Enhancement of *Streptococcus faecalis* Infection and Complement Depletion in Yeast-treated Mice

By GUSTAVO NEVES NOBRE* and ANTONÍO FRAZÃO FERREIRA

Instituto Bacteriológico Câmara Pestana, University of Lisbon, Rua do Instituto Bacteriológico, 1100 Lisbon, Portugal

(Received 21 January 1985; revised 27 September 1985)

Enhancement of *Streptococcus faecalis* infection and lowering of the complement level have been demonstrated in mice injected with a heat-treated suspension of baker's yeast (*Saccharomyces cerevisiae*). The leucocyte response to the infection was not affected. The yeast preparation showed, in vitro, an intense anti-complementary activity on mouse serum and interfered with the microbial killing function of the mouse peritoneal macrophages. No significant stimulation of the growth of *S. faecalis* in vitro in the presence of the yeast was observed. The enhancement of the infection in mice treated with the yeast seems to be mediated, mainly, by complement depletion.

INTRODUCTION

*Candida albicans* stimulates infections caused by various bacteria, e.g. *Staphylococcus aureus, Escherichia coli, Serratia marcescens* and *Streptococcus faecalis* (Gale & Sandoval, 1957; Carlson, 1982, 1983). Also, heat-killed baker's yeast (*Saccharomyces cerevisiae*) has been used as a virulence-enhancing agent in the active mouse protection test to assay the potency of *Salmonella typhi* vaccines because of the low virulence of *Salmonella typhi* to mice under normal conditions (Joó & Zsidai, 1982). Thus, these yeasts seem to have an amplifying effect on infections caused by other organisms, although the mechanism involved is not known. It is possible, however, that such amplifying effects are due to some components of the yeasts with anti-complementary activity, known as zymosan (Ecker *et al.*, 1943), since genetic and acquired complement deficiencies, in man and animals, are responsible for various bacterial infections.

Repeated pyogenic infections have been reported in patients with C3 (Alper *et al.*, 1970a, b, 1972) and C5 (Dick *et al.*, 1983) deficiencies of complement. One of the cases was a 25-year-old man who had had 28 hospital admissions for otitis media, mastoiditis, posterior auricular abscess, sinusitis, bronchopneumonia, pneumonia, septicaemia, meningooccaemia and skin infections. The bacteria isolated with highest frequency were *Staphylococcus aureus, Proteus vulgaris* and *Pseudomonas aeruginosa*. The concentrations of serum complement components were normal, except for that of C3, which was less than one-third of normal. Various complement-mediated functions, such as bactericidal activity for smooth Gram-negative organisms, chemotaxis for leucocytes and the enhancement of the phagocytosis of pneumococci by normal leucocytes, were deficient (Alper *et al.*, 1970a, b). Another case of C3 deficiency was a 15-year-old girl who had a history of 20 hospital admissions for pneumonia, meningitis, otitis and skin infections, and was found to have 1/1000th or less of the normal serum concentration of C3. The bacteria isolated included *Streptococcus pneumoniae, Streptococcus pyogenes* and *Klebsiella pneumoniae* (Alper *et al.*, 1972). Patients with C6, C7 and C8 deficiencies of complement are particularly prone to systemic infections by *Neisseria gonorrhoeae* and *N. meningitidis* (Lee *et al.*, 1978; Ragnaud *et al.*, 1984). Among laboratory animals, C5-deficient

**Abbreviations:** YS, yeast suspension; i.p., intraperitoneally.
mouse strains are particularly susceptible to *Streptococcus pneumoniae* (Shin et al., 1969) and to a bacterium pathogenic for mice, *Corynebacterium kutscheri*, and C₅-deficient rabbits have a low serum bactericidal activity against salmonellae (Eisen, 1980).

In the work described here we demonstrated the enhancing effect of heat-treated baker's yeast suspension on the virulence of *Streptococcus faecalis* to mice and investigated the mechanism involved in this effect.

**METHODS**

**Yeast suspension (YS).** Commercial baker's yeast suspended at 5% (wt/wt) concentration in isotonic saline solution was heated at 100 °C for 30 min on two consecutive days.

**Infection in mice.** *Streptococcus faecalis* strain 5620, isolated from the genital tract of a woman, and identified according to the scheme of Noble (1978) was used.

The enhancing effect of YS in the infection of mice was evaluated by determining both the rate of death and the number of viable bacteria appearing in the peritoneum. To study the rate of death we inoculated, intra-peritoneally (i.p.), four groups of 16 mice with, respectively, 2.5 x 10⁷, 5 x 10⁷, 1.1 x 10⁸ and 2.2 x 10⁸ cells of *S. faecalis* in 1 ml YS and another four groups of 16 mice with, respectively, 1.3 x 10⁶, 2.5 x 10⁶, 5 x 10⁶ and 10¹¹ cells in 1 ml of isotonic saline solution. The inoculum was prepared from plate cultures of Columbia Blood Agar Base (Difco) with 5% (v/v) horse blood incubated for 24 h at 37 °C. The mice were observed for 6 d. The data obtained in these tests were evaluated by regression analysis after probit transformation according to the method of Finney (1978).

To determine the number of bacteria in the peritoneum, two groups of nine mice were injected i.p. with 1.3 x 10⁸ cells of *S. faecalis* in 1 ml YS and in 1 ml of an isotonic saline solution, respectively. At 2, 6 and 24 h after injection of bacteria, the number of viable organisms remaining in the peritoneal cavity of each of three mice from each group was determined by viable counts of peritoneal washes (5 ml isotonic saline injected i.p. followed by abdominal massage and aspiration).

**Complement assay.** We studied the effect of YS on mouse serum complement in *vitro* and in vivo. Complement was titrated by the method of Gewurz & Suyehira (1980), with some changes. Erythrocytes of ox (not of sheep) were used, and at one-tenth the concentration used to titrate the complement in human serum (McGhee, 1952). The method assessed the activity of the classical complement pathway.

**In vitro,** we titrated, in parallel, the complement in serum treated with YS and in serum not submitted to such treatment. The serum was treated according to the technique of Lachmann & Hobart (1978). Undiluted serum was treated with the yeast [0.2 ml of 50% (wt/wt) heat-killed YS per ml serum] at 37 °C for 45 min and the yeast removed by centrifugation.

For the study in *vitro,* we titrated the complement in 18 samples of serum obtained from mice that had been inoculated i.p. with 1 ml YS 6 h before, and in a similar number of serum samples from non-inoculated mice. To study the time-course of the complement titre in mice after intraperitoneal injection of YS, complement was titrated in groups of mice challenged with YS, 2, 6, 24, 48 and 72 h before. Blood samples were obtained by collection in the thoracic cavity after removal of the heart and lungs under chloroform anaesthesia. Each titration was performed on a pool of serum from 2 to 10 mice. All the experiments were randomized and the statistical evaluation of the results was made by variance analysis (Lison, 1968).

**Microbial killing function of peritoneal macrophages.** We used the technique of Territo & Cline (1980). Peritoneal cells were collected from normal mice by peritoneal washing with Hanks' balanced salts solution without antibiotics. The same strain of *S. faecalis* was used in the experiments and serum from normal mice was the source of opsonins. We compared the percentage of bacteria killed by the macrophages, after 1 h incubation at 37 °C, in the presence and in the absence of YS (5%, v/v). The trial was done 12 times and always randomized. The statistical evaluation of results was made by variance analysis (Lison, 1968).

**Leucocyte response to the infection.** The number of leucocytes in peripheral blood was counted in three groups, each of about 30 mice. One group comprised normal mice, one group was inoculated i.p. with 10⁸ *S. faecalis* in 1 ml of an isotonic saline solution, and one group was inoculated i.p. with the same number of bacteria in 1 ml YS. Blood was sampled from the tail 6 h after inoculation with *S. faecalis*, and leucocytes were counted after lysis of erythrocytes with 1% (v/v) acetic acid.

**In vitro growth stimulation.** We studied the growth of *S. faecalis* strain 5620 in Nutrient Broth no. 2 (Oxoid), in the presence and absence of YS (5%, wet wt/v). Three simultaneous trials were done. Viable bacteria were counted in samples at 3, 8 and 24 h.

**RESULTS**

**Infection in mice.** The majority of mice died within 24–28 h of inoculation. Probit regression lines relating the death rate to the inoculum of *S. faecalis* are presented in Fig. 1.
Infection in complement-depleted mice

Fig. 1. Corrected probit regression lines for virulence in mice of *S. faecalis* with (●) and without (○) YS.

Fig. 2. Complement titres in pooled sera of groups of 10 mice before, and 2, 6, 24, 48 and 72 h after, inoculation with YS.

The number of *S. faecalis* found in the peritoneal cavity, expressed as the number of per ml of peritoneal washing, was very different in the two groups, after inoculation of $1 \times 10^8$ bacteria, being (means ± sd, $n = 3$) $(5.5 \pm 1.7) \times 10^6$ at 2 h, $(4.0 \pm 2.9) \times 10^5$ at 6 h and $(4.6 \pm 3.3) \times 10^4$ at 24 h, in the group challenged with *S. faecalis* only, and $(3.1 \pm 2.2) \times 10^7$ at 2 h, $(6.4 \pm 2.4) \times 10^4$ at 6 h, and $(3.8 \pm 3.5) \times 10^7$ at 24 h, in the group challenged with a mixture of *S. faecalis* and YS.

Complement assay. The YS used appeared to have intense anti-complement activity, *in vitro*, on the mouse serum: the titre of the assayed serum (in complement units, CH$_{50}$), initially 7.8 U, fell to zero after YS treatment.

In randomized experiments *in vivo* also, there were highly significant differences ($P < 0.01$) in the titres of complement between non-inoculated mice (4.1 ± 2.4 U, $n = 18$), and mice inoculated with YS 6 h before, (1.9 ± 1.6 U, $n = 18$). The time-course of complement titres within a period of 72 h after inoculation of YS is shown in Fig. 2.
Macrophage function. The study of the killing effect of mouse peritoneal macrophage on \textit{S. faecalis} in vitro showed that the percentage of bacteria killed was significantly higher ($P \leqslant 0.01$) in the absence ($62.6 \pm 18.4\%$, $n = 12$) than in the presence ($28.8 \pm 21.8\%$, $n = 12$) of YS (Fig. 3).

Leucocyte response to the infection. The number of leucocytes in peripheral blood (mean no. $\mu l^{-1}$, $\pm$ SD) of mice was $6014 \pm 2348$ in non-inoculated mice, $8460 \pm 7393$ in mice inoculated with \textit{S. faecalis} in saline and $8406 \pm 6146$ in mice inoculated with \textit{S. faecalis} in YS (Fig. 4). In the last two groups, mice with high leucocytosis were found, but the results were quite similar and no animal suffered leucopenia. According to Schaffner \textit{et al.} (1983) we can consider leucopenia to be present in mice only when there are fewer than 700 leucocytes per $\mu l$ of blood.

\textit{In vitro} growth stimulation. In three trials we found no significant difference between growth in the presence and absence of YS.

**DISCUSSION**

The components of treated yeasts that have an anti-complementary action (Pillemer \& Ecker, 1941), and were called zymosan by Ecker \textit{et al.} (1943), effect depletion of the C$_3$ component of the complement, as a consequence of activation of the alternative pathway, and they slightly affect the C$_1$, C$_4$ and C$_2$ components of complement (Lachman \& Hobart, 1978). The yeast preparation that we used clearly exerted a relevant anti-complementary activity both \textit{in vitro} and \textit{in vivo}, also by activation of the alternative pathway. In the animals treated with YS, the values of CH$_{50}$ fell significantly as a consequence of C$_3$ consumption. \textit{In vitro}, YS also interfered with macrophage killing function. This effect was probably mediated by its action on the complement because the killing function of macrophages depends on the opsonization of microorganisms by IgG and by the C$_3$ complement fraction (MacGowan \textit{et al.}, 1983).

Although various evidence indicates that yeasts or yeast components depress the specific T-cell-mediated immune response (Rogers \& Balish, 1980), we have not done experiments to evaluate the capacity of YS to inhibit the functions of activated macrophage and/or T-lymphocytes. \textit{In vitro}, in microbial killing tests, we have used only macrophages of normal mice, which were probably not activated. \textit{In vivo}, our experimental model was not adequate to explore the T-cell-mediated immunity. \textit{S. faecalis} infection in mice is an acute process, mortality ensuing within a few days of challenge; Schaffner \textit{et al.} (1983) demonstrated that in mice infected by \textit{Listeria monocytogenes}, early mortality associated with administration of cortisone resulted from inhibition of the antimicrobial activity of nonimmune macrophages, and only the late mortality associated with the administration of cyclosporin A was a consequence of the inhibition of specific T-cell-mediated immunity.

Although complement fragments detached as a consequence of activation by zymosan are chemotactic attractants for neutrophils (Ward, 1971), the pronounced leucocytosis that we observed in some animals inoculated i.p. with \textit{S. faecalis} and YS was probably due only to the bacterial infection, because the generation of C$_{5\alpha}$ and C$_{3\alpha}$ in the peritoneal cavity would be expected to lead to blood neutrophil migration to this inflammatory site with consequent neutropenia, 6 h after injection of YS.

No stimulation of the growth of \textit{S. faecalis} \textit{in vitro} was found in the presence of the baker's yeast preparation, which contradicts the hypothetical mechanism postulated by Carlson (1983) for the amplifying effect of \textit{Candida albicans} upon infections caused by \textit{Staphylococcus aureus}.

In conclusion, it seems that the enhancement of \textit{S. faecalis} infection produced by baker's yeast is mainly due to its action on complement and can be compared to the higher susceptibility to pyogenic cocci observed in man and laboratory animals with C$_3$ and C$_5$ deficiencies of complement.

We are indebted to Mrs Olivia Cruz for technical assistance.
Infection in complement-depleted mice

REFERENCES


