Analysis of the Specific Immune Response against Capsular Polysaccharides of Two Patients with Systemic Enterococcal Infections

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Abstract

Systemic enterococcal infections often lead to life-threatening disease. By analyzing the immune response of two patients with systemic enterococcal infections against enterococcal polysaccharide antigens, we found that both patients had antibodies against all four of the capsular serotypes identified to date. Antibody concentrations against the causative capsular serotype were in the same range as antibodies against the other three capsular protoserotypes. Interestingly, we noted a difference between the two patients with respect to opsonic activity in the killing assay: one patient showed better killing of all four capsular prototypes than the other. However, killing against the infecting serotype was not increased in comparison to killing of the other serotypes in the two patients. This finding supports previously published data that most healthy humans possess preexisting, naturally acquired, anti-enterococcal antibodies. We conclude, therefore, that systemic infection with enterococci does not lead to higher antibody concentrations or better opsonic killing against the causative capsular serotype.

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Introduction

Systemic infections with enterococci are a major problem in hospitals worldwide: First, enterococci are the third most common pathogen isolated from bloodstream infections [1]. Second, the multiple antibiotic resistance of many strains results in rising hospital costs and in increased mortality, especially for infections due to vancomycin-resistant bacteria [2].

Little is known about the normal immune response to enterococcal infections [3]. *Rakita* et al. [4, 5] have observed that proteins of the complement system play an important role in the elimination of enterococci, but these investigators and others [6–10] have also emphasized the role of specific antibodies that promote opsonic killing. The targets of these opsonic antibodies were unknown until recently, when *Hufnagel* et al. [11] demonstrated that healthy human sera contain opsonic antibodies that are — at least partially — directed against capsular polysaccharides of four *Enterococcus faecalis* prototype strains. These authors used a capsular polysaccharide (CPS) enzyme-linked immunosorbent assay (ELISA) to detect IgG and IgM antibodies against four *E. faecalis* prototype strains (i.e. CPS-A to CPS-D [12]) in human sera from 12 healthy humans. These naturally acquired antibodies were further tested for their opsonic killing activity in an opsonophagocytosis assay (OPA) [11]. The healthy individuals possessed higher titers of opsonic antibodies directed more specifically against CPS-A and CPS-B strains than against CPS-C and CPS-D strains [11]. The present study was done to investigate whether patients with systemic enterococcal infections also possess specific antibodies against enterococcal capsular polysaccharides, and whether these antibodies promote serotype-specific opsonic killing.

Patients and Methods

The bacteria used in the present study are described in detail elsewhere [12]. The four capsular prototype strains (CPS-A, CPS-B, CPS-C, and CPS-D [12]) were grown without agitation at 37 °C either in Todd-Hewitt broth (THB, Becton Dickinson, Sparks, MD), or, for the capsular polysaccharide-specific ELISA (CPS-ELISA), in Columbia broth with the addition of 0.5% glucose (CBG, Difco Laboratories, Detroit, MI).

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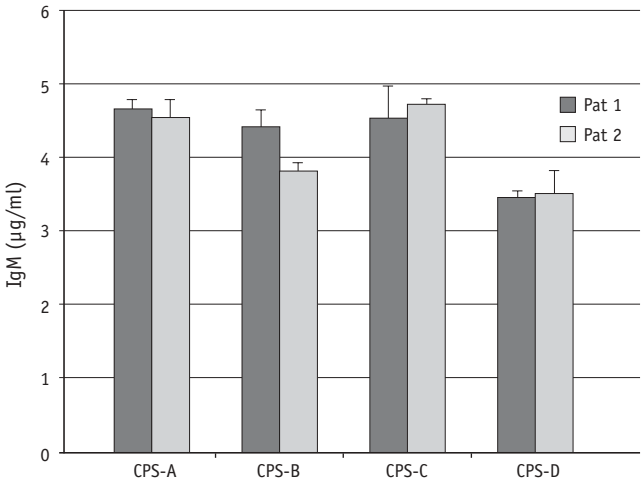

Figure 1. IgG concentrations in the CPS-ELISA against capsular polysaccharides of four *E. faecalis* prototype strains in two patient sera (normal values, 1 to 10 µg/ml [11]).

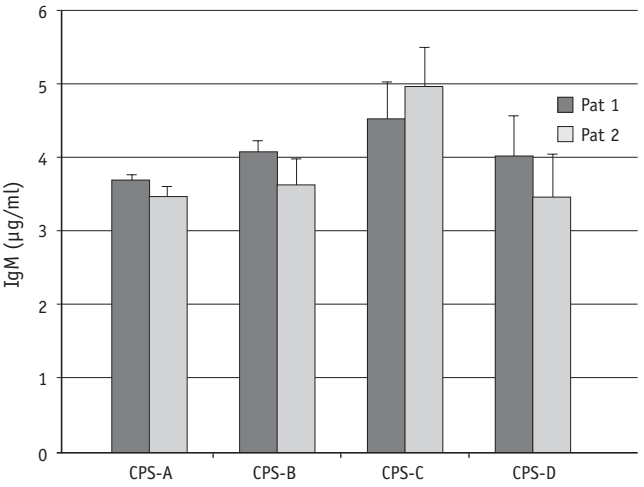

Figure 2. IgM concentrations in the CPS-ELISA against capsular polysaccharides of four *E. faecalis* prototype strains in two patient sera (normal values, 1 to 30 µg/ml [11]).

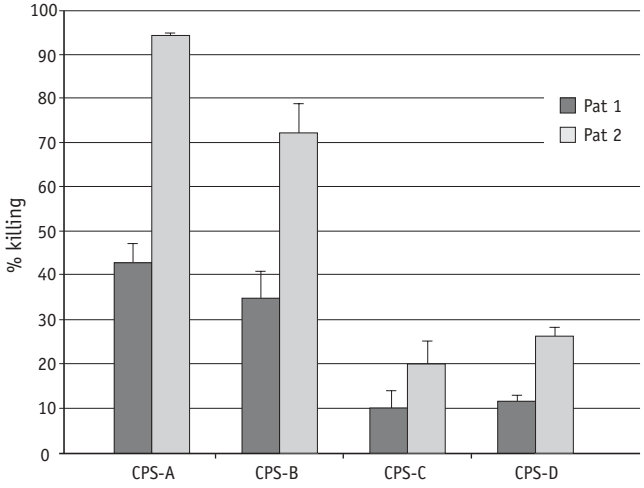

Figure 3. Opsonophagocytic killing of two patient sera against the four *E. faecalis* capsular prototype strains.

Reconvalescent sera and bacterial strains were taken from two patients with systemic *E. faecalis* infections (i.e. positive blood cultures). Sera and bacteria were stored at –80 °C before use.

Capsular polysaccharide-specific antibodies were measured using an ELISA format as described elsewhere [11]. Crude polysaccharide extracts to coat ELISA microtiter plates were prepared by an extraction method using trichloroacetic acid (TCA). The sugar content of the polysaccharide extract was quantified by the hexose assay [13]. 96-well microtiter plates (Immulon 2HB, Dynex Technologies, Chantilly, VA) were sensitized with 1 µg antigen per well. The patient sera were used in dilutions of 1:100, 1:1,000, and 1:5,000. In a row parallel to the primary antibodies, an IgG or IgM standard was added in different concentrations (from 0 ng/ml to 250 ng/ml). The secondary antibody (i.e. goat anti-human IgG whole molecule or IgM whole molecule, alkaline phosphatase-conjugated, Sigma Chemicals, St. Louis, MO) was used at a dilution of 1:1,000. The detection reaction was done with p-nitrophenyl-phosphate (Sigma Chemicals, St. Louis, MO). Plates were read in a Bio-Tek EL 309 ELISA reader (Bio-Tek Instruments, Winooski, VT). Resulting titers at an optical density at 405 nm were normalized against negative controls. Antibody concentrations were calculated by linear regression using the respective IgG and IgM standard.

The opsonophagocytosis assay was performed as described elsewhere [9, 10]. The sera were heated at 56 °C for 30 min to inactivate complement components. The complement was absorbed with the homologous enterococcal isolate before use, as described elsewhere [11]. The patient sera were tested against the four *E. faecalis* prototype strains (CPS-A, CPS-B, CPS-C, and CPS-D [12]).

To assign the two *E. faecalis* isolates to one of the four capsular prototype strains, serotype-specific immune sera from rabbits were used in a CPS-specific ELISA as described elsewhere [12].

Statistics

For statistical analysis, Microsoft Excel and GraphPad PRISM V.3 were used. Correlations were calculated using linear regression models and comparisons between two groups were made with Student's t test. Comparisons among three or more groups were performed by ANOVA using Tukey's Multiple Comparison test for pair-wise comparisons. A p-value of $< .05$ was considered to be statistically significant.

Results and Discussion

The results of the CPS ELISA indicated that the enterococcal pathogen isolated from patient 1 was serologically reactive with serotype-specific immune rabbit serum [12] raised against CPS-D (96% reactivity vs serotype-specific immune serum), while patient 2 was infected with a bacterial strain belonging to the CPS-B serogroup (89% reactivity vs serotype-specific immune serum, data not shown).

The CPS-ELISA results of the sera from the two patients are shown in figure 1 (IgG concentrations) and figure 2 (IgM concentrations). Both patients had IgG and IgM antibodies against all four capsular polysaccharide antigens. Neither patient showed higher amounts of antibodies against the infecting serotype in comparison to the other three serotypes.

Opsonic killing of the serum from patient 2 was better than that from patient 1 (Figure 3). Again, there was no increased killing activity against the respective infecting serotype (CPS-D for patient 1 and CPS-B for patient 2). The values for IgG and IgM concentrations, as well as for opsonic killing, were all within the range of those obtained from healthy human controls (1 µg/ml to 30 µg/ml for IgG or IgM concentrations) [11]. The better opsonic killing of the serum from patient 2 in comparison to that from patient 1 is similar to the findings in healthy human sera, where better killing against CPS-A and CPS-B strains could be depicted in comparison to poorer killing against CPS-C and CPS-D strains [11]. Whether this difference between the two serotype groups is caused by induction of lower frequency of opsonic antibodies or by increased resistance to opsonophagocytic killing remains to be determined.

Our data confirm that individuals, either healthy or reconvalescing from systemic enterococcal infections, possess antibodies against enterococcal capsular polysaccharide antigens. This may be related to occasional translocation of bacteria from the gastrointestinal tract into the bloodstream [14, 15], which occurs even in otherwise healthy individuals. The fact that patients with systemic infections do not mount a more profound antibody response in the CPS-ELISA against specific polysaccharide antigens of the infecting strain is surprising, but similar observations also have been made with other bacterial pathogens [16]. It may be possible that only a small fraction of the antibodies binding to enterococcal polysaccharides in the CPS-ELISA are responsible for opsonization and protection. We [17] and others [18–20] have shown that several different polysaccharide antigens exist within enterococci. Antibody to some or all of these antigens may be measured in the CPS-ELISA [11].

Finally, our data indicate that quantitative differences in opsonic activity among patient sera exist. Whether these differences correlate with unequal susceptibility to infection, or, whether they correspond to a more mitigated course of the disease, should be assessed in a larger prospective study.

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