Group G Streptococcal Lymphadenitis in Rats

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Group G streptococci which have been isolated from the oral flora of rats are also normal inhabitants of the human skin, oropharynx, gastrointestinal tract, and female genital tract. This group of streptococci can cause a wide variety of clinical diseases in humans, including septicemia, pharyngitis, endocarditis, pneumonia, and meningitis. Ten days after oral gavage with 7,12-dimethylbenz(a)anthracene, 12 of 22 two-month-old, female, outbred, viral-antibody-free rats presented with red ocular and nasal discharges and marked swelling of the cervical region. Various degrees of firm, nonpitting edema in the region of the cervical lymph nodes and salivary glands as well as pale mucous membranes and dehydration were observed. Pure cultures of beta-hemolytic streptococci were obtained from the cervical lymph nodes of three rats that were necropsied. A rapid latex test system identified the isolates to have group G-specific antigen. These streptococcal isolates fermented trehalose and lactose but not sorbitol and inulin and did not hydrolyze sodium hippurate or bile esculin. A Voges-Proskauer test was negative for all six isolates. Serologic tests to detect the presence of immunoglobulin G antibody to rat viral pathogens and Mycoplasma pulmonis were negative. Histopathologic changes included acute necrotizing inflammation of the cervical lymph nodes with multiple large colonies of coccoid bacteria at the perimeter of the necrotic zone. To our knowledge, this is the first report of naturally occurring disease attributed to group G streptococci in rats.

The most common streptococcal species encountered in rats is alpha-hemolytic Streptococcus pneumoniae, which often colonizes the oral cavity. In cases of overt clinical disease caused by S. pneumoniae, bacteremia and bronchopneumonia often develop and may lead to pulmonary consolidation, fibrinopurulent pleuritis, pericarditis, and peritonitis (10).

Group G beta-hemolytic streptococci have also been isolated from the oral flora of rats (13). Their location in the oral cavity presented an opportunity for this organism to be used to experimentally induce valvular endocarditis in rats (13). After the presence of periodontal disease and catheter-induced endocarditis was confirmed, tooth extractions were performed. Group G streptococci as well as other organisms were isolated from blood cultures drawn immediately after tooth extraction. Group G streptococci were cultured from the aortic valves of 15 of 18 animals with experimental endocarditis (13).

Asymptomatic pharyngeal carriage of group G streptococci is found in up to 23% of humans (15). These bacteria are normal inhabitants of the human skin, oropharynx, gastrointestinal tract, and female genital tract. However, group G streptococci can cause a wide variety of clinical diseases in humans, including septicemia, pharyngitis, endocarditis, pneumonia, and meningitis (6). They are also the major streptococcal type isolated as commensal flora from the skin and mucosa of dogs (2) but can also cause abscesses, dermatitis, polyarthritis, mastitis, and other genital infections (8).

Contagious streptococcal lymphadenitis caused by group G beta-hemolytic streptococci in laboratory cats has been reported (7, 17). Cervical lymphadenitis, also caused by group C streptococci, in horses (18), swine (11), and guinea pigs (14) has been documented. To our knowledge, naturally occurring disease attributed to group G streptococci in rats has not been reported. The purpose of this report is to describe an outbreak of group G streptococcal cervical lymphadenitis in a colony of rats treated with the carcinogenic agent 7,12-dimethylbenz(a)anthracene (DMBA).

MATERIALS AND METHODS

A group of 24 two-month-old, female, outbred, viral-antibody-free rats were obtained from a commercial vendor for the purpose of studying the efficacy of a chemotherapeutic agent used to treat mammary carcinomas. Animals were housed in an American Association for the Accreditation of Laboratory Animal Care-approved facility in groups of two to four in polycarbonate cages (19 by 10 1/2 by 8 in. [1 in. = 2.54 cm]) with Micro-Isolator (Lab Products, Inc., Maywood, N.J.) lids. They received an alpha-tocopherol-free synthetic diet, high in unsaturated fat, for the duration of the experiment. Water was provided ad libitum. Hardwood chip bedding was used and was changed twice weekly.

Eleven days after arrival, all rats were orally administered 20 mg of DMBA in a sesame oil base by gavage. The same gavage needle was used for each rat. The procedure was performed under the observation of trained personnel and was without incident. Two rats were found dead, one each on days one and five following oral gavage. Necropsy of these animals was not performed.

Ten days after oral gavage, 12 of the 22 treated animals had red ocular and nasal discharges and marked swelling of the cervical region. Various degrees of firm, nonpitting edema in the region of the cervical lymph nodes and salivary glands of several rats were observed. Pale mucous membranes and dehydration in some rats were also observed. Two of the most severely affected rats were submitted for necropsy.

Four to five days after clinical signs were first observed,
all rats showed significant reduction in the amount of cervical swelling. Food and water consumption appeared normal. One anorectic, thin rat was euthanatized and submitted for necropsy. There have been no subsequent deaths or apparent recrudescence of symptoms.

Pharyngeal cultures were taken from all remaining rats of the affected group and from two technical personnel 7 days after symptoms were first observed. Fourteen days after clinical signs in the DMBA-treated rats were noted, pharyngeal cultures were taken from twelve rats on other experimental protocols, including two sentinel rats, housed in the same room. All rats were of the same strain, originating from the same vendor.

Diagnostic studies. Three rats were submitted for complete necropsy. They were anesthetized with carbon dioxide, bled via cardiac puncture, and euthanatized with an overdose of CO₂. Samples of all body organs and tissues were placed in neutral buffered 10% formalin for histopathologic evaluation. The tissues were processed by conventional methods and embedded in paraffin; sections were cut at 5 μm and stained with hematoxylin and eosin. Selected tissues were stained with Giemsa and Gram’s stains.

Serum was evaluated by utilizing an enzyme-linked immunosorbent assay for detection of immunoglobulin G antibody to the following agents: Sendai virus, sialodacryoadenitis virus, pneumonia virus of mice, reovirus 3, Mycoplasma pulmonis, Toolan H-1 virus, and Kilham rat virus.

Samples of heart blood, cervical lymph node, and pharyngeal swabs were collected aseptically for microbiological examination, placed onto sheep blood agar plates and into Trypticase soy broth, and incubated at 35°C. Cultures that produced mixed flora were subcultured onto phenylethanol media selective for the isolation of gram-positive cocci; isolates from this media were then transferred to blood agar plates. Streptococcal group-specific antigens were identified with a rapid latex test system (Wellcome Diagnostics, Dartford, England).

Biochemical tests were performed with six isolates of streptococci. Isolates 1 to 3 were cultured from the affected lymph nodes of the three rats which were necropsied. The remaining isolates, of pharyngeal origin, were selected at random from other, DMBA-treated, asymptomatic rats housed in the same room. Fermentation of trehalose, sorbitol, lactose, and inulin; hydrolysis of sodium hippurate and bile esculin; and production of acetylmethylcarbinol (Voges-Proskauer) were examined.

Antimicrobial susceptibility tests were performed with the same six isolates of group G streptococci. Amoxicillin with clavulanic acid (10 μg), chloramphenicol (30 μg), sulfamethoxazole-trimethoprim (25 μg), cephalothin (30 μg), erythromycin (15 μg), gentamicin (10 μg), bacitracin (10 U), neomycin (30 μg), and polymyxin (300 U) were employed in the disc method of Bauer et al. by using discs prepared by Difco Laboratories, Detroit, Mich. (1).

RESULTS

Pure cultures of beta-hemolytic streptococci were obtained from the cervical lymph nodes of the three rats that were necropsied. The rapid latex test system identified these isolates to have group G-specific antigen.

Gross postmortem findings from the first two rats necropsied were similar. These included multiple petechiae and ecchymoses in the subcutaneous tissues of the thorax and abdomen, and a suffuse hemorrhage in the medial muscularity of the right rear leg of one rat. Sublumbar and mesenteric lymph nodes were swollen and congested. Adrenal glands were also congested. Cervical lymph nodes and the salivary glands in the cervical area were swollen and hemorrhagic and adhered to one another, forming a single firm mass (Fig. 1). Gross abnormalities in the third rat, in addition to inanition, were limited to congestion of the cervical and inguinal lymph nodes.

Histopathologic changes in the first two rats included acute necrotizing inflammation of the cervical lymph nodes, with multiple large colonies of coccoid bacteria at the perimeter of the necrotic zone (Fig. 2). The inflammatory process extended to the adjacent salivary glands and connective tissue, where it was characterized by edema, fibrin deposition, and a predominantly polymorphonuclear cell infiltration. Edema and mild polymorphonuclear cell infiltration extended throughout the subcutaneous tissues of the head. Because of thrombosis of the adrenal vein, one adrenal gland of one rat had extensive necrosis and hemorrhage that destroyed all but a small portion of the medulla and a thin rim of subcapsular cortex. Histopathologic changes in the third rat were limited to accumulations of hemosiderin in the cervical lymph nodes.

Group G beta-hemolytic streptococci were isolated from the pharynges of 16 of 22 rats from the same experimental group. Pharyngeal samples from 3 of 12 rats housed in the same room but not from this experimental group (no DMBA administered) were also positive for this organism. Pharyngeal...
Serologic tests to detect the presence of immunoglobulin G antibody to rat viral pathogens and M. pulmonis were negative for the three rats necropsied. Heart blood cultures from the necropsied rats were also negative.

All six streptococcal isolates fermented trehalose and lactose but not sorbitol and inulin and did not hydrolyze sodium hippurate or bile esculin. All isolates were negative for the Voges-Proskauer reaction in which Streptococcus anginosus was employed as a positive control. Antimicrobial susceptibility tests for the same isolates of group G streptococci showed susceptibility to amoxicillin with clavulanic acid, bacitracin, cephalexin, chloramphenicol, and erythromycin. All isolates were resistant to gentamicin and neomycin and partially or completely resistant to polymyxin B and tetracycline. Four of the six isolates were resistant to sulfamethoxazole-trimethoprim, and two were susceptible.

DISCUSSION

Since first classified by Lancefield (12), group G streptococci have been isolated from dogs (8); cats (7); nonhuman primates (21); cattle, sheep, and swine (11); and mice (20). Streptococcus canis is the proposed official name for group G beta-hemolytic streptococci of animal origin (4). The information from biotyping and DNA hybridization studies of animal and human group G streptococci indicates that they are not identical and probably do not have a high zoonotic potential (3, 16). The biochemical test results for the six isolates examined are identical to those published elsewhere (18).

All rats in the room were from the same vendor but arrived at the facility at different times over a 10-month period. It seems unlikely to find 73% of the DMBA-dosed rats naturally harboring this organism, considering the 25% prevalence noted for other rats from the same vendor. However, the lack of documentation in the literature identifying group G streptococci in rats may also reflect a reluctance to serogroup beta-hemolytic streptococci routinely in the past because of the lengthy procedures involved. Readily available kits now permit fast, reliable serogrouping.

With the prevalence of group G beta-hemolytic streptococci in rats not currently known, it is only speculative whether all of the affected animals were harboring the organism at the time of the oral gavage procedure. Alternatively, the gavage needle may have served as a fomite for transmission. Stress of the experimental protocol, potential scarification of the oral mucosa at the time of the gavage procedure in the presence of the group G streptococci, and the powdered synthetic diet also may have contributed to the initiation and progression of the cervical lymphadenitis. Trauma of the oral mucosa was not a determining factor for successful infection of cats with group G streptococci (17) or for the induction of cervical lymphadenitis in guinea pigs with group C streptococci (14). The most frequently documented portal of entry of group G streptococci in humans is the skin, although the upper respiratory tract can also serve as a site (6). It is interesting that the cervical lymphadenitis was self-limiting in the majority of these rats, unlike streptococcal lymphadenitis in cats (17) and guinea pigs (14).

The induction of periodontal disease in rats is frequently accomplished by feeding them a soft synthetic diet high in carbohydrates (13). The synthetic diet used for these rats may have contributed to the proliferation of the streptococci in the gingival tissue.

Administration of DMBA may have played an important role in the pathogenesis of the disease. Oral administration of DMBA in sesame oil to 50-day-old female Sprague-Dawley rats results in the rapid induction of breast cancer; this model is influenced by endocrine control, as is the disease in human females (19). The side effects or toxic reactions associated with this compound may have accounted for the two unexplained deaths shortly after the gavage procedure, as well as for the pale mucous membranes seen in several of the rats with lymphadenitis. Importantly, DMBA has been reported to induce leukopenia as well as carcinoma from a single 20-mg feeding (9). The leukopenic

FIG. 2. Hematoxylin and eosin stain showing acute necrotizing inflammation of a cervical lymph node (N), due to group G streptococcus infection, with colonies of bacteria at the perimeter of the necrotic zone (arrowheads). The inflammatory process extends to the perinodal connective tissue (C) and adjacent salivary glands (S). Bar = 120 μm.
stage lasted approximately 10 days in the rats before signs of recovery from this symptom were evident (9). The clinical disease observed in this outbreak corresponded to the expected duration of the leukopenic response previously noted for DMBA-treated rats (9). A decrease in resistance to the streptococcal infection occurring in the leukopenic rats may have contributed to the development of the cervical lymphadenitis and may provide an explanation for the transient nature of the clinical symptoms. Increased susceptibility to streptococcal disease as a result of neutropenia in alcoholics with folate deficiency has been identified (6).

Group B beta-hemolytic streptococcus was isolated from one animal in the surveillance group. Commonly associated with mastitis in livestock, group B Streptococcus agalactiae has also been isolated and has been known to cause disease in humans, dogs, and mice (6, 8, 20). Its importance in the oral flora of rats is unknown.

The oral flora cultured from rats, or other species, may vary depending on the place of origin or environmental factors. Under the appropriate conditions, opportunistic pathogens can cause disease in the host. Although group G streptococci have previously been identified as normal oral flora in rats, they can under certain conditions cause cervical lymphadenitis and should be included in the differential diagnosis, along with sialodacryoadenitis virus, as a cause of cervical swelling in rats (10). Experimental transmission studies with the streptococcus group G isolates from this outbreak are needed to elucidate the pathogenic potential of this organism in the immunocompetent rat.

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REFERENCES