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Effect of *Mycobacterium tuberculosis* BCG Infection on the Resistance of Mice to Ectromelia Virus Infection: Participation of Interferon in Enhanced Resistance

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Mice infected with *Mycobacterium tuberculosis* BCG were more resistant than normal mice to ectromelia virus infection. It is suggested that enhanced interferon production in peritoneal exudate cells and spleen cells of BCG-infected mice plays an important role in this resistance.

The effect of the administration of agents affecting the reticuloendothelial system on a number of virus infections has been studied extensively, and enhancement of resistance to viral infection has been demonstrated (4-6, 10; L. J. Old, D. A. Clarke, and B. Benacerraf, Fed. Proc. **19**:208, 1960). However, the mechanism of increased host resistance is not understood. The purpose of this study was to determine the extent of the role that interferon plays in the nonspecific defense mechanism in BCG-infected mice.

The Ishibashi strain of ectromelia virus (EV) was kindly supplied by Y. Ichihashi, Department of Virology, Niigata University School of Medicine, and was grown in mouse embryo cell primary cultures. The infectivity titer was $10^{7.2}$ 50% tissue culture infectious doses/ml in mouse embryo cells and $10^{6.8}$ 50% lethal doses/ml in mice. The BCG strain of *Mycobacterium tuberculosis* was kindly supplied by J. Arima, Institute of Immunological Science, Hokkaido University, and was grown in Dubos liquid medium.

Eight-week-old female mice of the ddN strain were used. One group of mice was inoculated intraperitoneally with 1 mg (wet weight) of BCG, and another group of mice was left uninfected. Three weeks later, these mice were used in the experiment. The preparation and assay of interferon were carried out as described previously (1). Normal and BCG-infected mice were inoculated intraperitoneally with 0.2 ml of 10-fold-diluted EV, and subsequent deaths were recorded. The BCG-infected mice were more resistant to EV than normal mice (Table 1), in agreement with an earlier report (5).

To obtain some information on the distribution and propagation of EV in mice, normal and BCG-infected mice were inoculated intraperitoneally with EV ($10^{3.0}$ 50% lethal dose) and sacrificed daily. Peritoneal exudate cells, livers, spleens, and blood were collected and pooled. The peritoneal exudate cells were washed with 0.01 M phosphate-buffered saline solution (pH 7.4) and suspended in phosphate-buffered saline at a concentration of 4×10^6 cells/per ml. The livers and spleens were homogenized by pestle and mortar and suspended in phosphate-buffered saline at a concentration of 20% (wt/vol). All samples were subjected to three cycles of freezing and thawing and were centrifuged at 2,500 rpm for 15 min. The infectivity titers of supernatant fluids were assayed in tube cultures of mouse embryo cells. In normal mice, EV initially propagated in the peritoneal exudate cells and spleen cells and then appeared in the liver and blood (Fig. 1). In BCG-infected mice, the growth of EV was greatly suppressed in these organs. These results suggest that peritoneal exudate cells and spleens are primary target organs for intraperitoneally inoculated EV.

Interferon produced in the peritoneal exudate, spleen, liver, and blood was measured. The interferon titer in peritoneal exudate was significantly higher in BCG-infected mice than in normal mice (Fig. 2). In other organs of BCG-infected mice, lower titers of interferon were shown; this result may reflect low dissemination and suppressed growth of EV in these organs, which may be caused by an initial suppression of growth of the virus in the peritoneal exudate cells.

Experiments were then performed to determine the extent of interferon production by peritoneal exudate cells and spleen cells in vitro. The spleen cells were prepared by straining the minced spleen tissues through a stainless-steel mesh. These cells were washed with phosphate-buffered saline and suspended in Eagle mini-

mum essential medium supplemented with 5% fetal bovine serum (MEM-FS5%) at a concentration of 2×10^6 cells per ml. The cell suspensions were inoculated with EV (2 50% tissue culture infective doses/cell). After adsorption at 37°C for 2 h, the cells were washed three times with phosphate-buffered saline and replenished with MEM-FS5%. Interferon titers in culture fluids were assayed. The peritoneal exudate cells and spleen cells derived from BCG-infected mice showed about an eightfold increase in the yield of interferon as compared with the interferon produced by cells from normal mice (Table 2).

Numerous studies have demonstrated the important role of macrophages in the primary defense against various virus infections (3, 7-9, 11-14). Hirsch et al. (7) confirmed the lack of

TABLE 1. *Effect of BCG treatment on EV infection*

Virus dilution inoculated[a]	Mortality[b]	
	Normal mice	BCG-infected mice
10^{-2}	3/3	3/3
10^{-3}	3/3	2/3
10^{-4}	3/3	1/3
10^{-5}	3/3	0/3
10^{-6}	2/3	0/3
10^{-7}	0/3	ND[c]
Log_{10} LD_{50}[d]	−6.2	−3.5

[a] Diluted EV (0.2 ml) was inoculated intraperitoneally.
[b] Number of mice died/number of mice inoculated.
[c] Not done.
[d] LD_{50}, 50% lethal dose.

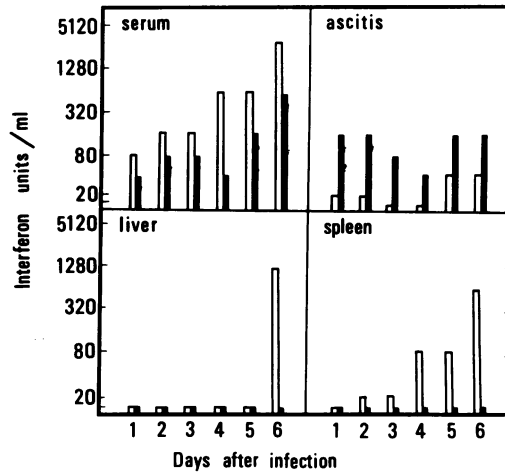

FIG. 2. *Interferon production in normal mice and BCG-infected mice. Symbols: ▭, normal mice; ▬, BCG-infected mice.*

TABLE 2. *Interferon production induced by EV in peritoneal exudate cells and spleen cells from normal and BCG-infected mice*

Cells	Interferon (U/ml) at time postinduction of:	
	12 h	24 h
Normal PEC[a]	20	20
BCG-infected PEC	160	40
Normal SC[b]	20	20
BCG-infected SC	80	160

[a] PEC, peritoneal exudate cells.
[b] SC, spleen cells.

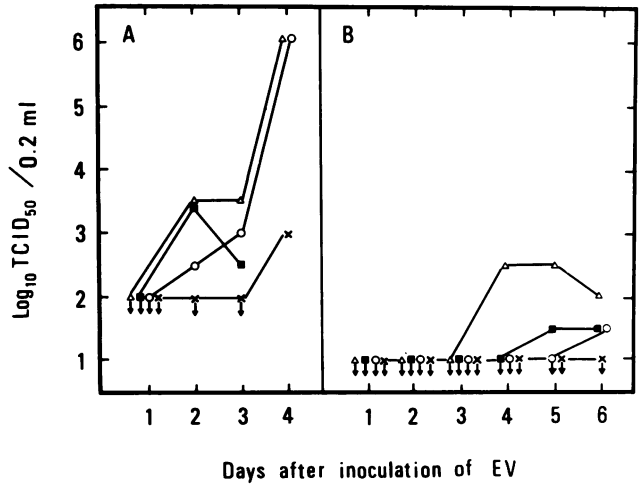

FIG. 1. *Growth of EV in normal mice (A) and BCG-infected mice (B). Symbols: △, spleen; ■, peritoneal exudate cells; ○, liver; ×, blood. Arrows represent lower infectivity titer than the titer indicated. $TCID_{50}$, 50% tissue culture infective dose.*

dissemination of virus from mature macrophages. But the basic nature of the restriction of virus replication in the macrophages has not been revealed. The results reported here show that EV inoculated intraperitoneally grew initially in peritoneal exudate cells and spleen cells and that these cells from BCG-infected mice produced interferon at a higher rate than cells from normal mice. These results suggest that the increased interferon production in the peritoneal exudate cells and spleen cells plays a role in the enhanced resistance of BCG-infected mice to EV infection. The mechanism of enhancement of interferon production in cells from BCG-infected mice is now being investigated in connection with the mechanism of interferon induction by Newcastle disease virus reported previously (2). Further experiments should be undertaken to determine what kind of cells in the peritoneal exudate and spleen cell populations actually function in the enhanced interferon production, and what activity of the cells besides enhanced interferon production also relates to this nonspecific resistance in BCG-infected mice.

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