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MINIREVIEW

Phlogistic Properties of Peptidoglycan-Polysaccharide Polymers from Cell Walls of Pathogenic and Normal-Flora Bacteria Which Colonize Humans

JOHN H. SCHWAB

Department of Microbiology and Immunology, University of North Carolina School of Medicine, CB 7290, Chapel Hill, North Carolina 27599

INTRODUCTION

The localization in tissue of peptidoglycan-polysaccharide (PG-PS) polymers derived from bacterial cell walls leads to an acute inflammatory response which can evolve into a remittent chronic inflammatory process with a prolonged course (54). The PG-PS from several bacterial species, including normal flora as well as pathogenic bacteria, can induce such responses. The capacity to initiate and to sustain a chronic destructive inflammation is dependent upon resistance to biodegradation and consequent persistence in tissue. The structural basis for resistance to host muralytic enzymes, and other relevant biological properties, form part of this review.

The target tissue and severity of disease in the various experimental models described by ourselves and others depend upon the immunocompetence of the host, the species and genetic strain of animal, and route of injection (49). The experimental chronic diseases induced by PG-PS include remittent, nodular lesions of rabbit skin (41, 52); carditis in mice resembling rheumatic carditis (11); synovitis in rabbit knee joints (53, 64); granulomatous hepatitis in Lewis rats (71); uveitis in rats (72); granulomatous enterocolitis in rats which resembles Crohn's disease (47); intestinal hemorrhage in rats (45); trilineage hemopoietic hyperplasia (21); and chronic, remittent, erosive arthritis in rats (12). It is evident from this summary that PG-PS can induce multisystem disease.

The preparation of bacterial cell walls, isolation and fractionation of PG-PS, injection of animals, and monitoring of disease have been summarized in detail (68). Much of our work has employed PG-PS isolated from the cell walls of group A streptococci (*Streptococcus pyogenes*), which we label PG-APS, but the arthropathogenic activities of numerous other bacterial species also have been analyzed. The cellular and molecular mechanisms of tissue injury and regulation of remission and recurrence in experimental models of arthritis and intestinal inflammation have recently been reviewed (44, 51). The balance of this review will examine the evidence that PG-PS capable of inducing experimental arthritis and enteritis can be identified in intestinal contents and be isolated from pure cultures of normal-flora bacterial species and that it can be systemically distributed to joints and other tissue.

RELEVANT STRUCTURAL AND BIOLOGICAL PROPERTIES OF PG-PS

Properties of PG. PG and associated PS polymers of bacterial cell walls are unique chemical structures which are ubiquitous in our environment and have a broad range of biological activities (9, 24). A basically similar PG structure is present in almost all bacterial species, with important differences in details of cross-linking and certain amino acid components (24). PG can modulate the immune system (15, 50), and this is probably an important part of the pathogenesis of the experimental chronic inflammatory disorders listed above (49). The PG-APS, which we have studied most extensively, is toxic, immunogenic, and only slowly eliminated from tissue (17, 41). The basis of the toxicity is due, in part, to stimulation of secretion of interleukin-1, tumor necrosis factor alpha, and other cytokines, as well as oxygen radicals, nitric oxide, and eicosanoids, from a variety of cells, including macrophages, granulocytes, and lymphocytes (51). PG-APS has a well-defined chemical structure with at least four characterized immunodeterminant groups on the PG (48) and two on the PS (10) moiety. The antibody response of the rat to these epitopes on PG-APS has been analyzed by Esser et al. (19). The PG was shown to be immunogenic (1) and to be the primary toxic moiety (2). PG shares many biological activities with endotoxin (9), and both may use the same binding site on lymphocytes (16). Numerous other biological properties of PG have been described, including activation of the alternate and classical complement pathways (18, 23) and activation of macrophages to become cytotoxic (60), to release lysosomal enzymes (14), and to resorb bone (4).

Many of these and other activities have been ascribed to the muramyl dipeptide (*N*-acetyl-muramyl-L-alanyl-D-isoglutamine) structure of PG (6, 70); and certain analogs of muramyl dipeptide were reported to induce polyarthritis in rats (30). Aqueous suspensions of PG-PS or synthetic disaccharide-dipeptide have also been reported to induce an acute arthritis in certain inbred strains of mice (29). Fleming et al. have shown that PG produced by *Neisseria gonorrhoeae*, especially a lysozyme-resistant O-acetylated form produced by some strains, could be involved in gonococcal arthritis (20). The gonococcal PG injected into Lewis rats in a water-in-oil emulsion induced a severe synovitis with pannus which progressed to erosion of cartilage and bone (61).

Properties of the PS and enzymatic degradation of PG-PS. The PS moiety, covalently bound to the PG, modulates PG activity and is essential for the capacity of PG-PS complexes to persist and to induce chronic inflammation. The PS moiety can be any PS which is covalently bound to PG, including the ribitol teichoic acids. This excludes the capsular PSs. The group A streptococcal polysaccharide (APS) component of PG-APS is especially effective in protecting PG from tissue muralytic enzymes, and it also masks the acute

toxicity of PG, thereby permitting chronic irritation (2, 41). The cell wall of group A streptococci and the isolated PG-APS are very resistant to avian or mammalian lysozyme, and this is the reason for the persistence of PG-APS in tissue for prolonged periods (17, 22, 66). This extreme resistance of PG-APS is largely due to the APS moiety, since PG-PS polymers from many other bacteria, which differ in the details of composition and structure of the PS, are more susceptible to in vitro degradation by lysozyme. The relative in vitro resistance to lysozyme correlates with their persistence in tissue and their capacity to induce prolonged chronic inflammation (65). Structural features of the PG are also important determinants of lysozyme degradation, as shown by the effect of N or O acylation on resistance to lysozyme and arthropathic activity (67). Spitznagel et al. (63) reported that treatment of cell walls from group A, B, or D streptococci with lysozyme exposed different amounts of amino sugar reducing groups on the PG, and this correlated with alteration of complement activation by the PG. In addition to lysozyme, other mammalian enzymes such as serum *N*-acetylmuramyl-L-alanine amidase and endopeptidase can degrade isolated PG or MDP, but their significance in "detoxification" or removal of PG-PS in vivo is uncertain (22).

Although PG-APS is very resistant to mammalian and avian lysozymes, it can be degraded in vitro by mutanolysin (endo-*N*-acetylmuramidase) produced by *Streptomyces globisporus* or by group C streptococcal phage-associated lysin (*N*-acetylmuramyl-L-alanine amidase) to fragments with M_rs of less than 5×10^6, which cannot induce chronic erosive arthritis (8). Mutanolysin can also function in vivo to prevent or treat arthritis induced by PG-APS. A single intravenous injection of 0.4 mg/kg protects against development of chronic arthritis, even if treatment is delayed until arthritis is established (25, 27). Part of the in vivo protective effect of mutanolysin is due to the loss of complement activation by PG-APS (26).

We have also shown that the purified APS has relevant biological activity. This substance is able to induce rapid, transient edema in the limbs of rats (7), and this is due to the selective degranulation of mast cells in the limbs (13). Low-molecular-weight PG-APS fragments derived by cleavage with muramidase enzymes, which are too small to induce arthritis, can also induce early microvascular changes and edema in rat limbs (8), but this is more prolonged than the response elicited by PS and is not associated with mast cell degranulation (13). Cell wall derivatives with this effect on microvasculature could be a part of the pathogenesis of experimental arthritis by influencing distribution of arthropathic PG-APS fragments into synovial tissue or by effecting expression of leukocyte adhesion molecules or secreted mediators by endothelial cells (73).

ARTHROPATHOGENIC ACTIVITY OF INTACT CELLS OR PG-PS FROM CELL WALLS OF NORMAL BACTERIAL FLORA

As noted in the first description of bacterial cell wall-induced arthritis, cell wall fragments from some bacterial species other than group A streptococci can also induce acute arthritis following systemic injection, and with some of these the disease progresses into a chronic, erosive synovitis (12). These species include beta-hemolytic streptococci of groups B, C, and H. It is important that intact heat-killed group A and group B cells, at equivalent doses of cell wall rhamnose, could also induce chronic arthritis, but in the case of group A streptococci, only after a lag time of 56 to 120 days after intraperitoneal injection (12, 62). Stimpson et al. (65, 69) showed that PG-PS isolated from group D streptococci (*Streptococcus faecium*) and *Peptostreptococcus productus*, both normal-flora species, could also induce chronic arthritis, whereas PG-PS from *Propionibacterium acnes* and the pseudomurein-PS complex isolated from *Methanobacterium formicium* could not. However, PG-PS from group D streptococci could only induce both acute and chronic phases of the arthritis if care was taken to stabilize it by heating and extracting with detergent to eliminate autolytic enzyme activity (65). This is consistent with the concept that prolonged inflammation requires persistence of PG-PS, which in turn is dependent upon resistance to muralytic enzymes, either host lysozyme or microbial autolytic enzymes (22, 53). Cell wall fragments from some *Lactobacillus* species can also induce chronic inflammation (33).

In our experience, cell wall fragments from group A streptococci produce the most impressive chronic recurrent erosive arthritis, and disease remains active for at least 4 months after intraperitoneal injection. Disease induced with material from most other species, although often severe, subsides after 2 to 3 months (65). We have associated this with in vitro susceptibility to lysozyme and persistence in tissue (66, 67), but others have reported that this association of in vitro resistance to lysozyme and severe chronic arthritis is not seen with the strict anaerobes *Eubacterium aerofaciens* and *Coprococcus ramosum* (57). In a series of studies Severijnen and coworkers showed that arthropathic cell walls could be isolated from two strains of *Eubacterium contortum*, which are gram-positive anaerobic organisms that are prominent in the stools of patients with Crohn's disease (56). Arthropathic cell wall fragments were also isolated from anaerobic species common in the normal human intestine (58, 59), including *E. aerofaciens* and *Bifidobacterium* sp., but not from some other *Eubacterium* or *Clostridium* species examined (56). However, cell walls isolated from species of autochthonous intestinal flora of rats, which had resemblance to human strains of *E. aerofaciens*, did not induce chronic arthritis upon intraperitoneal injection (32).

ASSOCIATION OF INTESTINAL INFLAMMATION, SYSTEMIC DISTRIBUTION OF PG-PS, AND INFLAMMATION OF JOINTS AND LIVER

It is evident that arthropathogenic PG-PS polymers can be derived from the cell walls of a variety of bacteria, including many species making up the normal flora (58, 59), as well as bacteria which occur in increased numbers in the intestines of rheumatoid arthritis patients (57) and patients with Crohn's disease (56). The well-established association between arthritis and intestinal inflammation (43) has stimulated efforts to determine if, in fact, potentially arthropathogenic PG-PS products of bacterial degradation exist in the intestinal lumen and if they are systemically translocated to tissues such as the joints and liver. Kool et al. (31) have demonstrated that PG-PS complexes can be isolated from the feces of healthy humans and from ileostomy fluid. Material from an ileostomy fluid, but not that isolated from feces, could induce arthritis when injected subcutaneously in incomplete Freund's adjuvant. However, it is difficult for such studies to be definitive since much of the PG-PS in the intestine must be nonarthropathogenic because of properties related to structure or fragment size (59, 65), and this would greatly increase the dose of total cell wall required in order

to be effective. As a further complication, we have shown that the presence of relatively small (M_r of $<10^4$) nonarthropathogenic, enzyme-degraded PG-PS fragments can suppress development of arthritis (69).

Sartor and coworkers (46) have demonstrated that arthropathogenic, ^{125}I-labeled PG-APS fragments injected into rat cecum which had been inflamed with acetic acid could be detected in the liver, spleen, and mesenteric lymph nodes. In parallel assays, APS antigen, which is not found in the rat microbial flora, was also measured in the same tissues by enzyme-linked immunosorbent assay.

In another approach, jejunal self-filling blind loops, which cause small bowel bacterial overgrowth with very elevated numbers of anaerobic bacteria, were surgically created in rats (35). With this model, indirect evidence for the translocation of PG across the intestinal wall was provided by the increased level of anti-PG antibodies in the sera of rats with self-filling blind loops compared with that in sham-operated control rats or rats treated with metronidazole. In addition, following intraluminal injection of the inflamed loop with PG-APS, the APS antigen could be detected in the plasma and liver (35). Related studies also showed that circulating PG-PS can be eliminated in the bile (38). Evidence that the hepatobiliary injury that occurs in rats with small bowel bacterial overgrowth is initiated by PG-PS is provided by the protection of these animals by intravenous injection of mutanolysin, an enzyme whose only known activity is degradation of PG (37). About 50% of the PG-PS transported to the liver is localized in Kupffer cells, with the rest in parenchymal and endothelial cells (36). Evidence that PG-PS stored in the liver retains arthropathogenic activity and could provide a depot for induction of recurrent arthritis comes from experiments showing that transplantation of PG-PS-laden livers can cause reactivation of monoarticular arthritis in recipient rats that had been injected intra-articularly with PG-APS (34).

The significance of these findings for human disease is indicated by the serological evidence that immunoglobulin G and M antibodies to PG are elevated in sera of rheumatoid arthritis and juvenile rheumatoid arthritis patients (5, 28, 39, 40, 42), suggesting that immunogenic bacterial PG complexes can be systemically translocated in humans. That this may be related to transient or chronic changes in the mucosal barrier is indicated by studies summarized by Sartor (43). Bjarnason et al. (3) reported that two-thirds of rheumatoid arthritis patients have ileocecal inflammation; and although he associated this with mucosal injury caused by nonsteroidal anti-inflammatory drugs, others have proposed a primary relationship of intestinal inflammation and rheumatoid arthritis (55).

SUMMARY

PG-PS polymers which can induce experimental chronic inflammation in joints and other tissues can be isolated from the cell walls of human pathogens, such as group A streptococci, as well as from certain indigenous bacterial species which colonize the human intestinal tract. The structural and biological properties that are required for cell wall fragments to express this remarkable activity are still not well defined, but polymer size, resistance to tissue enzymes, and capacity to sustain activation of complement, macrophages, neutrophils, and T cells are properties associated with the most active preparations. There is increasing evidence that PG-PS structures with arthropathogenic activity occur in the human intestinal lumen and that these polymers can be translocated systemically. These observations support the concept that PG-PS, derived from a variety of bacterial species, can be part of the etiology of rheumatoid arthritis and other chronic inflammatory diseases. Since the PG component provides a common element to which all individuals are exposed, it follows that susceptibility is related to efficiency of disposal of bacterial cell wall debris, as well as to cytokine networks and immune cell function (51).

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