MODELS OF INFECTION

Influence of various immunosuppressive agents on the occurrence of endogenous bacteraemia in mice

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Summary. The influence of six immunosuppressive agents on the occurrence of endogenous bacteraemia in mice was evaluated. The mortality rates in conventional ddY mice given cyclophosphamide (CY), fluorouracil (5-FU), methotrexate (MTX), cisplatin (CDDP) or FK-506 intraperitoneally, or dexamethasone (DXM) subcutaneously were 70, 100, 100, 100, 0 and 0%, respectively. *Pseudomonas aeruginosa* was isolated from 70% of mice treated with CY but from only 10% of mice treated with 5-FU and 30% treated with MTX. Enterobacteria were isolated from 90% of mice treated with 5-FU. Specific-pathogen-free (SPF) mice fed *P. aeruginosa* were also treated with these agents. All mice in the CY, 5-FU, MTX and CDDP groups died whereas mice treated with DXM and FK-506 showed 20% and 0% mortality, respectively. Pure cultures of *P. aeruginosa* were obtained from all of the mice treated with CY. Polymicrobial bacteraemia with *P. aeruginosa* and enterobacteria occurred in 5, 25, 5 and 5% of mice treated with 5-FU, MTX, CDDP and DXM, respectively. Enterobacterial bacteraemia was observed in 70% of mice treated with CDDP and in 5% of the DXM group. Different types of bacteraemia were induced by different immunosuppressive agents. The mechanism of immunosuppression may affect the frequency of bacteraemia and the causative organism.

Introduction

Bacteraemia in immunocompromised hosts frequently arises endogenously from the patient's own flora.^{1–3} *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Candida albicans* are typical causes of such infections, especially in bacteraemias originating in the intestinal flora.^{2–7} An animal model that resembles septicaemia in man is valuable for the evaluation of prophylaxis and treatment.

P. aeruginosa frequently causes endogenous systemic bacteraemia in leucopenic mice treated with cyclophosphamide (CY)⁸ and bacteraemia with specific *P. aeruginosa* strains can be induced by treating mice fed the organisms with CY and ampicillin.⁹ These models have been used to investigate the pathogenic mechanism of endogenous bacteraemia, virulence factors and treatment.^{9–12} *Escherichia coli*, other enterobacteria and enterococci often cause portal bacteraemia only and are not isolated from cardiac blood.^{8,13}

In many animal studies, CY is used to induce a compromised state.^{8–12,14–18} It is not well known

whether other immunosuppressive agents induce bacteraemia or other infections in animals, nor is it clear if *P. aeruginosa* bacteraemia can be induced by other immunosuppressive agents.

Six immunosuppressive agents were administered to conventional mice and specific-pathogen-free (SPF) mice fed *P. aeruginosa* strain D4 to compare mortality rates, frequency of occurrence of bacteraemia and causative organisms.

Materials and methods

Reagents

Sodium ampicillin (Viccillin; Meiji Seika Kaisha Ltd, Tokyo, Japan), cyclophosphamide (CY) (Endoxan; Shionogi and Co. Ltd, Osaka, Japan), fluorouracil (5-FU) (Kyowa Co. Ltd, Tokyo), cisplatin (CDDP) (Randa Inj.; Japan Chemical Co. Ltd, Tokyo), and dexamethasone (DXM) (Wako Pure Chemical Industries, Tokyo) were purchased from commercial sources. Methotrexate (MTX) and FK-506 were kindly provided by Lederle Japan Co. Ltd, Tokyo, and Fujisawa Pharmaceutical Co. Ltd, Tokyo respectively.

Mice

Conventional and SPF male ddY mice (Japan S.L.C. Co. Ltd, Shizuoka, Japan) weighing 20–24 g were used in the experiments. Mice were fed a sterile diet and were given sterile distilled water except during the period of oral administration of the bacteria.

Bacterial strain and media

P. aeruginosa strain D4 was isolated from cardiac blood of a mouse with systemic bacteraemia induced by CY as described previously.⁸ The organism was grown on Trypticase Soy Agar (BBL Microbiology Systems, Cockeysville, MD, USA) at 37°C for 18 h. Bacteria were suspended in sterile saline 0.45% and adjusted to 10^7 cfu/ml by spectrophotometry (UVIDEK-40; Jasco, Tokyo).

Determination of endogenous bacteraemia

Normal conventional mice. Endogenous bacteraemia was induced as described previously,⁸ with some modifications. Groups of 10 mice received ampicillin 200 mg/kg/day intraperitoneally for 15 days. Simultaneously, immunosuppressive agents were administered as follows: CY, 5-FU or MTX 200 mg/kg/day; FK-506 100 mg/kg/day; CDDP 20 mg/kg every other day. DXM 10 mg/kg was given subcutaneously every other day.

Mice were observed four times each day and the number of deaths was recorded up to day 15. Cultures from cardiac blood were performed immediately after

death. Survivors were killed by cervical dislocation and cultures were done at the end of the experiments. All samples were cultured immediately aerobically on sheep blood 5% agar (Nissui Pharmaceutical Co. Ltd, Tokyo) and trypticase soy agar at 37°C in humidified air for 24 h. The isolates were identified by Vitek Identification Cards (Vitek Systems, Inc., Hazelwood, MO, USA).

SPF mice fed *P. aeruginosa* D4. Faecal specimens from SPF mice were obtained before the study and examined to confirm the absence of *P. aeruginosa*. Mice were fed *P. aeruginosa* D4 in drinking water for 4 days and also received ampicillin 200 mg/kg/day during this period to disrupt the balance of the normal gastro-intestinal flora. Each immunosuppressive agent was administered on days 5, 7, 9 and 11 as described above. Mice were monitored until day 15 and cultures of samples and identification of bacterial isolates were performed as described above.

Results

Survival of mice treated with immunosuppressive agents

Conventional mice. Mice treated with CY began to die on day 6 and their final mortality rate was 70%. Mice treated with CDDP also started to die on day 6 and by day 8 all had died. Mice treated with 5-FU or MTX began to die later, but all mice died within the 15 days. In contrast, none of the mice treated with DXM or FK-506 died (fig. 1).

SPF mice fed *P. aeruginosa* D4. Mice treated with

Fig. 1. Survival of groups of 10 conventional mice treated with immunosuppressive agents: ○, CY; ●, 5-FU; △, MTX; ▲, CDDP; □, DXM; ■, FK-506.

Fig. 2. Survival of groups of 20 SPF mice fed *P. aeruginosa* D4 and treated with immunosuppressive agents: ○, CY; ●, 5-FU; △, MTX; ▲, CDDP; □, DXM; ■, FK-506.

Fig. 3. Organisms isolated from cardiac blood of conventional mice treated with ampicillin and immunosuppressive agents and dying before day 15: ■, *P. aeruginosa*; ▨, enterobacteria; □, no growth.

Table I. Enterobacteria isolated from cardiac blood of conventional mice treated with immunosuppressive agents

Species	Number of mice treated with		
	5-FU	MTX	CDDP
<i>K. pneumoniae</i>	4	0	0
<i>K. oxytoca</i>	3	3	1
<i>Enterobacter cloacae</i>	2	1	0
<i>M. morganii</i>	0	1	4
<i>M. morganii</i> and <i>K. oxytoca</i>	0	1	1

CY died between days 9 and 13. Mice treated with 5-FU, MTX or CDDP also showed a similar pattern of mortality, whereas most of the mice treated with DXM or FK-506 survived (fig. 2).

Table II. Enterobacteria isolated together with *P. aeruginosa* from cardiac blood of SPF mice treated with immunosuppressive agents

Species	Number of mice treated with			
	5-FU	MTX	CDDP	DXM
<i>K. pneumoniae</i>	1	1	0	0
<i>K. oxytoca</i>	1	0	1	0
<i>Pr. mirabilis</i>	0	5	0	1
<i>M. morganii</i> and <i>Pr. mirabilis</i>	0	1	0	0

Table III. Enterobacteria isolated without *P. aeruginosa* from cardiac blood of SPF mice treated with immunosuppressive agents

Species	Number of mice treated with	
	CDDP	DXM
<i>E. coli</i>	1	0
<i>M. morganii</i>	8	0
<i>Pr. mirabilis</i>	5	1

Organism isolated from cardiac blood

Conventional mice. *P. aeruginosa* strains were isolated from seven, one and three mice treated with CY, 5-FU and MTX, respectively. Enterobacteria were isolated from nine, six and six mice treated with 5-FU, MTX, CDDP (fig. 3). Table I shows the species of enterobacteria isolated from these mice. *Klebsiella* spp. were the commonest cause of endogenous bacteraemia in mice treated with 5-FU or MTX. No bacteria were isolated from 10% of mice in the MTX group nor from 40% treated with CDDP. Amongst mice killed after 15 days, *Morganella morganii* was isolated from

Fig. 4. Organisms isolated from cardiac blood of SPF mice fed *P. aeruginosa* D4 and treated with ampicillin and immunosuppressive agents and dying before day 15: ■, *P. aeruginosa*; ▨, *P. aeruginosa* and enterobacteria; ▩, enterobacteria; □, no growth.

two out of three mice treated with CY but cardiac blood from mice treated with DXM or FK-506 was sterile.

SPF mice fed *P. aeruginosa* D4. Pure cultures of *P. aeruginosa* were obtained from 100, 90, 65, 20 and 5% of mice treated with CY, 5-FU, MTX, CDDP and DXM, respectively. Polymicrobial bacteraemia (*P. aeruginosa* and enterobacteria) occurred in 5, 25, 5 and 5% of mice treated with 5-FU, MTX, CDDP and DXM. The species isolated most frequently from mice treated with MTX was *Proteus mirabilis* (table II). Enterobacterial bacteraemia without *P. aeruginosa* was observed in 70 and 5% of mice treated with CDDP and DXM (table III). *M. morganii* and *Pr. mirabilis* were isolated more frequently from CDDP-treated mice. Only one mouse each in the groups treated with 5-FU, CDDP and DXM and dying before day 15 remained free of bacteraemia (fig. 4).

Discussion

K. pneumoniae, *E. coli* and *P. aeruginosa* are frequently involved in nosocomial septicaemia.^{3,19} Several outbreaks of infections including septicaemia by multi-resistant *K. pneumoniae* strains have been reported.^{5,20,21} Epidemiological investigations have revealed that the reservoir for *K. pneumoniae* is the patients gastro-intestinal tract.^{5,6}

We have reported previously that CY often induces bacteraemia due to endogenous *P. aeruginosa* in mice.⁸ Moreover, bacteraemia with specific *P. aeruginosa* strains was induced by administering CY and ampicillin to mice fed the organism.⁹ These animal models are useful for the investigation of the pathophysiology of endogenous bacteraemia, virulence factors of *P.*

aeruginosa, and therapeutic interventions.^{9,10-12} Collins *et al.*¹⁴ and Opal *et al.*¹⁵ described a model of endogenous *P. aeruginosa* bacteraemia in rats for the evaluation of antibiotic treatments and immunotherapy. Unlike most models of infection, which employ intraperitoneal or intravenous routes of infection, these models incorporated other steps, such as bacterial colonisation, overgrowth and invasion. Consequently, they closely mimic the pathophysiology of septicaemia in man.^{9,11,14}

Enterobacteria such as *K. pneumoniae* and *E. coli* often cause only portal bacteraemia in CY-treated mice because they are easily cleared from blood by the reticulo-endothelial system, especially by Kupffer cells in the liver.^{8,13} Therefore, suitable immunosuppressive agents other than CY should be used to establish a model of endogenous bacteraemia due to enterobacteria. Braude *et al.*²² and Ziegler *et al.*²³ induced endogenous bacteraemia by feeding *E. coli* and *K. pneumoniae* to coliform-free rabbits conditioned with nitrogen mustard and inserting a temperature probe into the rectum. These animal models resemble the overwhelming septic shock observed after pelvic instrumentation. However, it is difficult to perform experiments with sufficient numbers of animals because of the size and cost of rabbits.

In this study, significant differences in mortality rates, frequency of bacteraemia and causative organisms were observed among mice treated with various immunosuppressive agents. CY frequently induced *P. aeruginosa* bacteraemia in both conventional mice and SPF mice fed *P. aeruginosa* D4. In contrast, CDDP often caused enterobacterial bacteraemia but mice treated with CDDP frequently died without bacteraemia, suggesting that the dose employed killed mice by acute toxicity before bacteraemia developed. DXM and FK-506 seldom induced bacteraemia. These immunosuppressive agents may not provide suitable models of endogenous bacteraemia. 5-FU induced different types of bacteraemia in conventional and SPF mice fed *P. aeruginosa* D4. Conventional mice frequently suffered septicaemia due to enterobacteria whereas SPF mice fed *P. aeruginosa* frequently suffered *P. aeruginosa* bacteraemia. This may be due to the difference in concentration of *P. aeruginosa* in the intestinal tracts of these mice. CY may induce *P. aeruginosa* bacteraemia selectively even when the concentration of *P. aeruginosa* is relatively low. The ability of MTX to induce bacteraemia was intermediate between CY and 5-FU.

The mechanisms of these differences are still to be clarified, but variation in target cells, degree of mucosal damage and the resident intestinal flora may account for the findings. The results may lead to the establishment of a new model of endogenous bacteraemia since immunosuppressive agents seem to be capable of selecting for certain bacteraemias.

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