**

One and 3 out of 15 nude mice, which had received daily doses of 0.1 to 10 µg rIL-2, respectively, died within Day 14 postinoculation (p. i.) and all treated with a daily dose of 1 µg survived (Table 1).

In the livers of the 10 µg group as well as non-treated one, virus titers reached to 10⁶ PFU/0.2g on Days 7 to 21 p.i. (Fig. 1). In the 1 µg-group, however, virus titers were 1 log lower than those of the non-treated control. The 0.1 µg-group showed virus titers between the two groups.

In the 0.1 µg-group and non-treated controls the liver showed a number of necrotic foci diffusely extending into the surrounding parenchyma. However, in the liver of 1 µg- and 10 µg-groups of mice killed on Day 14 or 21, multiple round and circumscribed necrotizing foci were produced. The lesions varied in size and some of them were confluent to each other. No hepatocytes remained within these lesions.
and eosinophilic reticular networks were formed (Fig. 2a). At the periphery of the lesions there were many infiltrating lymphoid cells located closely to eosinophilic or hyalinized hepatocytes (Fig. 3a). Some of these lymphoid cells appeared to be large granular lymphocytes (Fig. 4) by electron microscopy and invaded into degenerated hepatocytes (Fig. 5). Mitosis was frequent in the hepatocytes around the lesions.

Infiltrating lymphoid cells contained some asialo-GMI positive cells and a very small number of Thy 1.2 positive ones, whereas no Lyt 2.2-positive cells were detected. The type of other lymphoid cells could not be identified. There were a small number of macrophages with a few fibroblasts among those lymphoid cells. Neutrophils and some lymphoid cells accumulated in and around the hepatic inflammatory foci of non-treated control groups.

Viral antigen was detected within the cytoplasm of hyalinized hepatocytes at the periphery of the lesion (Fig. 6). Number of virions were observed in the cytoplasm of degenerated hepatocytes as well as intercellular spaces by electron microscopy.

**DISCUSSION**

The therapeutic effects of rIL-2 have been shown in various infectious diseases. In the lethal acute bacterial infections, increase in survival rate or induction of complete recovery was induced by rIL-2 using various dose and route of administration [2]. In the experimental toxoplasmosis rIL-2 was reported to induce lower mortality rate after challenge infection [16]. In the present study 1 μg daily dose of rIL-2 suppressed the viral growth in the liver of athymic mice. However, virus titers of the liver from 10 μg and 0.1 μg-groups, were rather higher than those of the 1 μg-group, though infection was not fatal. In the case of herpes simplex virus infection, a large dose of rIL-2 enhanced infection in guinea pigs, while smaller doses showed suppressive effect [23]. The affected liver of athymic mice infected with low virulence MHV was characterized by eosinophilic and necrotic changes with inflammatory cells, followed by fibrosis [4, 20]. Within the necrotic foci there remained some degenerated or necrotic hepatocyte. In those treated with rIL-2, however, necrotic foci appeared more distinct containing no degenerated and surviving parenchymatous cells. This suggested that cytotoxic activity of lymphocyte might be enhanced by rIL-2 treatment.

These histopathological findings showed that rIL-2 prevented the enlargement of necrotic foci. In 10 μg-group, which showed characteristic hepatic lesions, cytotoxic or cytolytic effects of lymphocytes were so active that infected hepatocytes were degenerated rapidly. Higher virus titer of this experimental group might be due to the fact that virions replicated increasingly together with the progress of hepatocyte degeneration (unpublished data).

The rIL-2 treatment might have enhanced the natural killer (NK) cell activity and generated cytotoxic T-lymphocytes in the course of infectious diseases [6]. Present study showed that the accumulation of lymphocytes was prominent in the hepatic lesions of rIL-2 treated mice. Some of these infiltrating cells were found to be large granular lymphocytes [11] by electron microscopy. And also asialo-GMI positive cells were present. This suggested that NK cells participated in these hepatic lesions of rIL-2 treated mice. The activation of NK cells has been well documented in the defense mechanisms in viral infection [1, 11]. The NK cells, which were shown to be more active in nude mice [19], might be active in the present MHV-2-CC infection not only as effector cells [1] but also as activators for gamma interferon [5, 10] or antibody-dependent cytotoxicity [17].
Fig. 2. Circumscribed inflammatory foci in the liver of infected nude mice treated with rIL-2 (10 μg/day) (a). Diffuse and confluent lesions in the liver of a mouse non-treated (b). ×25 HE stain.

Fig. 3. Higher magnification of Fig. 2 a and b, showing degenerated hepatocytes closely contacted with lymphoid cells in the rIL-2 treated case (a). Many neutrophils and some lymphoid cells accumulated in and around the inflammatory foci (b). ×320.
Fig. 4. Electron micrograph of a lymphoid cell appeared to be a large granular lymphocyte around the inflammatory foci of 10 μg-group. ×7,600.

Fig. 5. Several lymphoid cells closely contacting with or possibly invading into degenerated hepatocytes (arrows) of 10 μg-group. Epoxy resin embedded semi-thin section stained with toluidine blue. ×390.

Fig. 6. MHV antigen within a limited number of hepatocytes at the periphery of inflammatory foci of a case of 10 μg-group (a), and more abundant antigen within hepatocytes of non-treated control (b). ×320.
These findings suggested that the suitable dose of rIL-2 was effective for suppressing the progress of hepatic lesions in the present experimental viral infection.

REFERENCES


弱毒マウス肝炎ウイルス MHV-2·CC 株による胸腺欠損ヌードマウスの活動性肝炎に対する組換え型インターロイキン 2 の効果: 後藤直彰・土居和久1)・井上 武2)・村井洋子・藤原公策（東京大学農学部家畜病理学教室、1)山口大学農学部家畜病理学教室、2)家畜衛生学教室）—弱毒マウス肝炎ウイルス MHV-2·CC 株による胸腺欠損ヌードマウスの活動性肝炎に対する組換え型インターロイキン 2 (rIL-2) の効果について検討した。ヌードマウスに MHV-2·CC, 6 ×10⁵PFU を腹腔内接種後, rIL-2, 0.1, 1, 10μg を毎日腹腔内に投与した。接種後 7-21日に 1μg 投与マウスの肝臓のウイルス価は rIL-2 無投与対照マウスに比べて 1/10であった。rIL-2 投与マウスに特微的な病変は 1 および10μg 投与群にみられ、肝臓の多発性、円形、境界不明瞭の壊死病変で、病変内に肝細胞の残存および炎症細胞の集簇はみられなかった。病変周縁には多数の変性に陥ったウイルス抗原陽性の肝細胞があり、多数の単核細胞がとりまっていた。これら単核細胞中には asiato-GM1 抗原陽性のものも認められた。