SHORT COMMUNICATION

Importance of Polymorphonuclear Leucocytes in Protection of Mice against *Escherichia coli*

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Bacterial growth and lethality of *Escherichia coli* infection of mice were enhanced by X-irradiation but not by treatment with carrageenan. Since carrageenan depletes macrophages but not polymorphonuclear leucocytes, it is concluded that protection against *E. coli*, at least in the early phases, depends mainly on polymorphonuclear leucocytes.

INTRODUCTION

Polymorphonuclear leucocytes (PMN) and cells of the macrophage series are believed to contribute to protection against a variety of micro-organisms. In our previous papers (Mitsuyama *et al.*, 1978; Tatsukawa *et al.*, 1979), *Listeria monocytogenes* and *Pseudomonas aeruginosa* were shown to be eliminated mainly by macrophages and by PMN, respectively, at least at an early stage of infection. Thus, the phagocytes protecting against infection may differ for various bacteria.

Escherichia coli is a micro-organism of relatively low virulence. PMN are reported to phagocytize and kill this bacterium readily *in vitro* (Leijh *et al.*, 1979; Proctor, 1979). Severe infection caused by *E. coli* is rare, but fatal systemic infection may occur in so-called 'compromised hosts' (Bodey *et al.*, 1978).

The present work was conducted to determine the main cells effective in protection against *E. coli* infection, using phagocyte-depleted mice. X-irradiation was used to deplete the total population of phagocytes except for fixed macrophages and carrageenan was used to deplete selectively cells of the macrophage series (Catanzaro *et al.*, 1971; Tatsukawa *et al.*, 1979).

METHODS

Animals. Eight-week-old female mice of an outbred ddN strain (CLEA Japan Inc., Tokyo) were used.

Micro-organisms. *Escherichia coli* (strain E77156, O6:H1) was kindly donated by Dr Ohashi, Department of Microbiology, Tokyo Metropolitan Research Laboratory of Public Health. The bacteria were maintained by serial passage in ddN mice. Fresh isolates were obtained from spleens, subcultured once in Tryptic Soy Broth (Difco) and used for infection. The LD₅₀ of this strain was about 8×10^7 viable bacteria by the intravenous route and about 2×10^7 by the intramuscular route. *Listeria monocytogenes* (strain EGD), used in each experiment as a control to confirm the effects of X-irradiation and carrageenan treatment, was maintained and inoculated as described previously (Mitsuyama *et al.*, 1978).

Determination of bacterial growth. Mice were inoculated intravenously with 5×10^5 viable *E. coli* suspended in phosphate-buffered saline (PBS). At various times after inoculation, they were bled to death and their livers and spleens were removed aseptically. The organs were homogenized in PBS and dilutions were plated for colony counts as described previously (Tatsukawa *et al.*, 1979) except that nutrient agar without glucose was used. To

observe bacterial growth in a local infection, bacterial suspension (5×10^5 or 5×10^6 viable bacteria) was inoculated intramuscularly into the middle of the thigh. The complete infected muscle was subsequently removed and treated in the same way as for the estimation of bacterial growth in other organs.

X-irradiation and carrageenan treatment. These were carried out as described previously (Tatsukawa *et al.*, 1979).

RESULTS

Mortality and bacterial growth after systemic infection. X-irradiated mice died within 4 d of inoculation with 5×10^5 *E. coli*, while normal and carrageenan-treated mice survived. Both X-irradiated and carrageenan-treated mice died within 7 d of inoculation with 1×10^4 *L. monocytogenes* (non-lethal to normal mice).

Control and carrageenan-treated mice completely cleared their blood of *E. coli* within 1 d. In the livers of untreated controls, bacteria decreased progressively in number and became almost undetectable after 4 d (Fig. 1). In the livers of carrageenan-treated mice, bacterial growth was observed up to 2 d after infection, but elimination of bacteria followed thereafter. Progressive growth of bacteria was observed in X-irradiated mice. The patterns of bacterial growth were similar in the spleen.

Mortality and bacterial growth after local infection. All of the carrageenan-treated mice survived after intramuscular inoculation of 5×10^5 *E. coli*, while none of the X-irradiated mice survived more than 3 d. Bacterial growth was not found in the muscle of either control or carrageenan-treated mice, and the inoculated bacteria were completely eliminated by 96 h (Fig. 2*a*). In X-irradiated mice, however, the number of bacteria increased rapidly to reach over 10^9 within 48 h of infection. Large numbers of bacteria were recovered from the blood of irradiated mice 2 d after infection.

Even at a dose of 5×10^6 *E. coli* none of the carrageenan-treated mice died during the period of observation (Fig. 2*b*). X-irradiated mice died within 48 h of local infection with 5×10^6 bacteria. The multiplication of *E. coli* in X-irradiated mice was more rapid after inoculation of 5×10^6 *E. coli* than 5×10^5 . Bacterial elimination was poor in controls infected with 5×10^6 bacteria, but fulminating bacterial growth as observed in the X-irradiated mice was obviously inhibited. The carrageenan-treated mice eliminated bacteria somewhat faster than the control mice.

Fig. 1. Bacterial growth in the liver after intravenous challenge with 5×10^5 *E. coli*: ○, control mice; ●, mice treated with carrageenan (200 mg kg^{-1}) 1 d before challenge; □, mice X-irradiated (8 J kg^{-1}) 2 d before challenge; †, death. Each point and bar indicates the mean result for five animals $\pm$ S.E.M.

Fig. 2. Bacterial growth in thigh muscle after intramuscular inoculation of (a) 5×10^5 or (b) 5×10^6 *E. coli*: ○, control mice; ●, carrageenan-treated mice; □, X-irradiated mice; †, death. Each point and bar indicates the mean result for five animals $\pm$ S.E.M.

DISCUSSION

Many micro-organisms cause severe infections in hosts whose phagocyte functions are depressed artificially by X-irradiation (Kaplan *et al.*, 1952; Gordee & Simpson, 1967; Gillette & Lance, 1973; Mitsuyama *et al.*, 1978). X-irradiation depresses PMN, macrophages and lymphocytes, so that it is difficult to reach any conclusion as to which cells make the main contribution to protection against individual micro-organisms. Carrageenan selectively damages cells of the macrophage series but not PMN; carrageenan-induced loss of host defence may be mainly attributed to the depletion of macrophages (Mitsuyama *et al.*, 1978; Tatsukawa *et al.*, 1979).

X-irradiation markedly increased mortality of mice after inoculation of a normally sublethal dose of *E. coli*, irrespective of the route of infection. Carrageenan did not affect mortality. This carrageenan-resistant, X-irradiation-susceptible host defence implies that protection against *E. coli*, like that against *Pseudomonas aeruginosa* (Tatsukawa *et al.*, 1979), depends mainly on PMN, and not on macrophages. Such an interpretation is also supported by the pattern of bacterial growth in the liver or muscle. In systemic infection with *E. coli* the recovery of viable bacteria from the liver was always greater in carrageenan-treated mice than in control mice, but fatal bacterial growth was inhibited and elimination ultimately occurred; X-irradiated mice showed progressive bacterial growth in the liver. After local infection fulminant and fatal bacterial growth was observed only in the group of mice exposed to X-irradiation. The resistance at the site of local infection depends on the function of accumulating phagocytic cells. Carrageenan treatment did not affect this resistance. After local inoculation of a large dose of *E. coli* (5×10^6), the recovery of viable bacteria from the site was always smaller in carrageenan-treated mice than in controls. Carrageenan is known to increase the number of PMN for several days (Tatsukawa *et al.*, 1979), so the smaller recovery of bacteria might be attributed to an increase of PMN accumulation into the local sites.

In the present study, the contribution of antibody to resistance against *E. coli* infection is unknown. Transfer of antibody can protect mice from a lethal effect after systemic or local infection of large numbers of *E. coli* and *P. aeruginosa* (unpublished results), but the protective effects of antibodies are exhibited only in the presence of intact PMN.

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