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PRELIMINARY REPORT

ENHANCED PRODUCTION OF INTERFERON IN MICE INFECTED WITH *MYCOBACTERIUM BOVIS* BCG

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Mycobacterium bovis BCG or its components, which were recently used as immunotherapeutic agents, activate the functions of immunocompetent cells (Akagawa and Tokunaga, 1979; Mackaness et al., 1974) and macrophages (Masuno et al., 1979; Chikuma et al., 1979), and lymphokines, such as macrophage migration inhibitory factor (MIF), blastogenic factor (BF) or lymphotoxin (LT), were released from BCG-sensitized lymphocytes stimulated by purified protein derivatives of tuberculin (PPD) (David, 1971). Interferon (IFN), which was named immune IFN or IFN-γ, was released in the same manner as other lymphokines (Youngner and Salvin, 1973; Green et al., 1969). Furthermore, it is known that BCG enhanced IFN production in mice injected with bacterial endotoxin (Kato et al., 1979). However, there is no report about the effect of BCG on IFN production by IFN inducers other than endotoxin. In general, it is thought that the mechanism of IFN production on induction of endotoxin is different from that on induction of viral or other IFN inducers. If BCG enhances IFN production by induction of IFN inducers, combined therapy of viral diseases and tumors with IFN inducer and BCG may be possible. Therefore, we examined the effect of BCG on IFN production in mice stimulated by polyriboinosinic polyribocytidylic acid (poly I: C) and Newcastle disease virus (NDV).

Female ICR mice, female C3H/He N mice and female DBA/2 mice of 6 weeks old were purchased from Clea Japan, Inc., Osaka, Japan. The mice were infected with 10^7 cells of *Mycobacterium bovis* BCG, which was provided by the Japan BCG laboratory, Tokyo, Japan. Three weeks later, sensitization to BCG were confirmed by the footpad test with PPD. One week later, 10^8 PFU of Newcastle disease virus (NDV) or 100 μg of poly I: C (purchased from P-L Biochemicals, Inc., Milwaukee, Wis., USA, which is thought not to contain the endotoxin) was dissolved in redistilled water and injected intravenously. Blood was withdrawn and pooled 1, 3, 6, 12 and 24 h later, and the serum was isolated and stocked at −80 C until IFN assay. IFN activity in the serum was titrated by the microassay method using murine L_{929} cells and the New Jersey strain of vesicular stomatitis virus (VSV) (Imanishi et al., 1977).

Mice infected with BCG produced more IFN than control mice when 10^8 PFU of NDV was injected intravenously (Fig. 1A). When 100 μg of poly I: C was injected intravenously,

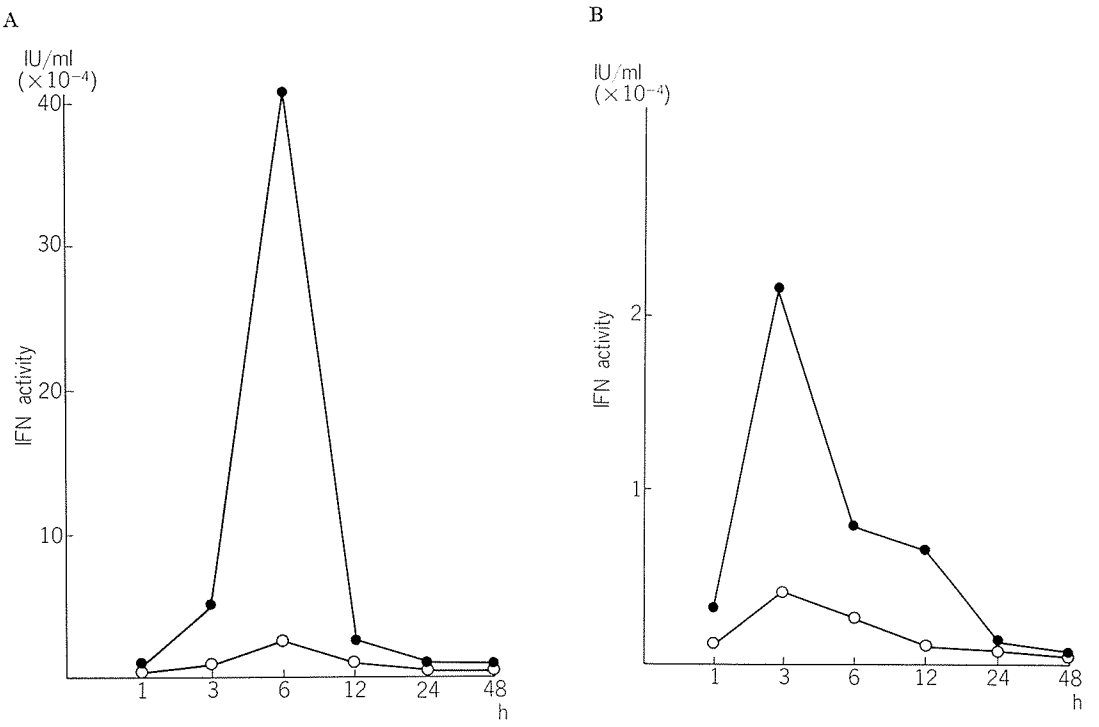

FIGURE 1. IFN production by NDV and poly I: C in ICR mice infected with BCG.

10^8 PFU of NDV was injected intravenously into ICR mice infected with BCG (A: ●——●) or normal mice (A: ○——○).

100 μg of poly I: C was injected intravenously into mice infected with BCG (B: ●——●) or normal mice (B: ○——○).

higher IFN activity was detected in the serum of mice infected with BCG than in that of control mice (Fig. 1B). No IFN activity was detected in the serum of BCG-infected mice when IFN inducers were not injected or when normal saline was injected instead of poly I: C or NDV. There were no differences between BCG-infected mice and control mice in the stabilities to pH 2, heating at 56 C for 1 h or trypsinization of poly I: C-induced and NDV-induced IFNs. Moreover there were differences in the sensitivities of heterologous human FL cell to poly I: C-induced or NDV-induced IFN of BCG-infected mice and control mice (data not shown).

C3H/He N mice and DBA/2 mice were infected with BCG, and NDV was administered intravenously after confirming sensitization to BCG by the footpad test. The BCG-infected mice produced more IFN than the control mice (Fig. 2A, 3A). The IFN activities in the sera of C3H/He N and DBA/2 mice infected with BCG were also higher than those in control mice (Fig. 2B, 3B). Thus, enhanced IFN production on infection with BCG was confirmed in several strains of mice.

Lymphocytes and/or macrophages were probably closely related to the production of IFN in mice stimulated with poly I: C or NDV (Kolot et al., 1976; Maehara et al., 1977; De Maeyer et al., 1969; De Maeyer-G. and De Maeyer, 1971). BCG activates the functions of lymphocytes (Akagawa and Tokunaga, 1979; Mackaness et al., 1974; Masuno et al.,

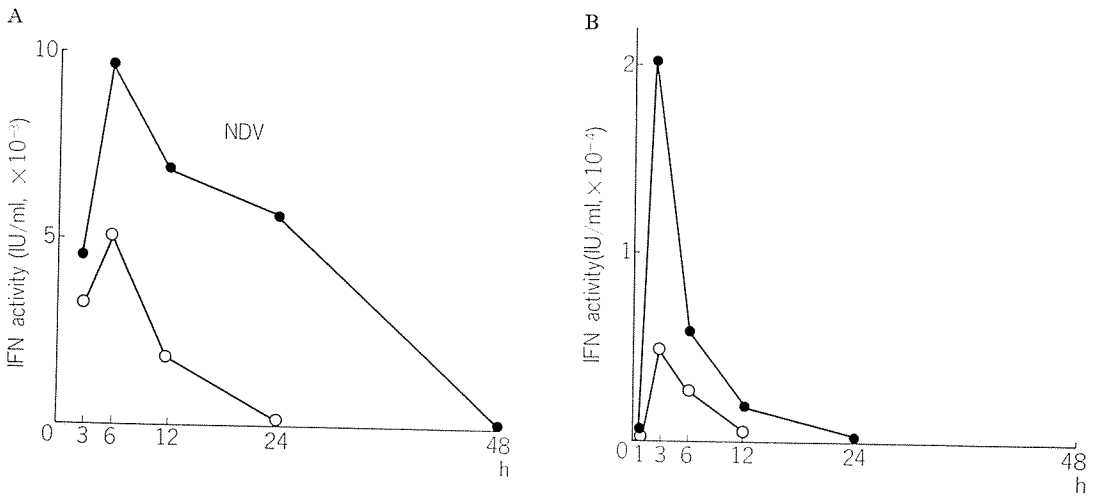

FIGURE 2. IFN production by NDV and poly I: C in C3H/He N mice infected with BCG.
NDV was injected into C3H/He N mice infected with BCG (A: ●——●) or normal mice (A: ○——○).
Poly I: C was injected into mice infected with BCG (B: ●——●) or normal mice (B: ○——○).

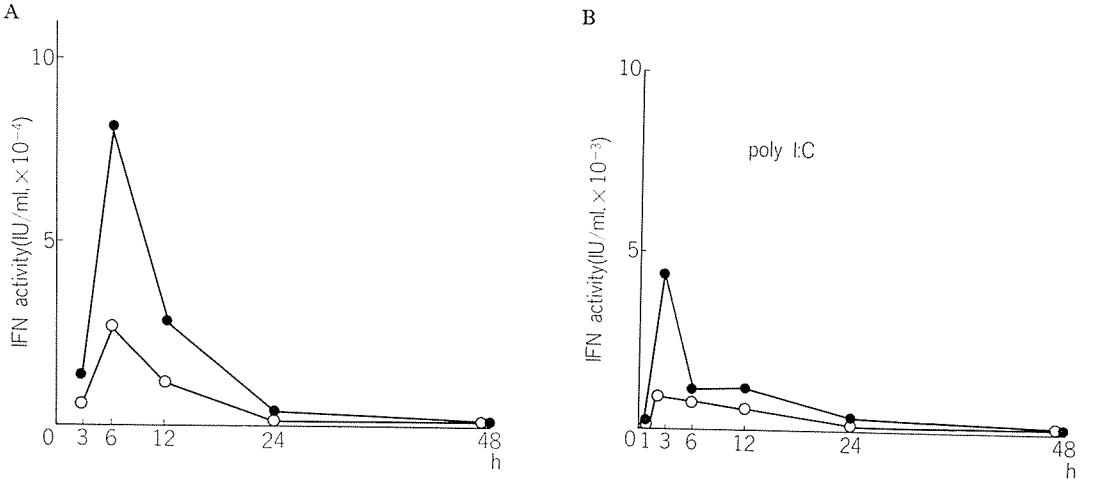

FIGURE 3. IFN production by NDV and poly I: C in DBA/2 mice infected with BCG.
NDV was injected into DBA/2 mice infected with BCG (A: ●——●) or normal mice (A: ○——○).
Poly I: C was injected into mice infected with BCG (B: ●——●) or normal mice (B: ○——○).

1979; Chikuma et al., 1979) and macrophages. Enhanced IFN production may be caused by activation of macrophages and lymphocytes by BCG. Furthermore, various serum factors that modify the immunological activity are released in mice infected with BCG (David, 1971) and these factors may be related to the enhanced production of IFN in BCG-infected mice.

On the basis of enhanced production of IFN, combined therapy of viral and malignant diseases with IFN inducer and BCG may be possible.

ACKNOWLEDGMENTS

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