Enhanced neutrophil emigration and Porphyromonas gingivalis reduction following PGG-glucan treatment of mice

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Abstract

Periodontal disease is the consequence of a mixed Gram-negative infection in the gingival sulcus and has been associated with deficits in the neutrophil response. A novel, and heretofore untested, alternative approach to therapy is the use of biological-response modulators that enhance the neutrophil response. Poly-$
\beta_1$-$\beta_6$-glucotriosyl-$\beta$-$\beta_1$-$\beta_3$-glucopyranose glucan (PGG-glucan) is an immunomodulator, derived from yeast, which specifically enhances neutrophil priming, phagocytosis and bacterial killing while failing to induce inflammatory cytokine expression. The hypothesis tested was that PGG-glucan could enhance host resistance to a Gram-negative periodontal pathogen, Porphyromonas gingivalis. Chambers were implanted subcutaneously in the dorsolumbar region of C57BL/6J mice and allowed to heal for 14 days. PGG-glucan was administered subcutaneously to one-half of the animals and saline to the other half. In the first set of experiments the chambers were inoculated with $P$. gingivalis (A7436) at $4 \times 10^6$, $4 \times 10^7$, and $4 \times 10^8$ colony-forming units (CFU). In the second set of experiments the chambers were inoculated with $5 \times 10^8$ CFU of either P. gingivalis or Streptococcus sanguis, a Gram-positive oral microbe that is not periodontopathic. Chambers were sampled over the following 2 weeks. The results demonstrated that: (1) bacterial CFU and neutrophils increased with increasing bacterial inoculum ($P < 0.02$); (2) bacterial CFU were lower in the PGG-glucan-treated animals than in the saline controls ($P < 0.02$); and (3) neutrophil counts were higher in the PGG-glucan-treated animals than in the saline controls ($P < 0.01$). These results indicate that PGG-glucan significantly enhances neutrophil emigration and bacterial killing, thus decreasing the bacterial infection in this model system.

Keywords: Neutrophils, Immunomodulation, Bacterial infection, P. gingivalis

1. Introduction

Oral infection with Porphyromonas gingivalis and Bacteroides forsythus has been associated with periodontal disease (Holt et al., 1988; Haffajee and Socransky, 1994; Socransky et al., 1998). Variations in bacterial virulence (Holt and Bratthall, 1991), the cellular host response (Schepkin and Van Dyke, 1994; Novak and Novak, 1996) and cytokine expression (Page and Kornman, 1997; Kornman et al., 1997) may account for variations in susceptibility to periodontal disease and lack of response to therapy. Treatment of these infections is mechanical, with or without antimicrobial therapy to reduce or eliminate the infection. The use of antimicrobials is, however, of concern in relation to accurate diagnosis, appropriate selection, microbial resistance and allergic reactions (Neu, 1992).

A potentially powerful, and novel, alternative approach to the problem of infection control is the use of biological-response modifiers that enhance the host phagocyte response without the expression of damaging inflammatory cytokines. A polysaccharide derived from yeast, PGG-glucan...
2. Materials and methods

2.1. Animals

Two sets of experiments were carried out on 8–12-week-old female C57BL/6J mice (n = 30 and 52, respectively) (Charles River Laboratories, Wilmington, MA). Animals were kept in the Forsyth Institute animal facility under specific pathogen-free conditions. Their general health, and the number and severity of postsurgical infections (Babineau et al., 1995). We have now examined the effect of PGG-glucan on a local P. gingivalis infection, using a chamber implanted subcutaneously in the rat.

2.2. Chamber implantation

Coil-shaped subcutaneous chambers were prepared from 0.028 in., stainless steel round wire (GAC International Inc., Manati, PR). Over the following 2 weeks, the external incision healed completely in all mice.

2.3. PGG-glucan administration

Animals were randomly divided into two groups. One group was injected subcutaneously on the contralateral side of the body from the chamber with 0.5 mg/kg PGG-glucan (Biopolymer Engineering Inc., Minneapolis, MN) 24 h before, and on the day of bacterial challenge. This dose was maximally effective in previous studies (Patchen and MacVittie, 1986; Babineau et al., 1992). The other group served as controls and received injections of sterile saline in the same location.

2.4. Bacterial strains

The periodontopathogen P. gingivalis A7436 was originally isolated from a patient with refractory periodontitis. Streptococcus sanguis, a Gram-positive strain associated with oral health, was obtained from the Forsyth Institute collection. Both strains were grown on anaerobic blood-agar plates in an atmosphere of 80% N2, 10% H2 and 10% CO2. Two days before inoculation, the bacteria were placed in supplemented Schaedler broth (Difco Laboratories, Detroit, MD). Following suspension culture, the bacteria were centrifuged at 2500 × g for 10 min at room temperature, resuspended in 10-fold dilutions of the original volume in prereduced Schaedler broth, and their concentration estimated at OD A745 = 1.2, corresponding to approximately 109 CFU/ml. The actual bacterial inoculum was confirmed by serial dilution and plating.

2.5. Infection

In the first experiment, animals (n = 30) were divided into three groups of 10 each to determine the dose-response to three inocula of P. gingivalis (4 × 107, 4 × 108, 4 × 109 CFU) in the presence of PGG-glucan or saline (n = 5/group each). In the second experiment, animals (n = 42) were divided into three groups of 14 each to determine the response to two bacteria, S. sanguis (5 × 108 CFU) and P. gingivalis (5 × 109 CFU), and to Schaedler broth. Each group of 14 was also subdivided into two groups of seven each to compare the effects of PGG-glucan with saline.

2.6. Chamber fluid sampling

Fluid was aseptically aspirated from implanted chambers with a 25G hypodermic needle at days −1, 1, 4, 7 and 14 relative to bacterial infection. A 10 μl sample was obtained from each chamber and diluted 1:50 in Hank’s balanced salt solution. Samples were used for bacteriological culture, total white blood cell and differential counts.

2.7. Microbial analysis

A portion (100 μl) of the diluted chamber fluid was streaked on to anaerobic blood-agar plates (Remel, Lenexa KA) and cultured in an anaerobic chamber. The CFU/ml was determined by direct colony counting.

2.8. White blood-cell analysis

A portion (200 μl) of the diluted sample was centrifuged in a Cytospin apparatus (Shandon Inc., Pittsburgh, PA)
for 3 min at 850 rpm/min. Following cytocentrifugation, slides were stained with Wright-Giemsa for differential cell counts, which were done by light microscopy at 40× magnification. All leucocytes in three random fields were counted using an ocular micrometer and the number of granulocytes determined.

2.9. Statistical analysis

Kruskal–Wallis ANOVA was used to compare responses to bacterial CFU and leucocyte emigration at day 1. Repeated-measures ANOVA was used to compare the effects of PGG-glucan and saline over the entire time course.

3. Results

3.1. Effect of PGG-glucan on bacterial infection

The initial experiments examined the ability of PGG-glucan-treated mice to lessen *P. gingivalis* infections from subcutaneously implanted stainless steel chambers. As shown in Fig. 1A–C, at day 1 the peak number of recoverable viable bacteria increased with increasing bacterial inoculum for all animals (*P* = 0.02 for PGG-glucan, *P* = 0.004 for saline; both Kruskal–Wallis ANOVA) and decreased thereafter. Injection with PGG-glucan also effected a reduction in the chamber bacterial CFU when compared to saline.
Following infection with $4 \times 10^8$ CFU there was a significant reduction in bacteria in the PGG-glucan-treated group when compared to the saline-treated group ($P = 0.048$; repeated-measures ANOVA). Although there was a clear trend toward a reduction in PGG-glucan-treated animals inoculated with $4 \times 10^6$ and $4 \times 10^7$ CFU, the differences were not statistically significant. Similar reductions following PGG-glucan administration were seen in four additional experiments (data not shown).

3.2. Effect of PGG-glucan on leucocyte emigration

The ability of PGG-glucan to modulate the number of polymorphonuclear leucocytes emigrating into the chambers following infection with $P$. gingivalis was then examined. As shown in Fig. 1(D–F), the number of leucocytes increased with increasing bacterial infection in all animals ($P = 0.02$ for PGG-glucan, $P = 0.008$ for saline; both Kruskal–Wallis ANOVA). Emigration peaked at day 1 and declined thereafter. The number of leucocytes was higher and remained elevated longer in the animals treated with PGG-glucan than in those treated with saline. These differences were only statistically significant in the group inoculated with $4 \times 10^8$ CFU ($P = 0.007$, repeated-measures ANOVA). Similar qualitative, but not identical quantitative, results were obtained in four additional experiments (data not shown).

3.3. PGG-glucan effects following infection with a pathogenic or non-pathogenic oral species

In the second series of experiments, PGG-glucan- and saline-treated mice were infected with $P$. gingivalis or

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with *S. sanguis*, a non-periodontopathic species. As shown in Fig. 2A–C, the bacterial CFU for both *P. gingivalis* and *S. sanguis*-infected animals was significantly lower than for saline-treated control animals (both \( P < 0.002 \); repeated-measures ANOVA). PGG-glucan-treated animals were able to clear completely the *P. gingivalis* infection whereas the infection persisted in the saline-treated animals. The animals injected with Schaedler broth as a sterility control exhibited no bacterial growth in either the PGG-glucan- or saline-treated groups.

Fig. 2D–F shows how PGG-glucan affected the emigration of polymorphonuclear leucocytes. In the presence of PGG-glucan the leucocyte count increased significantly, compared to baseline, following infection with both *P. gingivalis* and *S. sanguis* (both \( P < 0.001 \); Kruskal–Wallis ANOVA). Counts were also significantly higher than in the saline-treated controls for *P. gingivalis* \( (P = 0.001; \) repeated-measures ANOVA) but not for *S. sanguis*. The leucocyte count in the PGG-glucan-treated, *P. gingivalis*-infected animals was significantly higher than in the PGG-glucan-treated, *S. sanguis*-infected animals \( (P = 0.001; \) repeated-measures ANOVA). Notably, the count did not increase after the chamber was injected with sterile Schaedler broth, indicating that the leucocyte immigration was not randomly stimulated by PGG-glucan in the absence of infection. Taken together, these results suggest that PGG-glucan increases the responses of polymorphonuclear leucocytes to both pathogenic and non-pathogenic species, and that the magnitude of this response is a function of the infecting microorganism.

4. Discussion

Our results support the hypothesis that an immunomodulator, PGG-glucan, can increase host resistance to pathogenic bacterial infections. This protective response appeared to be mediated by polymorphonuclear leucocytes, as an inverse relation between the number of infecting bacteria and the number of polymorphonuclear leucocytes was consistently observed (Fig. 2C and F). The response indicates that in addition to the previously noted effects of PGG-glucan on leucocyte production, priming, bacterial phagocytosis and killing (Onderdonk et al., 1992; Babineau et al., 1993; Stashenko et al., 1995), PGG-glucan also enhances leucocyte emigration to sites of infection.

Aggressive, early-onset or refractory periodontal disease has been consistently associated with a decrease in either the number or function of polymorphonuclear leucocytes (Schenkein and Van Dyke, 1994). Our findings with PGG-glucan, which enhances both leucocyte number and functional activity (Stashenko et al., 1995), demonstrate that this immunomodulator can increase resistance to a specific periodontal pathogen. The molecular mechanism(s) that underlie these effects are not well understood but are under investigation.

An interesting observation was that the PGG-glucan-stimulated leucocyte response, rather than being generically enhanced, appeared to be specifically regulated by the host only to the extent necessary to combat the infection successfully. For example, following PGG-glucan administration, there was no leucocyte emigration into the sterile Schaedler broth-injected chambers (Fig. 2D), a modest but significant emigration into the *S. sanguis*-infected chambers (Fig. 2E) and a vigorous emigration into the *P. gingivalis*-infected chambers (Fig. 2F). Thus, PGG-glucan does not appear to stimulate random leucocyte emigration throughout the body, but rather enhances a response that is regulated by the host. Of interest, the magnitude of the leucocyte response might be dependent on the potential pathogenicity of the infection and be modulated to the extent necessary to bring the infections under similar control.

When comparing the first and second series of experiments (e.g. Fig. 1C and Fig. 2C), we found that in the first series the total recoverable bacterial CFU was lower, and the bacteria persisted in the chambers for a shorter time, than in the second series. This interexperiment variation was observed in four other subsequent replicates. The relative relation between saline and PGG-glucan animals was, however, always very similar, i.e. PGG-glucan-treated animals always had significantly lower bacterial CFU and higher leucocyte counts than the saline-treated animals, indicating the reproducibility of the effect.

Taken together, these data point to the possibility that PGG-glucan may provide an entirely new therapeutic approach to preventing and treating bacterial infections, including those of the periodontium. This host-oriented approach may be especially timely, given the increasing use of systemic antibiotics to treat infections and the concern about the rise in bacterial resistance to these agents (Nes, 1992). In the oral environment, this approach could prove particularly beneficial for patients who are susceptible to periodontal disease due to a number of predisposing factors. These include a reduced phagocyte response (Schenkein and Van Dyke, 1994; Novak and Novak, 1996), increased cytokine response (Stashenko et al., 1991; Page and Kornman, 1997; Kornman et al., 1997), the persistence of bacterial pathogens (Haffajee and Socransky, 1994; Socransky et al., 1998), the presence of antibiotic-resistant bacteria (Slots and Rams, 1990), autoimmune disorders that affect the host response, environmental factors such as smoking, patients who require premedication for dental procedures, or those who are at risk of systemic or local infections after undergoing oral surgery or chemotherapy.

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References


