SHORT COMMUNICATIONS

Growth of Two Strains of *Mycobacterium bovis* (BCG) in Athymic Mice

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INTRODUCTION

Acquired resistance to tuberculous infection is mediated mainly by T lymphocytes. Both neonatally thymectomized mice and adult mice that had been thymectomized, lethally irradiated and reconstituted with bone marrow cells (THXB mice) were incapable of resisting the vigorous growth of *Mycobacterium tuberculosis* (Takeya *et al.*, 1967; North, 1973). The Montreal strain of BCG (TMC1012, obtained from the Trudeau Mycobacterial Culture Bank, Saranac Lake, New York, U.S.A.) was reported to cause severe systemic infection in THXB mice (Collins, Congdon & Morrison, 1975), although the Japanese strain of BCG failed to induce progressive infection in neonatally thymectomized mice as we reported previously (Takeya *et al.*, 1967). To explore further the contribution of T lymphocytes in resistance to different BCG strains, growth of the Japanese and a French strain of BCG in athymic nude mice was compared.

METHODS

Females of congenitally athymic nude mice (nu/nu) and their normal littermates (nu/+) of BALB/c background were raised in specific pathogen-free conditions, maintained on sterilized bedding in a clean (but not sterile) room and given sterilized pellets and chlorinated water. Japanese and French strains of BCG were obtained from the Japan BCG Laboratory in Tokyo and the Pasteur Institute in Paris respectively. They were grown on 1% Ogawa's egg medium (containing: KH_2PO_4 , 1.0 g; sodium glutamate, 1.0 g; glycerol, 6 ml; distilled water, 100 ml; homogenized whole eggs, 200 ml; and 2% (w/v) aqueous malachite green solution, 6 ml) at 37 °C for 10 days. The numbers of viable bacteria in suspensions were counted after incubation on Ogawa's egg medium for 4 weeks. Eight-week-old mice were injected intravenously with 2.0×10^6 and 3.5×10^5 viable cells, respectively, of the Japanese and French strains. Three mice from each group were sacrificed at intervals of 2 or 3 weeks. The liver, spleen, kidney and lung were homogenized separately in 10 ml of 4% (w/v) NaOH within 5 min, a treatment which did not affect the viability of BCG. After 10-fold serial dilutions, 0.1 ml of individual samples was inoculated on to Ogawa's egg medium and the numbers of viable organisms were determined from counts after 6 weeks incubation.

Fig. 1. Numbers of viable bacteria in different organs [liver (○, ●); lung (△, ▲); spleen (▽, ▼); kidney (□, ■)] at various times after intravenous inoculation of mice with (a, b) 2.0×10^6 viable cells of the Japanese strain of BCG, or (c, d) 3.5×10^5 viable cells of the French strain of BCG. Open symbols indicate results for nu/nu mice; filled symbols, for nu/+ mice. Bars indicate the range of results for three mice.

RESULTS AND DISCUSSION

The numbers of viable organisms after inoculation with the Japanese strain of BCG increased only slightly in all organs of nu/nu and nu/+ mice during the observation period of 10 weeks (Fig. 1a, b). No significant differences were found between nu/nu and nu/+ mice, although a small increase in the numbers of viable bacteria was detected in the lung and kidney of nu/nu mice at 10 weeks.

Two weeks after injection with the French strain of BCG, no difference was detectable between nu/nu and nu/+ mice (Fig. 1c, d). Four weeks after injection, greater numbers of viable bacteria were detected in the liver and kidney of nu/nu mice than in those of nu/+ mice. Thereafter the numbers in each organ decreased in nu/+ mice, but increased progressively in nu/nu mice.

These results suggest that the Japanese strain of BCG is more attenuated than the French strain. The former appeared to be resisted by a non-specific defence mechanism composed mainly of normal macrophages, since there was no difference in bacterial growth between T cell-deprived mice and controls. On the other hand, specific immunity mediated by T lymphocytes appeared to be required for resistance against the more virulent French strain. This interpretation is supported by the results of D'Arcy Hart & Armstrong (1974) and Armstrong & D'Arcy Hart (1975) in their studies of virulence and lysosomal responses in macrophages infected with different strains of *M. tuberculosis*. The Phipps strain of BCG

obtained from the Trudeau Mycobacterial Culture Bank appeared to be comparable to the French strain in virulence, since it grew in nude mice but not in normal littermates (Sher *et al.*, 1975). The Montreal strain studied by Collins *et al.* (1975) should probably be assigned to the same group as the French and the Phipps strains.

Variation in the virulence of strains of BCG has previously been indicated by others using intact or immunosuppressed animals (Bunch-Christensen, Ladefoged & Guld, 1968; Sawada & Hashimoto, 1970; Sher *et al.*, 1973). Our work suggests that athymic nude mice may be more suitable for investigating the differences in virulence between attenuated strains of BCG. We also believe that for the manufacture of vaccine, the Japanese strain of BCG may be safer than the French, the Montreal or the Phipps strains, especially if used in immunodeficient subjects.

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