A SKIN TEST FOR DETECTING GROUP C HEMOLYTIC STREPTOCOCCAL INFECTION CAUSING EPIZOOTIC LYMPHADENITIS IN GUINEA PIGS

Applications in Selecting Breeding Stock

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(Received for publication, May 13, 1936)

Epizootic lymphadenitis of guinea pigs was first reported by Boxmeyer (1). The disease is characterized by large buboes involving any of the peripheral lymph nodes, but most frequently those of the cervical chain. The lymph node enlargement is usually so marked that the involved glands frequently reach several centimeters in diameter. They are filled with thick yellowish white pus from which, almost invariably, pure cultures of hemolytic streptococci can be isolated. The spontaneous disease may be widespread in an infected herd; Boxmeyer found over 50 per cent of the animals diseased in a group of 3,000 guinea pigs. The naturally acquired disease usually runs a chronic benign course. The abscessed nodes may rupture spontaneously, drain their contents and heal. In other animals, the huge nodes cause death indirectly by mechanical pressure which interferes with vital functions. All strains of hemolytic streptococci isolated from this spontaneous guinea pig lymphadenitis fall into group C of Lancefield's serological classification (2).

During the course of tissue culture experiments on bacterial hypersensitivity induced infections with group C hemolytic streptococci in guinea pigs were studied (3). The strain of hemolytic streptococcus used was K104, originally isolated from an epidemic of spontaneous guinea pig lymphadenitis by Hardenbergh (4). The infection, induced by subcutaneous injection of broth culture, was characterized by a chronic course with the development of local abscesses, regional and distant lymphadenopathy and occasionally internal visceral involvement.

A bacterial extract, made as described below, was used in a study of cutaneous hyperreactivity, and for testing tissue cultures. The skin test dose of 0.1 cc. was injected intradermally into the shaved abdominal surface. Normal animals failed to react to this dose of bacterial extract. Infected animals, on the other hand, gave definite cutaneous reactions in all instances. A delayed inflammatory skin response similar to a positive tuberculin reaction occurred, and was characterized by redness, swelling, edema, induration and in many instances by areas of central necrosis. The skin reactions reached their height at about 24 hours and then gradually decreased in intensity over a period of several days. Positive reactions were at least 10 mm. in diameter, and many were 30 to 40 mm. in diameter with variable thicknesses and occasional areas of central necrosis. A persistent scar resulted when such necrosis occurred. Reactions were noted as early as 5 days after infection, persisted throughout the course of the disease, and for variable lengths of time in instances where apparent recovery had taken place. The intensity of the cutaneous hyperreactivity varied, depending to a certain extent on the stage of the infection. The larger and more intense reactions occurred during the acute phase, while in the chronic stage, reactions of moderate intensity were obtained.

Conditions other than active infection may induce skin hypersensitivity. Guinea pigs inoculated with repeated large doses of formolized strain K104 hemolytic streptococcal vaccines, reacted to intradermal injection of the extract. Repeated skin testing in an originally negative reactor resulted in cutaneous hyperreactivity to subsequent skin tests with the same extract. Since the bacterial extract is a crude product containing many different chemical fractions, it seems that animals infected with closely related strains or those having similar chemical components might give positive skin reactions. In a preceding study (3) it was noted that guinea pigs with induced group C hemolytic streptococcal infection gave positive skin reactions when tested with crude bacterial extracts prepared from a group B hemolytic streptococcus and from a *Streptococcus viridans* of guinea pig origin. The reactions, however, were not as intense as those obtained with the extract prepared from the homologous streptococcus.

Using the data obtained by our study (3) of the skin reactivity of infected and normal animals to bacterial extract prepared from group C

hemolytic streptococcus, the following conclusions seemed justified: first, all animals infected with group C hemolytic streptococci give a positive cutaneous reaction to a bacterial extract prepared from microorganisms of this group; second, animals recently recovered from streptococcal infection give positive reactions, but of diminished intensity; third, animals not reacting to the intracutaneous dose of streptococcal extract are free of infection with the group C hemolytic streptococcus.

The prevalence of epizootic lymphadenitis of guinea pigs is apparently widespread since infected animals were frequently obtained from various commercial dealers. The Rockefeller Institute guinea pig breeding stock also had variable numbers of infected animals. Although obviously diseased animals with large peripheral nodes were removed from the stock, this did not control the spread of the infection. It seemed possible that animals with very small lesions or undetectable internal lesions might act as reservoirs of the etiological agent and thus spread the infection. Such supposedly normal animals were found to react positively to the skin test with bacterial extract, and on autopsy deep seated lesions containing group C hemolytic streptococci were discovered. This skin test therefore showed its value in detecting disease of the internal viscera due to group C hemolytic streptococci when external evidences of the disease were lacking.

In an effort to obtain a guinea pig breeding stock free of this particular streptococcal infection it was planned to test the cutaneous reactivity with streptococcal extract and discard the positive reactors. It was presumed that a negative reactor would be free of this infection.

Preparation of Group C Hemolytic Streptococcal Extract for Skin Testing

Strain K104 streptococcus grew well in neopeptone broth. Several liters of 18 hour cultures were thrown down by centrifugation; the packed organisms were washed by resuspending in normal saline, and again centrifuged. The packed bacteria were then frozen and dried by the method of Swift (5) and ground in a ball mill for 2 or 3 weeks to disrupt completely the cells. The resulting ground bacteria were stored, and solutions of bacterial extract were prepared as needed. Since our bacterial extract was also tested in tissue culture experiments, a highly buffered saline solution (Tyrode's) was used. A solution containing 5.0 mg. of the dried powdered bacteria per cc. was prepared by grinding in a mortar and by adding small amounts of Tyrode's until the required strength was reached. This solution was then centrifuged at 2,000 revolutions per minute for 45 to 60 minutes

to throw down the insoluble portion. The resulting clear, slightly opalescent solution was the bacterial extract used for skin testing. Each skin test dose of 0.1 cc. contained the soluble products of 0.5 mg. of the dried, powdered streptococci.

Selection of Animals for Breeding Stock

In January, 1935, guinea pigs were obtained from several commercial dealers. All animals were carefully inspected and palpated for lymph node enlargement, and any showing these lesions were discarded. 330 guinea pigs, 276 females and 54 males, free from external signs of streptococcal lymphadenitis, and isolated for several days were then skin tested with 0.1 cc. of bacterial extract. The skin reactions were read at 24 and 48 hours. Reactions greater than 10 mm. in diameter were considered positive. 20 positive reactors were found. The largest reaction was 33 x 18 x 2 mm. with moderate induration. The average diameter of the 20 reactions was about 20 mm. The positively reacting guinea pigs were autopsied. None showed macroscopic lesions suggestive of the hemolytic streptococcal type of infection. Several animals had areas of pulmonary consolidation from which pneumococci were cultured. 171 females and 24 males of the 310 negatively reacting guinea pigs were used for breeding purposes. They were transferred to sterilized pens in a thoroughly cleaned and disinfected room. Isolation technique was observed by the attendants. No new outside animals were added to the breeding stock.

During the 15 months from January, 1935, to April, 1936, 1,296 progeny resulted from the original negatively reacting breeding stock. No instance of hemolytic streptococcal lymphadenitis was observed in the original stock or their progeny. In April, 1936, 100 of the progeny were skin tested with a dose of streptococcal extract similar to that used originally. No positive reactions resulted.

DISCUSSION

Epizootic lymphadenitis of guinea pigs caused by hemolytic streptococcus (group C—Lancefield) is a frequent epidemic or endemic infection in herds of guinea pigs. Due to the chronic, relatively benign course of the disease, it has been difficult to eradicate the infection from large herds. Theobald Smith (6) studied the spontaneous disease in a small herd of guinea pigs in which the infection was spread by contact for a period of 9 years.

Infected animals with large peripheral nodes are readily recognized and easily eliminated. Animals with smaller foci of infection or with lesions in internal nodes or in their viscera are difficult of detection by ordinary observation, since these animals maintain good nutrition and

present no outward evidence of disease. They may, however, act as reservoirs or carriers of the infective agent, and thus maintain the infection in a herd.

The use of a group C hemolytic streptococcal extract for skin testing supposedly normal guinea pigs, and the separation of negative from positive reactors, has made it possible to obtain a breeding stock presumably free from the hemolytic streptococcus causing epizootic lymphadenitis. This presumption was strengthened by the fact that the negatively reacting breeding stock and their progeny, kept in isolation, have remained free of spontaneous lymphadenitis for a period of 15 months. Furthermore, a group of 100 of the progeny skin tested with bacterial extract gave entirely negative reactions.

The portion of the crude bacterial extract which induces cutaneous reactions is apparently stable since the dried powdered bacteria and the solutions made from them have been kept for months without losing their capacity of inducing reactions in animals known to be diseased. Caution should be emphasized in interpreting results of repeated skin tests in the same animal, since a negative reactor to the first test may eventually develop a positive reaction on repeated subsequent testings.

Skin testing with a bacterial extract prepared from a group C hemolytic streptococcus offers a method of detecting guinea pig hemolytic streptococcal carriers which show no obvious external evidence of this infection. Negative reactors are apparently free from infection with the etiological agent of epizootic lymphadenitis and may be used as a breeding stock for obtaining guinea pigs free from this infection.

SUMMARY

1. A skin test with a crude bacterial extract prepared from group C (Lancefield) hemolytic streptococci was used as a means of detecting possible carriers of the streptococcus causing epizootic lymphadenitis in guinea pigs. A positive test similar to a positive tuberculin reaction was considered presumptive evidence of present or recent infection with this streptococcus.

2. 20 positive reactors were found in 330 supposedly normal guinea pigs.

3. 195 negatively reacting animals were used as a breeding stock which yielded 1,296 progeny over a period of 15 months. None of the breeding stock or their progeny showed evidence of spontaneous lymphadenitis. Skin tests of 100 of the progeny were all negative.

4. The use of this skin test as a means of obtaining guinea pig breeding stock free of the streptococcus causing spontaneous lymphadenitis is suggested.

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