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Involvement of Macrophages in the Development of Encephalomyocarditis (EMC) Virus-Induced Diabetes in Mice

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Abstract: The role of macrophages in the development of diabetes following infection with encephalomyocarditis (EMC) virus was examined in 3 strains of mice (DBA/2 and BALB/c: susceptible, C57BL/6: resistant). After infection with 100 plaque forming units (PFU) / head of EMC-D (highly diabetogenic variant), the incidence of diabetes at 3 days post infection (DPI) (DBA/2: 7/8, BALB/c: 3/8, C57BL/6: 0/8) was well correlated with the severity of macrophage infiltration with β cell damage in the pancreatic islets (DBA/2: severe, BALB/c: moderate, C57BL/6: slight). Silica-pretreatment depleted macrophage infiltration in the pancreatic islets and decreased the incidence of diabetes at 7 DPI from 100% to 40% in DBA/2 and from 80% to 0% in BALB/c mice, respectively. These results suggest that macrophages play a critical role in the process of pancreatic β cell damage in EMC virus infection in mice.

Key words: diabetes, EMC virus, macrophage, mouse

Encephalomyocarditis (EMC) virus is a cardiovirus which belongs to the family Picornaviridae. EMC virus has been attracting a great attention as a useful tool for the investigation of viral diabetes since Craighead and McLane [2] showed that the M variant of EMC virus (EMC-M) isolated from pigs with myocarditis could produce diabetes mellitus-like syndrome in mice of particular strains. Today the highly diabetogenic variant (EMC-D) which was established by repeated plaque-purification of EMC-M [4] is widely used.

As to the pathogenesis of EMC virus-induced diabe-

tes in mice, we recently found in a preliminary study that macrophages were involved in the development of diabetes in BALB/c mice infected with a low dose (100 plaque forming units (PFU) /head) of EMC-D. The purpose of this study is to confirm this phenomenon using 3 strains of mice (DBA/2 and BALB/c: susceptible to EMC-D-induced diabetes; C57BL/6: resistant to EMC-D-induced diabetes).

First, blood glucose levels (BGLs) and the histopathology of pancreatic islets were examined. Twenty-four 8-week-old mice of each strain were infected intraperitoneally (i.p.) with 100 PFU/head of

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EMC-D, and 8 mice of each strain were killed at 1, 2, and 3 days post infection (DPI), respectively. Five mice of each strain inoculated with 0.1 ml of phosphate buffered saline (PBS) were served as controls and killed at 3 DPI.

BGLs were measured colorimetrically with a Glucose C-test kit (Wako Pure Chemical Industries Inc., Tokyo), and an infected mouse was scored as diabetes if the BGL was more than 3 standard deviations above the mean for controls. At 3 DPI, 3 of 8 BALB/c mice and 7 of 8 DBA/2 mice developed diabetes while none

of C57BL/6 mice did.

Small pieces of the pancreas obtained from each mouse at necropsy were frozen with dry ice-ethanol. Cryosections were fixed with acetone (for the detection of macrophages, CD4 and CD8-positive T cells) or with 4% paraformaldehyde/PBS (for the detection of β cells), and then they were stained immunohistochemically [3] with anti-Mac1, anti-L3T4 and anti-Lyt2 monoclonal antibodies (Boehringer Mannheim Yamanouchi, Tokyo) or anti-insulin polyclonal antibody (Funakoshi Co., Tokyo) as the first antibody, respectively.

Fig. 1. Cryosections of pancreas of EMC-D-infected mice stained with anti-Mac1 mAb. Pancreatic islet of DBA/2 mouse infected with EMC-D at 1(a), 2(b) and 3 DPI (c), of BALB/c mouse at 3 DPI (d) and of C57BL/6 mouse at 3 DPI (e), $\times 240$.

In DBA/2 mice, a few mononuclear cells appeared around and/or within the pancreatic islets at 1 DPI. After that, infiltration of mononuclear cells within the islets with β cell-destruction became prominent. Almost all of these mononuclear cells were Mac1-positive macrophages (Fig. 1 a, b, c), and neither CD4 nor CD8-positive T cells were detected (data not shown).

The nature and sequence of such pancreatic islet changes were common to mice of 3 strains, but the severity was marked in DBA/2 mice, moderate in BALB/c mice and slight in C57BL/6 mice (Fig. 1 c, d, e). Such difference in the severity of pancreatic islet change was well correlated with that in the incidence of diabetes.

Second, the effect of silica-pretreatment on the development of EMC-D-induced diabetes was examined in DBA/2 and BALB/c mice to confirm the role of macrophages. Long-term pretreatment with silica is known to deplete macrophages [1]. Ten 5-week-old mice of each strain were injected i.p. with 100 mg/kg B.W. of silica (Sigma Chemical Co., St. Louis, USA)

three times per week for 3 weeks from 5 to 8 weeks of age. They were then infected with 100 PFU/head of EMC-D at 8 weeks of age and killed at 7 DPI. Ten 8-week-old mice of each strain infected with 100 PFU/head of EMC-D without silica-pretreatment and 10 8-week-old mice of each strain inoculated with PBS alone were also killed in the same way. The measurement of BGLs and immunohistochemical examinations were done at 7 DPI.

As shown in Table 1, the incidence of diabetes was significantly decreased by silica-pretreatment. Namely, the incidence in DBA/2 mice was decreased from 100% to 40% and that in BALB/c mice from 80% to 0%. In addition, mononuclear cell infiltration and destruction of β cells were markedly depressed in the silica-pretreated mice as compared with those in the silica-unpretreated mice (Fig. 2). In addition, the number of anti-insulin-positive β cells was apparently larger in the silica-pretreated mice than in the silica-unpretreated ones (Fig. 3). There was no significant difference in the virus concentration of pancreas be-

Table 1. Effect of silica-pretreatment on the development of diabetes in EMC virus-infected DBA/2 and BALB/c mice. Blood glucose levels (BGLs) were measured at 7DPI.

	DBA/2		BALB/c	
	BGL (mg/dl)	Diabetes (%)	BGL (mg/dl)	Diabetes (%)
Silica+EMC	419 $\pm$ 180	40	162 $\pm$ 21	0
EMC	671 $\pm$ 96	100	576 $\pm$ 184	80
Control	186 $\pm$ 25		163 $\pm$ 13

Fig. 2. Pancreatic islet of a silica-pretreated mouse (a) and a silica-unpretreated mouse (b) infected with EMC-D at 7 DPI. Insulitis was markedly depressed in the silica-pretreated mouse, $\times 400$.

Fig. 3. Cryosections of pancreas of a silica-pretreated mouse (a) and a silica-unpretreated mouse (b) infected with EMC-D at 7 DPI stained with anti-insulin polyclonal antibody, $\times 400$.

tween the silica-pretreated mice and the untreated mice (data not shown).

The present study suggests that Mac1-positive macrophages may play a critical role in the development of EMC-D-induced diabetes. Further investigations are now under way to clarify how macrophages can induce β cell damage in mice infected with a low dose of EMC-D.

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