Protection Against Herpes Simplex Virus Infection in Mice by *Corynebacterium parvum*

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*Corynebacterium parvum* administered in mice prior to herpes simplex virus (HSV) infection significantly protected them against lethal encephalitis. This was seen both with a mouse strain highly susceptible to HSV and with one relatively resistant to HSV. Mice immunosuppressed by cyclophosphamide and showing an increased mortality after HSV infection were also protected by *C. parvum* pretreatment. However, *C. parvum* given simultaneously with or after HSV infection did not exert a therapeutic effect.

Infections with herpes simplex virus (HSV) are ubiquitous in humans and usually are locally restricted. However, they may generalize in immuno-incompetent newborns and in immunodeficient or immunosuppressed patients (for review, see references 8 and 12). HSV is a common cause of fatal, sporadic encephalitis (2). HSV is also suspected to be an oncogenic agent, particularly in the development of cervical carcinoma (9). A satisfactory therapy for HSV infection does not exist, and attempts to specifically immunize against HSV have not been encouraging in terms of effectiveness (4). Therefore, it appears useful to screen nonspecific stimulants of the immune system for their antiviral activity. A mouse model of HSV-induced encephalitis has been previously used to study the effect of immunostimulants on HSV infection (11). In the present investigation we have tested killed *Corynebacterium parvum*, an adjuvant that has received great interest because of its antitumoral effects in animal systems but has been little studied for antiviral protection (10).

**MATERIALS AND METHODS**

Inbred STU mice, originally obtained in 1961 from W. Schäfer, Tübingen, Federal Republic of Germany, were bred in our department by continuous brother-sister mating. C57BL/6J BOM (B6) mice were obtained from Gt. Bommelgardt, Ltd., Denmark. The weight of all mice was between 20 and 25 g, and they were 8 to 12 weeks of age when used in the experiments. HSV-1 (strain WAL) (7) has been adapted to mice by intracerebral passage in STU mice, and brain passage 35 was used in this study. Infected brains were homogenized in saline to obtain a 10% solution and stored at −70°C. This suspension was regarded as a 10−1 dilution in the titrations. Immediately before injection, the suspension was centrifuged to remove cellular material. The titer of the supernatant was 3.5 × 10^6 plaque-forming units (PFU)/ml when assayed in cultures of primary mouse embryo fibroblasts. The 50% lethal dose of HSV-1 in STU mice after intraperitoneal (i.p.) infection, as determined by the Spearman-Karber method, was 2 × 10^3 PFU, whereas it was 5 × 10^4 PFU in B6 mice. Formalin-killed *C. parvum* (CN 6134, Ba 3935/A; kindly provided by M. T. Scott, Department of Experimental Immunobiology, The Wellcome Research Laboratories, Beckenham, Kent, England) was injected i.p. at various times prior to and after HSV-1 infection.

**RESULTS**

In initial experiments, we have found that maximal protection occurred when *C. parvum* was given 6 to 8 days before HSV-1 infection and protection could be demonstrated with HSV-1 doses up to 100 50% lethal doses in STU mice. No protection was seen when *C. parvum* was given on the day of viral infection or when it was tested therapeutically after infection. In experiment A of Table 1 the effect of 210 μg of *C. parvum* given 7 days prior to the injection of 10^5 PFU of HSV-1 in STU mice is shown. Whereas 19 of 20 mice injected with virus alone died after 5 to 10 days with clinical signs of encephalitis, only 2 of 20 mice died in the group pretreated with *C. parvum*. Similar results were obtained in experiment B of Table 1. In both experiments the differences between experimental and control groups were significant at the *P* < 0.001 level. B6 mice, in accordance with previous data of others (6), were found to be considerably more resistant to i.p. infection with HSV-1 than were STU mice. When B6 mice were injected with 10^6 PFU of HSV-1, 9 of 19 mice died (experiment C, Table 1). In the experimental group pretreated with 210 μg of *C. parvum* 7 days earlier, only 1 of 25 mice died...
(difference significant at the $P < 0.005$ level). Mice surviving viral infection after pretreatment with C. parvum were found to be immune to a second viral challenge (data not shown). Treatment of B6 mice with 6 mg of cyclophosphamide (CY) on the day of HSV infection decreased the 50% lethal dose about 100-fold (Table 2). Mice pretreated with C. parvum 7 days previously were resistant to HSV given together with CY.

**DISCUSSION**

Systemic administration of C. parvum has been shown to have a variety of biological effects, including an adjuvant effect on humoral immunity, a stimulation of antitumor cellular immunity, and induction of increased antibacterial resistance (for review, see references 3 and 10). There have been little data on the effects of C. parvum on viral infections. Halpern and co-workers (3) have reported protective effects of C. parvum on Mengo virus infection in mice. In our experiments, we have shown a striking protective effect of C. parvum on HSV-1-induced encephalitis in two strains of mice, one that is highly susceptible to i.p. infection and one that is relatively resistant. In view of the severe complications of HSV infections seen in immunosuppressed patients (8), we believe that our demonstration of a protective effect of C. parvum on HSV infection of CY-treated mice is particularly noteworthy. The mechanisms by which C. parvum exerts its antiviral effect are still to be identified. However, in view of the large body of evidence showing that much of the antitumor effect of C. parvum is mediated through general activation of macrophages (10), it is reasonable to speculate that the antiviral effect is mediated by a similar mechanism. In many animal models a role of the macrophage in antiviral defense has been documented (1), particularly in studies of HSV-induced lethal encephalitis of mice (13). Preliminary evidence from our laboratory has indicated that spleen cells from C. parvum-injected mice produce increased levels of interferon (unpublished data), a mechanism that, in conjunction with macrophage activation, might be also operative in C. parvum-induced protection.

Data similar to ours have appeared during the completion of our experiments. In these, the effect of several "immunostimulants" on HSV-2-induced infection of newborn mice was studied (11). Among these substances (C. parvum was not tested), only BCG had a protective effect. Similarly, as in our study, only protective and not therapeutic effects were seen. Thus, it might be premature to suggest the use of C. parvum, which has been widely used in immunotherapy of human tumors (5), for antiviral clinical trials in humans. However, it has to be realized that in the animal model used here, the time required for a strong systemic activation by C. parvum is almost equivalent to the interval at which the animals start to die. In a clinical situation where infection is less fulminant, a positive effect still might be observed.

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**LITERATURE CITED**


