Uveitis and Arthritis Induced by Systemic Injection of Streptococcal Cell Walls

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A single injection of an aqueous suspension of group A streptococcal peptidoglycan-polysaccharide complexes (PG-PS) when injected intraperitoneally into Lewis rats induced a self-limiting bilateral uveitis with associated perpetuating polyarthritis. The uveitis was characterized clinically during the first 72 h by iritis and fibrin deposition. Acutely, there was infiltration of polymorphonuclear cells. The symptoms gradually subsided, and at the close of the experiment eyes were normally clinically and histologically. In contrast, perpetuating inflammation and severe tissue injury developed in the limb. Using an enzyme immunoassay with specificity for the group A streptococcal polysaccharide, the levels of PG-PS in tissues of animals that were killed 1 to 7 days post-injection were measured. The relative amounts of antigen in eye:limb:liver of PG-PS injected animals were 1:9:170. The differences in the amounts of antigen detected in the eye and limb may help explain the development of the acute uveitis in contrast to the perpetuating polyarthritis observed on PG-PS administration. The authors suggest that bacterial debris may act similarly in causing ocular inflammation in man. Invest Ophthalmol Vis Sci 27:921-925, 1986

Uveitis sometimes results from systemic infections with microorganisms; in these circumstances, viable microbial agents can sometimes be isolated from the eye,1,2 although this is not always the case. Uveitis can also occur following focal infections at sites distant from the eye including dysentery caused by Shigella, Salmonella, and Yersinia and is associated with polyarthritis and the histocompatibility antigen HLA-B27.3-8 Live bacteria have not been demonstrated to localize in the eye or joint in these cases. We have hypothesized that in HLA-B27 associated, and possibly other types of uveitis, the ocular inflammation may result from the dissemination of inflammatory bacterial debris to the eye.9 This might explain how ocular inflammation could occur in response to a bacterial infection, even though no live bacteria are ever present in the eye.

Certain bacterial products including Gram negative endotoxin10,11 and Freund's complete adjuvant (a suspension of heat-killed mycobacteria in mineral oil) when administered systemically in rats will elicit uveitis.12 In the latter animal model, polyarthritis is also a major disease feature, and it is commonly referred to as adjuvant arthritis. The active inflammatory constituent of the mycobacteria is the bacterial peptidoglycan.13 Another rat model of perpetuating polyarthritis involves the systemic administration of group A streptococcal peptidoglycan-polysaccharide in aqueous suspension.14 The arthritis has been noted to develop within 24-72 hr and to persist indefinitely. Uveitis has not been previously described in this animal model, although it does occur upon intravitreous injection of PG-PS in the rabbit.9 In the rat arthritis model, one major factor in the development of inflammation is deposition of PG-PS in inflamed tissue,15-18 although other factors are also of importance.19-23 In the present study we describe the elicitation of bilateral uveitis and polyarthritis on systemic administration of streptococcal cell wall* fragments in the Lewis rat. Studies on the dissemination of streptococcal antigens using a wheat germ lectin-immunoassay were performed.

Materials and Methods

Investigations utilizing animals conform to the ARVO Resolution on the Use of Animals in Research.

Bacterial Cell Wall Preparation

Streptococcus pyogenes (ATCC #10389) was grown overnight in 20 l of Todd Hewitt Broth (BBL Microbiology Systems; Cockeysville, MD) in a Biostat U20G fermenter (B. Braun Instruments; Burlington, CA). The cells were harvested using a Millipore Pellicon Cassette System (Millipore Corporation; Bedford, MA) and washed with sterile phosphate buffered saline (PBS). The cells (approximately 35 gm wet weight in 120-130 ml buffer) were sonicated in 45-ml batches
for 90 min (Heat Systems Ultrasonics, Inc.; Long Island, NY) and centrifuged for 30 min at 10,000 rpm. The pooled supernatants were sequentially treated with hyaluronidase (Sigma; St. Louis, MO) in 0.1 M phosphate/0.15 M NaCl (pH 5.3); deoxyribonuclease and ribonuclease (Calbiochem–Behring; La Jolla, CA) in 0.1 M phosphate buffer (pH 7.2); papain in 0.1 M phosphate buffer (pH 7.2) containing 0.001 M cysteine, 0.001 M EDTA and 0.05% sodium azide and pepsin (Sigma) in 0.012 N HCl, pH 2.0–0.0025 mg of each enzyme per mg wet weight of cells. The buffers were changed between each enzyme treatment by dialysis overnight at 4°C and the digestion itself was performed while dialysing at 37°C for 6 hr. The cell wall was collected and washed by repeated centrifugation at 11,000 g and extracted three times with chloroform: methanol:water (34:17:10) at room temperature. The water phase was then dialysed against water and lyophilized. The samples were analysed for their carbohydrate composition by gas chromatography-mass spectrometry (GC-MS) and were approximately 5.0% fucose as determined by GC-MS.

Dische and Shettles. (The LPS preparations were approximately 18–25% rhamnose.

The lyophilised cell wall preparation was sonicated at a concentration of 20 mg/ml in sterile phosphate buffered saline (pH 7.4) in a sonicator for 70 min. The sonicate was centrifuged at 10,000 r.p.m. for 30 min. The supernatant was removed and filtered using sterile acrodisc filters (Gelman Science; Ann Arbor, MI) in the following order: 3.0 µ, 1.2 µ, 0.8 µ, 0.45 µ, and 0.2 µ.

The concentration of cell wall sonicate or E. coli 0127:B8 lipopolysaccharide (LPS) (Sigma, lot #82F-4012) to be injected was based on rhamnose or fucose content respectively as determined by the method of Dische and Shettles. (The LPS preparations were approximately 5.0% fucose as determined by GC-MS).

Induction and Evaluation of Uveitis and Arthritis

One hundred gram Lewis rats purchased from Harlan Sprague–Dawley (Madison, WI) were anesthetized by intramuscular injection of a 0.1 ml combination of ketamine hydrochloride (Bristol Laboratories; Syracuse, NY) and xylazine (Haver–Lockhart; Shawnee, KS) (1:1). The animals were then injected intraperitoneally (i.p.) with 15 mg of sterile PG-PS or 150 µg of LPS in PBS (pH 7.3) or PBS alone. The animals were examined immediately prior to i.p. injection and then once a day for the first week and at least once a week thereafter for 60 or more days. An estimate of the severity of uveitis was made by grading on a scale of 1+ to 4+ anterior chamber haze, iris injection (using a slit lamp), ciliary injection and retinal and vitreal detail (using a dissecting microscope). Arthritis was assessed essentially as described by Wood, et al. A scale from 1+ to 4+ was used based on the degree of erythema, edema, and joint distortion of each of the four joints giving a maximum score of 16.

Animals were killed, and one eye from each animal was fixed in Kolmer's fluid, and a limb and a portion of liver were fixed in 10% neutral buffered formalin. All tissues were then sectioned and stained with hematoxylin and eosin. The other eye, a limb, and a portion of liver were stored at −20°C until use in an enzyme immunoassay.

Enzyme-Linked Immunoassay For Streptococcal Antigens

Rabbit antisera were elicited with a vaccine consisting of pepsinized heat-killed group A streptococci. Affinity purified antibodies against the streptococcal group-specific carbohydrate were prepared employing an agarose amylomlin β-D-N-acetyl glucosamine column (Pharmacia Fine Chemicals; Piscataway, NJ) and elution with N-acetyl glucosamine. Tissue samples were homogenized in PBS/0.05% Tween followed by boiling for 15 min. The suspensions were clarified by centrifugation and stored at −20°C until assayed. The enzyme immunoassay (EIA) was performed as follows: 200 µL wheat germ agglutinin (10 µg/ml) (Sigma) in PBS was added to the wells of a microtitre plate (NUNC-immunoplate 1 with certificate, Vanguard International; Neptune, NJ) incubated at 37°C for 1 hr, and the wells washed with PBS/Tween containing 0.5% bovine serum albumin (BSA), using a multi-channeled washing apparatus (Vanguard International). Three hundred fifty microliter 0.5% BSA in PBS was then added to each well, incubated for 60 min at 37°C followed by washing. Two hundred microliter of twofold serial dilutions of standard suspensions of PG-PS, buffer alone, or tissue homogenates were added and incubated overnight at 4°C and the wells washed. One hundred microliter of the affinity-purified antibody solution was added, incubated for 1 hr at 37°C and washing performed. One hundred microliter of a 1:500 dilution of biotin-labeled goat anti-rabbit IgG (Cappel Worthington Biochemicals; Malvern, PA) was added, incubated for 1 hr at 37°C, and the wells washed. Two hundred fifty microliter of PBS/BSA was added incubated for 1 h at 37°C, and washing performed. One hundred microliter of a 1:50,000 dilution of avidin–peroxidase conjugate (Cappel) was added, incubated for 1 hr at 37°C, and then removed by washing. One hundred microliter of o-phenylenediamine dihydrochloride (Sigma), (0.4 mg/ml, in phosphate citrate buffer pH 5.0 containing 0.012% H2O2) was then added incubated at 37°C for 45 min and the reaction terminated by adding 50 µl of 8N H2SO4. Absorbance of the samples was measured in a multichanneled photometer (Titertek Multiskan; Flow Laboratories).

Results

Clinical and Histological Observations

PG-PS elicited a bilateral anterior uveitis and polyarthritis. Both diseases developed within 24–72 hr.
Fig. 1. A, Acute inflammation of the rat eye and hind limb 2 days post-injection of PG-PS. B, Chronic inflammation of the rat hind limb 86 days post-injection with PG-PS. Note the normal appearance of the eye. C, Normal appearance of rat eye and hind limb 2 days after injection of PBS.

Although the uveitis was easily observable initially, the inflammation largely subsided within 1–2 wk. However, the arthritis became progressively more severe and persisted until the close of the experiments. Figure 1A demonstrates the appearance of animals 2 days post-injection with PG-PS. The anterior chamber contained fibrin deposits and cellular infiltrates with vessel engorgement and tortuosity of the irides. An associated arthritis was apparent. Figure 1B shows an animal 86 days post-injection with PG-PS showing the normal appearance of the eye and a highly inflamed limb. LPS also elicited an acute bilateral uveitis that peaked within 24–48 hr, and was clinically undetectable 1 wk post-injection. The animals did not develop arthritis. PBS did not elicit any clinical manifestations, which is demonstrated in Figure 1C. The course of the inflammatory response of the eyes is plotted in Figure 2, and the inflammation of the hind limbs in Figure 3.

Histological observations of animals killed at 1, 3, 5, 7, and 60 days post-injection with PG-PS are summarized as follows. On day 1, there were no signs of inflammation in the uvea. At day 3, polymorphonuclear cells were present in the uvea, anterior chamber, and in the anterior choroid. Anterior choroiditis and kerato-conjunctivitis were the major features at day 5. At day 7, the eyes of some animals were completely normal and indistinguishable from the controls, whereas others had a few polymorphonuclear cells in the vitreous body; however, there were no other signs
of inflammation in any regions of the eye. Although the eyes appeared normal at 60 days, the joints were chronically inflamed with both bone and cartilage erosion and pannus formation. Figure 4 shows a low power H & E stained section of part of the anterior segment of a rat eye 3 days post-injection with PG-PS. A severe acute inflammatory cell infiltrate is readily apparent in the iris and ciliary body with aggregates of granulocytes and fibrin in the anterior chamber. Focal limbal keratoconjunctivitis is also evident.

Enzyme Immunoassay For PG-PS

In initial experiments the specificity of the affinity-purified antibody was determined by an EIA. The binding of the antibody to PG-PS was inhibited almost totally by N-acetyl glucosamine. The antibody did not bind to N-acetyl mannosamine or N-acetyl galactosamine (isomers of N-acetyl glucosamine) or D-alanine-D-alanine (the major immunodeterminant of peptidoglycan). Figure 5 demonstrates these observations.

This antibody was used in an EIA to measure PG-PS levels in tissue. The antigen assay had a sensitivity of 600 pg/ml with purified PG-PS standards. However, components of tissue that we have not yet identified inhibited binding of PG-PS, decreasing the sensitivity of the assay by approximately 12-fold. The levels at day 4 in one experiment were eyes 1.4 μg/g wet weight of tissue, hind limbs 14 μg/g, and livers 138 μg/g. Antigen was detected in all samples. The mean PG-PS levels in tissues during the first 7 days in another experiment were as follows: eyes 0.69 μg/g, hind limbs 6.5 μg/g, livers 166 μg/g. The antigen was detected in each of the 16 livers and 15 of the 16 hind limbs assayed but only in 8 of the 16 eye samples, including none of the four eye samples from animals killed 7 days post-injection. The persistence of PG-PS in tissues for extended periods of time was confirmed. Levels in the livers from animals killed 60 days post-injection were about 1.6 μg/g. Antigen was detected in the limbs but at such a level that quantitation was not possible.

Discussion

Peptidoglycan-polysaccharide complexes isolated from gram-positive bacteria display many of the properties of gram-negative endotoxin (lipopolysaccharide). These include the ability to activate phagocytic cells to cause tissue injury. The activation of the alternate complement cascade, a well-established property of PG-PS is probably involved. The poor biodegradability of PG-PS helps explain how this bacterial debris persists in tissues to act as a chronic stimulus for tissue injury. PG-PS is also antigenic, and thus its continued presence in tissue could lead to the production of immune complexes and immunopathology.

We note for the first time that the Lewis rat develops concurrent uveitis and polyarthritis on intraperitoneal
administration of PG-PS. Although the joint inflammation is severe and persistent, the ocular inflammation is limited in duration.

We are interested in determining the explanation for the different patterns of inflammation observed in the eye versus the joint after systemic administration of PG-PS. It was hypothesized that the variation might be related to differential deposition and/or persistence of streptococcal antigens in different tissues. An EIA was developed to study this question. Using this assay, streptococcal antigen was detected in the eye during the first several days post-injection; however, the levels were considerably lower than in the limbs. It is possible that the differences in the amounts of PG-PS that initially disseminate to the different tissues may explain the different clinical patterns noted. We established in previous work that chronic inflammation in the joint was related to persistence of streptococcal antigens.\(^\text{14}\) It remains to be determined whether trace amounts of these materials also persist in the eye.

The study of the mechanism of PG-PS-mediated uveitis in rats may give insights into a possible role for bacterial debris in causing ocular disease in man.

Key words: uveitis, peptidoglycan, arthritis, streptococcus, enzyme immunoassay

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References