The Cervical Lymph Nodes in
Streptococcus pyogenes, Group A,
Type 50, Infection in Mice*

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Abstract. Streptococcus pyogenes, group A, type 50, one of the few group A streptococcal types naturally occurring in mice, proved highly virulent in this species after experimental infection. Intranasal infection of 96 mice (Swiss albino, NMRI, and CBA) with this microorganism induced profound reactions in the cervical lymph nodes of 69% of the animals. Histologically, two different forms of reaction were distinguishable. In 61 mice, the lymph nodes exhibited follicular and lymphoplasmacellular hyperplasia and in 9 animals suppurative lymphadenitis was present. The ability of type 50 streptococci to persist in the pharynx of mice, and the similarity of the morphological changes induced by this organism appear to make intranasal murine group A, type 50, streptococcal infection a suitable model for human streptococcal pharyngitis.

Introduction. Of the 60 or more serologic types of group A streptococci known, only two have so far been recognized as producing spontaneous streptococcal infections in mice. Thus, Bell et al. (1958) reported an A streptococcus epidemic among field mice in Oregon and Northern California. Lancefield (1972) discovered that acute infections by this A streptococcus, which is now designated type 51, occurred frequently in certain regions of the USA among mice. In contrast, A streptococci of type 50, isolated from Swiss albino and Princeton mice in the USA and in Europe, primarily induced chronic infections (Nelson, 1954; Hook et al., 1960). Such type 50 streptococci, persisting in the pharynx of the mouse under housing conditions unfavorable to the animals (temperature changes, low humidity) were observed to quickly spread through the stock (Lancefield, 1972).

In a previous paper, the bacteriologic characteristics and immunologic properties of Streptococcus pyogenes type 50 were reported (Wildfeuer et al., 1975). In this study the pathomorphologic picture of the inflammatory process as evoked by this organism after intranasal infection of mice is described.

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Materials and Methods

1. Animals. NMRI and CBA mice were obtained from Süddeutsche Versuchstierfarm KG, Tuttlingen, Germany. Swiss albino mice were derived from Gräßlich-Degenfeld-Schornburg'schen Rentamt, Eybach, Württemberg, Germany. At the beginning of the experiment, the mice, aged 3-5 weeks, weighed 18-22 g. They were kept in groups of five in Makrolon cages and fed dry food (R. Fundel KG, Ulm/Donau) and water ad libitum.

2. Bacteriologic Methods. Streptococcus pyogenes, group A, type 50, strain B 514, was cultured on blood agar at 37°C for 24 h. Bacterial suspensions were prepared with physiologic saline and adjusted to the germ numbers required at 650 nm using a spectrophotometer. Also, the infective dose was assessed by culture count. Each mouse under chloroform anesthesia received 0.05 ml streptococcus suspension intranasally through a tuberculin syringe as described by Hook et al. (1960). Mice that did not die spontaneously after infection were exsanguinated by cardiac puncture at various intervals (3-31 days after infection). The pharynx, brain, heart, lungs, kidneys, cervical lymph nodes, and blood of each animal were examined bacteriologically.

3. Histologic Methods. At autopsy, samples of the cervical and axillary lymph nodes, brain, heart, lungs, liver, spleen, and kidneys of all animals were fixed in 4% formalin and embedded in paraffin. Thereafter, histologic sections were prepared and stained with hematoxylin-eosin and by Gram's stain (Romeis, 1968).

4. Immunohistologic Methods. Antisera used in the studies were obtained from rabbits (New Zealand White, 2-3 kg in weight) as described earlier (Wildfeuer et al., 1975). Samples of the cervical lymph nodes, brain, heart, lungs, liver, spleen, and kidneys were frozen in liquid nitrogen immediately after removal and stored at -50°C. From the frozen tissue, 5-μ thick sections were prepared using a cryostat (WKF, Brandau), and air dried at room temperature. The sections were then fixed for 5 min in absolute ethanol at 4°C, dried, and rehydrated in phosphate-buffered saline (PBS), pH 7.2, at 4°C. Thereafter, employing the direct immunofluorescence method (Smith, 1965; Sellin et al., 1970), they were examined for the presence of both group A and A-variant streptococcal carbohydrate antigens (Heymer and Haferkamp, 1971).

To demonstrate type-specific protein antigens (type 50, M protein), the indirect immunofluorescence method (Sellin et al., 1970) with corresponding streptococcal antisera from rabbits and FITC-labeled sheep-antirabbit-γ globulin conjugates was used.

5. Serologic Methods. Antistreptolysin O (ASO) antibodies in the sera of mice intranasally infected with streptococcus type 50 were determined by a Latex agglutination test (Latex-ASL-test Behringwerke AG, Marburg/Lahn, Germany). In contrast to routine test procedure, partial saturation of serum samples with streptolysin O was omitted in order to detect small quantities of antibodies. Also, the sera were examined for antibodies to streptococcal C-carbohydrate using the capillary precipitation method (Swift et al., 1943) and for antibodies to peptidoglycan by the Latex agglutination test (Heymer et al., 1973). Sera of noninfected mice were used as controls.

6. Electron-microscopic Methods. For electron microscopy, tissue samples of the cervical lymph nodes were fixed for 90-120 min in 3.5% buffered glutaraldehyde (Serva Feinbiochemica, Heidelberg) and 1% osmium tetroxyde (Heraeus, Hanau) as described