reptilian endocrine pancreas appears to be even more complex with regard to peptide localizations than the islets of mammals. Thus, the elucidation of the functional significance of different pancreatic hormones occurring in the same cells is an exciting challenge for the future.

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Encephalomyocarditis (EMC) virus-induced diabetes mellitus prevented by *Corynebacterium parvum* in mice

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Summary. Corynebacterium parvum prevented the development of encephalomyocarditis virus-induced diabetes in mice, when it was given 3–14 days before the virus infection. This treatment inhibited virus replication in the pancreas of the infected mice at an early stage of the infection.
Key words. EMC virus; *Corynebacterium parvum;* diabetes mellitus; immunity.

Accumulating evidence suggests that some cases of insulin-dependent diabetes mellitus (IDDM) are triggered by viral infection, acting either alone or in concert, with an autoimmune response[1,2]. Viruses have been isolated from patients with IDDM and these viruses are diabetogenic in experimental animals[3,4]. Therefore, it is of interest to investigate mechanisms which may afford protection against the viral infection.

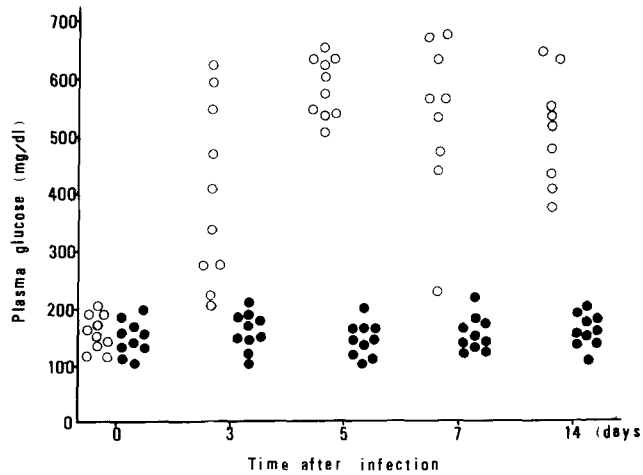
Encephalomyocarditis (EMC) virus strain D causes diabetes in certain strains of mice and is an excellent model for studying IDDM in humans[5,6]. We report here that *Corynebacterium parvum* (CP) provided complete protection for EMC-D virus infected mice and diabetes did not occur.

Materials and methods. EMC virus strain D was kindly provided by Dr A. L. Notkins, NIH, USA, and was propagated and titrated on a secondary culture of CD-1 mouse embryonic cells. Five hundred plaque forming units (p.f.u.) of the virus were inoculated intraperitoneally (i.p.) into each mouse on day 0. Six to eight-week-old male DBA/2 mice were purchased from Charles River Inc, Kanagawa, Japan and were fed food and water ad libitum, in an SPF environment. Plasma glucose levels were determined by glucose oxidase methods. Blood samples were obtained by retroorbital plexus puncture from non-fasted mice on 0, 3, 5, 7, 14 days after virus challenge. *Corynebacterium parvum* (CP) was purchased from Institut Merieux, Lyon, France. Each preparation, containing 4 mg of heat-inactivated dried CP (Lot No. 151) and 400 µg of formaldehyde was dissolved in 2 ml of phosphate buffered saline (PBS) and used throughout the study. For solvent controls, 400 µg of formalde-

Table 1. Protective effect of CP administered at various times before and after infection against EMC virus-induced DM

CP treatment (days)	Incidence of DM 5 days after infection
−42	4/4 (100%)
−28	3/6 (50%)
−21	5/6 (83%)
−14	0/7 (0%)
−7	0/7 (0%)
−5	0/7 (0%)
−3	0/7 (0%)
−1	3/6 (50%)
0	6/6 (100%)
+1	5/6 (83%)

Any mouse with a glucose level exceeding 250 mg/dl was defined as diabetic.



Protection against EMC-D virus-induced diabetes by *Corynebacterium parvum* (CP). One half milligram of CP was given to each mouse. Seven days after this treatment, the mice were infected with 500 p.f.u. of EMC-D virus. ○, control infected; ●, CP-pretreated and infected.

Table 2. Plasma glucose levels, virus titers and insulin contents of the infected pancreas in CP-pretreated and non-treated mice

Days after infection	Plasma glucose (mg/dl) (mean ± SEM)			IRI contents (× 10^3 μU/pancreas) (mean ± SEM)			Virus titers in the pancreas (p.f.u./g tissue) (mean)	
	C* (n = 6)	N* (n = 10)	CP* (n = 10)	C* (n = 6)	N* (n = 6)	CP* (n = 6)	N* (n = 3)	CP* (n = 3)
0	155 ± 12	152 ± 12	122 ± 5	107 ± 3.3	NT	NT	NT	NT
3	173 ± 15	576 ± 62	128 ± 8	NT	7.3 ± 3.3	60.0 ± 11.0	6.0×10^6	1.0×10^3
5	168 ± 6	553 ± 32	159 ± 7	NT	1.6 ± 0.3	62.4 ± 3.0	5.5×10^5	4.5×10^5
7	182 ± 8	575 ± 22	147 ± 11	NT	2.3 ± 3.8	59.3 ± 13.5	4.2×10^5	1.0×10^3

C*, Non-treated an non-infected; N*, Non-CP-treated and infected; CP*, CP-treated and infected; NT, Not tested.

hyde was dissolved in 2 ml of PBS. Control mice were given 0.25 ml i.p. of the solvent solution 7 days before the infection. One half milligram of CP in 0.25 ml solution was given to each mouse i.p. on the indicated days.

To determine the virus titers in the pancreas, the mice were killed and the glands aseptically removed and frozen at −80 °C with Dulbecco's modified Eagle's media (DMEM) containing 2% fetal calf serum (FCS), 300 μg/ml of gentamicin, and 5 μg/ml of amphotericin B, until virus titration. Organs were homogenized with 5 ml of DMEM and sonicated at 5 MC for 15 s. Following centrifugation at 3000 rpm for 30 min, the supernatants were assayed for virus titer by the ordinary plaque titration method. Insulin was extracted from the pancreas by acid-ethanol extraction and the concentrations measured by radioimmunoassay, as described[7].

Results. As shown in the figure, elevation of plasma glucose levels was observed as early as the 3rd day after infection and a plateau was reached at 5 days after infection in the infected control mice given solvent solution 7 days before the infection. In contrast, no such elevation in plasma glucose occurred in the CP-pretreated mice, throughout the study. Pretreatment of mice with CP 7 days before the infection completely inhibited the development of EMC-D virus-induced diabetes. One half milligram of CP was administered i.p. at 42, 28, 21, 14, 7, 5, 3, 1, 0, days before and 1 day after infection. As shown in table 1, the CP-mediated protection against EMC-D virus induced diabetes was evident when CP was administered between 3 and 14 days before the infection. CP was ineffective when administered either earlier than 21 days or later than one day before infection. When CP was given in a dose of over 0.1 mg/mouse 7 days before the virus challenge, the recipient mice did not develop diabetes (data not shown). Plasma glucose levels, virus titers and insulin contents of the infected pancreas in CP-pretreated and non-treated mice were determined. As shown in table 2, in the control infected mice, virus titers were significantly higher and IRI contents of the pancreas were greatly reduced at 3 days after infection, along with the elevation of plasma glucose levels. In contrast, in the CP-pretreated mice, virus growth was substantially inhibited and IRI contents of the pancreas were well preserved, thus inhibiting elevation of the plasma glucose levels. All this evidence suggests that CP-pretreatment prevents development of diabetes by reducing the growth potential of EMC-D virus and thus diminishing B cell damage in the pancreas.

Discussion. We found that *Corynebacterium parvum* prevented EMC-D virus-induced diabetes when given 3–14 days before the infection. Since CP was not effective when administered one day after the infection, CP is not likely to be directly toxic or inhibitory to the virus. Virus titers in the pancreas from the CP-pretreated mice were significantly inhibited over those in the control infected mice at 3 days after the infection, thereby suggesting that inhibitory mechanisms against virus replication are operative, at an early stage of infection.

The mechanisms involved in the anti-viral activity of CP were not clarified. CP is known to activate macrophages by both direct[8,9] and immunologically mediated[8–10] pathways, followed by an increased resistance to microbial infection[11–13]. It has also been reported that CP enhances the production of interferon, in vitro[14,15] and pretreatment of mice with CP greatly increases the responses by interferon inducers[16,17]. Therefore, these non-specific protective mechanisms are likely to be involved in the protection observed, operating alone or in combination, in our animal model. In case of EMC-D virus-induced diabetes, Yoon et al. reported the low interferon-inducing activity of EMC-D virus[5] and also the significant effect of interferon inducers against EMC-D virus-induced diabetes[6]. Studies to elucidate the protective mechanisms against the EMC-D virus-induced diabetes seen with CP are currently under way.

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