Effect of clearance of bacteria from the blood on the development of systemic bacteraemia in mice

Y. HIRAKATA, M. KAKU*, N. FURUYA, T. MATSUMOTO*, K. TATEDA, K. TOMONO† and K. YAMAGUCHI

Department of Microbiology, Toho University School of Medicine, Tokyo 143, and * Department of Laboratory Medicine, and † Department of 2nd Internal Medicine, Nagasaki University School of Medicine, Nagasaki 852, Japan

Summary. Clearance of various bacteria isolated from portal and systemic blood of mice was evaluated and compared. All portal blood strains, including *Escherichia coli* and enterococci were eliminated more rapidly from the circulation than were strains isolated from systemic blood, including *Pseudomonas aeruginosa*. With mannose-type lectin, mannose or fucose residues that mediated lectinophagocytosis were detected on the surfaces of most portal strains by agglutination tests. Blood clearance of *Esch. coli* H21 was inhibited by prior injection of mannose into mice, suggesting that the clearance of this strain was mediated by mannose-type lectin on the surface of tissue macrophages. However, no inhibition of clearance of any other strains was observed by the injection of mannose, galactose, or fucose into mice, nor by pre-incubation of bacteria with mannose. Blood clearance of some portal strains was significantly faster in CBA/J mice than in CBA/N mice with B cell immune deficiency, indicating that immunoglobulin was involved in their clearance. Among portal strains only enterococci showed high cell-surface hydrophobicity. These data suggest that initial bacterial blood clearance may be critical in determining whether latent portal bacteraemia progresses to systemic bacteraemia and that the rapid clearance of most strains is multifactorial.

Introduction

The reticuloendothelial system (RES) plays a key role in removing micro-organisms, endotoxins and other particles from the circulation,^{1–4} and hepatic phagocytosis by Kupffer cells accounts for > 80 % of RES function.^{5,6} Non-opsonic phagocytosis by Kupffer cells, such as lectinophagocytosis⁷ and hydrophobic interaction⁸, may be an important mechanism in clearance of bacteria from the bloodstream and in host defence against bacteraemia.⁹ Lectinophagocytosis can be mediated by both bacterial and macrophage surface lectins.⁷ Lectinophagocytosis of *Escherichia coli* mediated by the mannose-specific lectin associated with type 1 fimbriae has been investigated extensively.^{10,11} Mannose-specific lectin on the bacterial surface of some *Klebsiella pneumoniae* strains also mediates lectinophagocytosis.⁷ At least three types of lectin, i.e., mannose-, galactose-, and fucose-type lectin, expressed on the surfaces of tissue macrophages have been isolated and characterised.^{7,12} Although there have been reports on the mechanism of elimination of bacteria from blood,^{13–15} studies comparing blood clearance of particular species of bacteria have been rare.⁴

We have reported that *Pseudomonas aeruginosa* and *Morganella morganii* frequently cause systemic bac-

teraemia and that *Esch. coli*, enterococci, and other species caused portal bacteraemia only in cyclophosphamide-treated mice.¹⁶ The viability of *P. aeruginosa* D4 and *M. morganii* L48, that cause systemic bacteraemia, is not affected by the Kupffer cells and these bacteria are not eliminated from the blood. In contrast, *Esch. coli* L424, *Enterobacter cloacae* L522, *K. pneumoniae* H27, and *Enterococcus faecalis* L425, present in portal blood, are gathered up by the Kupffer cells, and eliminated rapidly from the circulation.

The present study evaluated and compared clearance from the bloodstream of various bacterial strains isolated from mice following their intravenous administration *via* the tail vein. Potential roles of cell-surface sugars and lectins and bacterial cell-surface hydrophobicity in mediating bacterial clearance from the blood of mice were also examined, as well as the influence of immunoglobulin on clearance.

Materials and methods

Bacterial strains

Bacterial strains were isolated from mice with bacteraemia produced by the administration of cyclophosphamide and ampicillin as described previously¹⁶ (table I). Strains were considered to be resistant to serum if 100 % viability was maintained after incubation for 60 min in fresh normal murine serum.

Table I. Bacterial strains

Systemic	Portal
<i>P. aeruginosa</i> D4	<i>Esch. coli</i> L33
<i>P. aeruginosa</i> D271	<i>Esch. coli</i> L424
<i>P. aeruginosa</i> H3	<i>Esch. coli</i> L529
<i>P. aeruginosa</i> H8	<i>Esch. coli</i> H21
<i>P. aeruginosa</i> H9	<i>Esch. coli</i> H25
<i>P. aeruginosa</i> H12	<i>E. cloacae</i> L31
<i>P. aeruginosa</i> H13	<i>E. cloacae</i> L317
<i>P. aeruginosa</i> H15	<i>E. cloacae</i> L522
<i>M. morganii</i> L48	<i>E. cloacae</i> L527
<i>M. morganii</i> L222	<i>K. pneumoniae</i> H29
<i>M. morganii</i> L322	<i>Ent. faecalis</i> L425
<i>M. morganii</i> L421	<i>Ent. faecium</i> L211
<i>M. morganii</i> H32	<i>Ent. faecium</i> L410
<i>M. morganii</i> H35	

Such strains could be separated into two groups. *P. aeruginosa* and *M. morganii* strains isolated from both cardiac and portal blood were defined as systemic strains. The other strains were isolated from portal blood only and were defined as portal strains.

Media

Bacteria were grown on Trypticase Soy Agar (TSA; BBL Microbiology Systems, Cockeysville, MD, USA) at 37°C for 24 h. Bacteria originating from a single

colony were incubated in Mueller-Hinton broth (Difco Laboratories, Detroit, MI, USA), washed with sterile saline, and adjusted to a suitable concentration for each experiment in saline by optical density measured with a spectrophotometer.

Clearance of bacteria from mouse blood

Clearance was evaluated as described previously.¹⁶ Into specific-pathogen-free (SPF) male ddY mice weighing 20–24 g, 0.2 ml of a bacterial suspension in saline containing 10^8 cfu/ml was injected in the tail vein. After injection, blood samples were obtained sequentially from the retro-orbital plexus into disposable heparinised capillary tubes, diluted in saline, and plated on TSA. The zero time values were calculated by estimating total blood volume as 8% v/w of each body weight of the mice used.¹³ Results are expressed as the mean recovery percentage in five mice.

Screening for mannose sensitivity and bacterial surface sugars

To examine the mannose sensitivity of bacterial strains, bacterial suspensions were mixed with mannosylated taxonobeads (E-Y Laboratories, Inc., San Mateo, CA, USA) in the absence or presence of 0.25 M

Fig. 1. Clearance of systemic strains from blood in mice. Results are expressed as the mean recovery percentages in five mice. (a) *P. aeruginosa* strains: ○, D4; ●, D271; ★, H3; ■, H8; △, H9; □, H12; ▲, H13; ☆, H15. (b) *M. morganii* strains: ○, L222; △, L322; ●, L48; ▲, L421; □, H32; ■, H35.

Fig. 2. Clearance of portal strains from blood in mice. Results are expressed as the mean recovery percentages in five mice. The clearance of portal strains was significantly faster than that of systemic strains, i.e., *P. aeruginosa* and *M. morganii* at all time intervals ($p < 0.01, 0.02, 0.05$ at 5, 15, 30 min after bacterial challenge, respectively): —□, *Esch. coli* L33; —★, *Esch. coli* L424; —□, *Esch. coli* L529; —■, *Esch. coli* H21; —■, *Esch. coli* H25; —△, *E. cloacae* L31; —△, *E. cloacae* L317; —☆, *E. cloacae* L522; —▲, *E. cloacae* L527; —⊙, *K. pneumoniae* H29; —○, *Ent. faecalis* L425; —●, *Ent. faecium* L211; —●, *Ent. faecium* L410.

D-mannose (Nacalai Tesque Inc., Kyoto, Japan) on a slide as described previously.¹⁶ Agglutination inhibited by mannose was considered to be a positive result for mannose sensitivity. To examine the mannose and fucose on the surface of the bacteria, agglutination was examined with Concanavalin A (Vector Laboratories, Inc., Burlingame, CO, USA) and *Ulex europaeus* lectin I (UEA-I; Vector), each at a concentration of 1 mg/ml.

Inhibition of blood clearance by sugars

Bacteria were incubated with or without 100 mM D-mannose at 37°C for 30 min. After washing with saline, bacteria were injected into mice as described above. In some experiments, 100 mg of D-mannose, D-galactose and D-fucose (Wako Pure Chemical Industries, Ltd, Osaka, Japan) was injected intravenously 10 min before bacterial challenge.

Assessment of bacterial cell-surface hydrophobicity

Cell-surface hydrophobicity was determined by adherence to liquid hydrocarbon as described previously.¹⁷ A bacterial suspension at an optical density of 1.2 at 400 nm (1-cm cell width) determined with a spectrophotometer was added to n-hexadecane (Nacalai) (1 in 6), and the mixtures were agitated uniformly for 2 min. After 15 min, when the hydrocarbon phase had separated completely, the optical density at 400 nm of the bottom aqueous phase was read. Results are expressed as the percentage decrease in the absorbance of the aqueous phase compared with that of the initial cell suspension.

Clearance of portal strains in CBA/J and CBA/N mice

Six-week-old male CBA/N (SPF) mice carrying an

Table II. Agglutination of bacterial strains with mannosylated beads. Con A and UEA-I

Bacterial strain	Agglutination with		
	mannosylated beads	Con A	UEA-I
<i>Esch. coli</i> L33	—	+	—
<i>Esch. coli</i> L424	+	—	—
<i>Esch. coli</i> L529	—	—	—
<i>Esch. coli</i> H21	±	+	—
<i>Esch. coli</i> H25	+	+	—
<i>E. cloacae</i> L31	±	+	—
<i>E. cloacae</i> L317	+	+	+
<i>E. cloacae</i> L522	±	—	—
<i>E. cloacae</i> L527	+	+	±
<i>K. pneumonia</i> H29	—	+	—
<i>Ent. faecalis</i> L425	—	+	—
<i>Ent. faecium</i> L211	+	+	—
<i>Ent. faecium</i> L410	±	+	—
<i>P. aeruginosa</i> D4	—	+	—
<i>M. morganii</i> L48	—	—	—

X-linked immune defect associated with the absence of mature or late developing B lymphocytes,^{18,19} and their normal counterpart, CBA/J (SPF) mice, were used to examine the influence of immunoglobulin on blood clearance. Recovery percentages of *Esch. coli* L424 and L529, *E. cloacae* L317, and *Ent. faecalis* L525 from blood at 30 min after injection were compared in CBA/J and CBA/N mice. In other experiments, heat-inactivated serum, heat-inactivated serum absorbed with heat-killed *Esch. coli* L424, and heat-inactivated serum absorbed with heat-killed *Esch. coli* L529 obtained from CBA/J mice were transferred (0.2 ml of serum/mouse) into CBA/N mice. After 30 min, blood clearance of *Esch. coli* L424 was compared in these mice.

Statistical analysis

Student's *t* test was used to compare means, and a *p* value of 0.05 was taken to be significant.

Fig. 3. Influence of pre-incubation of bacteria with D-mannose on clearance from blood in mice. Results are expressed as the mean recovery percentages (SD) in five mice. There was no significant difference between the clearance rates of mannose-treated *Esch. coli* L424 and those of the control; ●, *Esch. coli* L424 incubated with 100 mM D-mannose for 30 min at 37°C before injection; ○, control.

Fig. 4. Influence of initial injection of D-mannose into mice on clearance of bacteria from blood. Results are expressed as the mean recovery percentages (SD) in five mice. The clearance of *Esch. coli* H21 in mice given D-mannose before bacterial challenge was significantly slower than in control mice at all time intervals ($p < 0.01$ at 15 min; < 0.02 at 5 and 30 min after bacterial challenge): ○, mice given 100 mg of D-mannose 10 min before the bacterial challenge; ●, control.

Table III. Surface hydrophobicity of bacterial strains

Systemic		Portal	
Strain	Hydrophobicity* (%)	Strain	Hydrophobicity* (%)
<i>P. aeruginosa</i> D4	26.33	<i>Esch. coli</i> L33	3.76
<i>P. aeruginosa</i> D271	16.00	<i>Esch. coli</i> L424	0.49
<i>P. aeruginosa</i> H3	83.71	<i>Esch. coli</i> L529	4.49
<i>P. aeruginosa</i> H8	79.82	<i>Esch. coli</i> H21	0.92
<i>P. aeruginosa</i> H9	1.55	<i>Esch. coli</i> H25	0.03
<i>P. aeruginosa</i> H12	1.90	<i>E. cloacae</i> L31	-5.93
<i>P. aeruginosa</i> H13	83.25	<i>E. cloacae</i> L317	3.55
<i>P. aeruginosa</i> H15	4.42	<i>E. cloacae</i> L522	-0.44
<i>M. morganii</i> L48	66.73	<i>E. cloacae</i> L527	3.08
<i>M. morganii</i> L222	-3.45	<i>K. pneumoniae</i> H29	7.70
<i>M. morganii</i> L322	-1.80	<i>Ent. faecalis</i> L425	86.72
<i>M. morganii</i> L421	75.22	<i>Ent. faecium</i> L211	79.95
<i>M. morganii</i> H32	9.13	<i>Ent. faecium</i> L410	33.22
<i>M. morganii</i> H35	22.60		

* Results are expressed as means of three assays.

Fig. 5. Clearance of portal strains from blood in CBA/J (▨) and CBA/N (□) mice. Results are expressed as the mean recovery percentages (SD) in five mice 30 min after bacterial challenge. The clearance of *Esch. coli* L424 and *E. cloacae* L317 in CBA/N mice was significantly slower than in CBA/J mice ($p < 0.01$ and 0.05 , respectively). No differences were observed with *Esch. coli* L529 and *Ent. faecalis* L425.

Results

Blood clearance of bacteria in mice

Blood clearance rates of *P. aeruginosa*, and *M. morganii* strains are shown in figs. 1a and b. Blood clearance rates of portal strains are shown in fig. 2. The portal strains were cleared significantly more rapidly than the systemic strains at every time interval; 57.98% (SD 36.62) and 12.67% (SD 8.60) of viable bacteria remained in the blood of mice 5 min after injection of systemic and portal bacteraemic strains respectively ($p < 0.01$). At 30 min after injection, 18.25% (SD 26.38) of bacteria of systemic strains were recovered whereas only 0.10% (SD 0.07) of portal strains were recovered ($p < 0.05$).

Agglutination of bacterial strains with mannosylated beads, Con A, and UEA I

Table II shows that *Esch. coli* strains L424, H21 and H25, *E. cloacae* strains L31, L317, L522 and L527, and *Ent. faecium* strains L211 and L410 were mannose-sensitive, indicating that these strains carry mannose-type lectin on their surfaces. All other bacterial strains, including *P. aeruginosa* D4 and *M. morganii* L48 failed to agglutinate mannosylated beads and were considered not to be mannose sensitive. On the other hand, *Esch. coli* strains L33, H21 and H25, *E. cloacae* strains L31, L317 and L527, *K. pneumoniae* H29, *Ent.*

faecalis L425, and *Ent. faecium* strains L211 and L410 agglutinated with Con A, and agglutination was inhibited by mannose. *E. cloacae* strains L317 and L527 also showed agglutination with UEA-I that could be inhibited by glucose.

Inhibition of blood clearance by sugars

As shown in fig. 3, clearance of mannose-sensitive strain *Esch. coli* L424 was not inhibited by pre-incubation of D-mannose with bacteria to block their surface mannose-type lectin. No difference was found between blood clearance rates of D-mannose treated bacteria and controls with the other mannose-sensitive strains.

On the other hand, fig. 4 demonstrates that the blood clearance of *Esch. coli* H21 was inhibited by injection of D-mannose into mice before bacterial challenge. At 30 min after challenge, 0.77% (SD 0.17) of viable bacteria could be recovered from D-mannose-treated mice, compared with 0.08% (SD 0.01) from control mice ($p < 0.01$). However, the clearance of other strains that agglutinated with Con A or UEA-I was not inhibited by pre-treatment of mice with D-mannose, D-galactose, or D-fucose (data not shown).

Bacterial cell-surface hydrophobicity

As shown in table III, the hydrophobicity of systemic strains ranged from -3.45% to 83.71%. The

Fig. 6. Influence of transfer of serum from CBA/J mice into CBA/N mice on clearance of *Esch. coli* L424 from blood. Results are expressed as the mean recovery percentages (SD) in five mice. The clearance of *Esch. coli* L424 in CBA/N mice given heat-inactivated serum or heat-inactivated serum absorbed with *Esch. coli* L529 was significantly faster than in CBA/N mice that were untreated or had been given heat-inactivated serum absorbed with *Esch. coli* L424 ($p < 0.01$): ○, untreated CBA/N mice; ■, CBA/N mice given heat-inactivated serum; △, CBA/N mice given heat-inactivated serum absorbed with *Esch. coli* L529; ▲, CBA/N mice given heat-inactivated serum absorbed with *Esch. coli* L424; ●, CBA/J mice.

gram-negative bacteria causing portal bacteraemia showed little or no affinity with hexadecane. In contrast, *Ent. faecalis* L424 and *Ent. faecium* strains L211 and L410 showed significantly higher hydrophobicity compared with systemic strains and gram-negative portal strains ($p < 0.01$).

Blood clearance of portal bacteraemic strains in CBA/J and CBA/N mice

As shown in fig. 5, significantly greater numbers of *Esch. coli* L424 and *E. cloacae* L317 were recovered from the blood of CBA/N mice than from CBA/J mice ($p < 0.01$ and 0.05 , respectively). No differences were observed between CBA/J and CBA/N mice in blood clearance of *Esch. coli* L529 or *Ent. faecalis* L425.

Fig. 6 shows that transfer of heat-inactivated serum or heat-inactivated serum absorbed with *Esch. coli* L529 obtained from CBA/J mice into CBA/N mice significantly enhanced the blood clearance of *Esch. coli* L424 ($p < 0.01$) at all time intervals. However, heat-inactivated serum absorbed with *Esch. coli* L424 did not enhance the blood clearance of this strain in CBA/N mice.

Discussion

Results suggest that the blood clearance of bacteria isolated from the portal circulation of mice is generally more rapid than that of systemic strains. Opsonic phagocytosis and non-opsonic phagocytosis seem to act as complementary processes in protection against invading micro-organisms.

Some studies with *Salmonella typhimurium*,¹³ *Esch. coli*,¹⁴ and *Serratia marcescens*¹⁵ have shown that blood clearance or liver clearance of these micro-organisms is mediated by lectinophagocytosis. In this study most bacterial strains that were cleared rapidly from blood carried single or multiple factors on their surfaces facilitating lectinophagocytosis. Indeed, blood clearance of *Esch. coli* H21 could be inhibited by prior injection of mannose into the mice, indicating that the clearance of this strain was mediated by mannose-type lectin on the surface of tissue macrophages, but blood clearance of other strains was not inhibited by giving initial mannose, galactose or fucose to block corresponding lectins on the surfaces of macrophages, nor by incubation of bacteria with mannose to block their surface mannose-type lectin. These findings suggest that either lectinophagocytosis was ineffective or that additional factors may be involved in the clearance of these strains from the bloodstream.

Bacterial cell-surface hydrophobicity has been also cited as an important factor in mediating phagocytosis,⁸ but in this study no correlation was found between bacterial cell-surface hydrophobicity and blood clearance, except for enterococci. *P. aeruginosa* and *M. morgani* strains that resisted blood clearance showed greater hydrophobicity than did isolates that were eliminated rapidly from blood.

We demonstrated previously that normal murine serum enhanced bacterial association with Kupffer cells only for *Ent. faecalis* L425 but not for gram-

negative bacteria,¹⁶ and that bacterial association of these gram-negative bacteria did not decrease at 4°C *in vitro*. Such findings suggest that association of these strains with Kupffer cells may be mediated by non-opsonic phagocytosis. However, in this *in-vivo* study there were significant differences between blood clearance of some portal strains in CBA/N mice with X-linked immunodeficiency in B cells that lack normally differentiated B-1 cells,^{20,21} and clearance in CBA/J mice. Moreover, blood clearance of *Esch. coli* L424 in CBA/N mice was enhanced by transfer of serum obtained from CBA/J mice. The absorption of the serum with *Esch. coli* L424 before transfer strikingly suppressed the enhancement of blood clearance. These observations indicate that specific or cross-reactive antibodies against this organism may have existed in the mice used and contributed to elimination of this strain from the blood.

We have shown that the significant differences in blood clearance between the systemic and portal strains are common in the various strains used in this study. These findings indicate that elimination of bacteria may be critical in determining whether latent portal bacteraemia progresses to systemic bacteraemia. Our data suggest that opsonophagocytosis, lectinophagocytosis or hydrophobic interaction are involved in clearance from the circulation of mice of some bacterial strains, but other factors are probably involved also. Further studies are required to clarify the mechanism of the blood clearance of these strains.

References

- Katz S, Jimenez MA, Lehmkuhler WE, Grosfeld JL. Liver bacterial clearance following hepatic artery ligation and portacaval shunt. *J Surg Res* 1991; **51**: 267-270.
- Katz S, Plaisier BR, Folkening WJ, Grosfeld JL. Intralipid adversely affects reticuloendothelial bacterial clearance. *J Pediatr Surg* 1991; **26**: 921-924.
- Praaning-van Dalen DP, Brouwer A, Knook DL. Clearance capacity of rat liver Kupffer, endothelial, and parenchymal cells. *Gastroenterology* 1981; **81**: 1036-1044.
- Rogers DE. Host mechanisms which act to remove bacteria from the blood stream. *Bacteriol Rev* 1960; **24**: 50-66.
- Biozzi G, Stiffel C. The physiopathology of the reticulo-endothelial cells of the liver and spleen. In: Popper H, Schaffner F (eds) *Progress in liver diseases*, vol II. London, William Heinemann Medical Books. 1965: 166-191.
- Saba TM. Physiology and physiopathology of the reticulo-endothelial system. *Arch Intern Med* 1970; **126**: 1031-1052.
- Ofek I, Sharon N. Lectinophagocytosis: a molecular mechanism of recognition between cell surface sugars and lectins in the phagocytosis of bacteria. *Infect Immun* 1988; **56**: 539-547.
- Absolom DR. The role of bacterial hydrophobicity in infection: bacterial adhesion and phagocytic ingestion. *Can J Microbiol* 1988; **34**: 287-298.
- Speert DP, Eftekhari F, Puterman ML. Nonopsonic phagocytosis of strains of *Pseudomonas aeruginosa* from cystic fibrosis patients. *Infect Immun* 1984; **43**: 1006-1011.
- Boner G, Mhashilkar AM, Rodriguez-Ortega M, Sharon N. Lectin-mediated, nonopsonic phagocytosis of type 1 *Escherichia coli* by human peritoneal macrophages of uremic patients treated by peritoneal dialysis. *J Leukoc Biol* 1989; **46**: 239-245.
- Sharon N, Ofek I. Mannose-specific bacterial surface lectins. In: Mirelman D (ed) *Microbial lectins and agglutinins: properties and biological activity*. New York, John Wiley and Sons Inc. 1986; 55-81.
- Ashwell G, Harford J. Carbohydrate-specific receptors of the liver. *Annu Rev Biochem* 1982; **51**: 531-554.
- Leunk RD, Moon RJ. Association of type 1 pili with the ability of *Salmonella typhimurium* to clear *Salmonella typhimurium*. *Infect Immun* 1982; **36**: 1168-1174.
- Perry A, Ofek I. Inhibition of blood clearance and hepatic tissue binding of *Escherichia coli* by liver lectin-specific sugars and glycoproteins. *Infect Immun* 1984; **43**: 257-262.
- Rumelt S, Metzger Z, Kariv N, Rosenberg M. Clearance of *Serratia marcescens* from blood in mice: role of hydrophobic versus mannose-sensitive interactions. *Infect Immun* 1988; **56**: 1167-1170.
- Hirakata Y, Tomono K, Tateda K *et al.* Role of bacterial association with Kupffer cells in occurrence of endogenous systemic bacteremia. *Infect Immun* 1991; **59**: 289-294.
- Rosenberg M, Gutnick D, Rosenberg E. Adherence of bacteria to hydrocarbons: a simple method for measuring cell-surface hydrophobicity. *FEMS Microbiol Lett* 1980; **9**: 29-33.
- O'Brien AD, Scher I, Campbell GH, MacDermott RP, Formal SB. Susceptibility of CBA/N mice to infection with *Salmonella typhimurium*: influence of the X-linked gene controlling B lymphocyte function. *J Immunol* 1979; **123**: 720-724.
- Scher I. CBA/N immune defective mice: evidence for the failure of a B cell subpopulation to be expressed. *Immunol Rev* 1982; **64**: 117-136.
- Kantor A. A new nomenclature for B cells. *Immunol Today* 1991; **12**: 388.
- Pecquet SS, Ehrat C, Ernst PB. Enhancement of mucosal antibody responses to *Salmonella typhimurium* and the microbial hapten phosphorylation in mice with X-linked immunodeficiency by B-cell precursors from the peritoneal cavity. *Infect Immun* 1992; **60**: 503-509.