Cat scratch disease

An epidemiological and ultrastructural study of lymphadenitis caused by Warthin-Starry positive bacteria

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Summary. The aetiological agent of cat scratch disease (CSD) has been unknown for more than 30 years. Recently, a micro-organism clearly shown with Warthin-Starry silver (W-S) stain was found and thought to be a possible cause of the disease. In this study, 32 cases of regional lymphadenopathy histologically compatible with CSD and 20 contrasting cases of lymphadenopathy were examined retrospectively with W-S stain. W-S positive pleomorphic organisms were clearly demonstrated in 20 of the 32 suspected cases of CSD, but in none of the other cases. The onset of disease in these 20 cases with W-S positive organisms occurred between July and January. This seasonal variation in the onset of disease was highly significant (P<0.005) and was not due to a single epidemic. Moreover, some characteristic morphological features of the organism were found by electron microscopic observations. Ultrastructurally, the organism was a bacterium showing a chain-like arrangement, septal formation, branching and clubbed ends.

Key words: Cat scratch disease – Epidemiology – Ultrastructure – Bacterial morphology – Warthin-Starry stain

Introduction

Cat scratch disease (CSD), first recognized by Debré et al. in 1950 (Debré 1950; Debré et al. 1950a and b; Carithers 1970), is an infectious disease characterized by regional lymphadenopathy and a history of a scratch or bite by animals, or other skin puncture from various causes (Daniels and MacMurray 1954).

Several organisms have been proposed as possible aetiological agents of CSD, including the virus of Petzetakis’ disease (Fox 1952), Herpes simplex virus (Turner et al. 1960; Kalter et al. 1969), atypical mycobacteria (Boyd et al. 1961) and Chlamydia belonging to the Psittacosis-lymphogranuloma venereum group (Mollaret et al. 1951a and b; Gifford 1955; Kalter et al. 1955; Fowler and Bailey 1961). The actual causative agent is unknown. Therefore, three of the following four criteria are required for clinical diagnosis of CSD (Warwick 1967): (i) a positive skin reaction to Hanger-Rose antigen, (ii) negative laboratory findings for other causes of lymphadenopathy, (iii) a history of contact with animals and the presence of a scratch or primary dermal or eye lesion, and (iv) a characteristic histopathological appearance of a biopsied lymph node. In 1983, Wear et al. identified a bacterium that appeared pleomorphic with Warthin-Starry silver (W-S) stain, gram-negative with Brown-Hopps’ (B-H) gram stain and was not acid-fast in 34 of 39 excised lymph nodes from 39 patients with CSD (Wear et al. 1983). Subsequently, Gerber et al. (1985) reported culture of a bacterium from an excised lymph node of a patient with CSD, which contained a pleomorphic W-S stained organism. This cultured bacterium seemed to be Rothia dentocariosa. More recent reports confirmed the observations of Wear et al. (Margileth et al. 1984; Kitchell et al. 1985; Cotter et al. 1986; Korbi et al. 1986; Miller-Catchpole et al. 1986). To our knowledge, there has been no study of cases of lymphadenitis with a W-S stain-positive organism from an epidemiological point of view, and discrepancies in former electron microscopic observations on this organism (Gerber et al. 1985; Hadfield et al. 1985; Wear et al. 1985; Osborne et al. 1987) have not yet been resolved.
In the present study, we examined 32 cases of abscess-forming reticulohistiocytic lymphadenitis (ARHL) showing the histological features of CSD. For comparison, 20 cases of other kinds of regional lymphadenopathies were also examined. The difference in bacterium-positive and -negative cases of these diseases were studied, and the ultrastructural features of the bacteria in two cases were examined.

Materials and methods
Lymph node biopsy specimens from 52 patients with regional lymphadenopathy selected from the file of the Department of Pathology, Tokushima University for 1970 through 1987 were examined. All excised lymph nodes were fixed in 10% formalin and embedded in paraffin.

All sections stained with H&E were reexamined histologically, and the presence of abscess was confirmed by the Naphthol AS-D chloroacetate method (Katayama et al. 1983). The cases reevaluated were as follows: 32 cases of ARHL, 15 cases of histiocytic necrotizing lymphadenitis (HNL), three cases of mesenteric lymphadenitis, one case of lymphadenitis with rheumatoid arthritis and one case of lymphadenitis with systemic lupus erythematosus.

All specimens were stained with W-S stain (Bridges and Luna 1957) and B-H gram stain (Brown and Hopps 1973; Wear et al. 1983) and specimens in all 32 cases of ARHL were stained with Ziehl-Neelsen stain. Specimens from two cases in which bacilli were demonstrated with W-S stain were also stained with PAS stain and Grocott's stain.

The bacilli in specimens from two cases could be examined by electron microscopy. Portions of the 10% formalin-fixed lymph node of one case were excised and washed with water. Portions of the paraffin-embedded lymph node of the other case in which bacilli were demonstrated with W-S stain were excised and deparaffinized. Then each specimen was cut into small blocks, postfixed with 1% osmium tetroxide and embedded in Epon 812. Ultrathin sections were stained with uranyl acetate and lead citrate, and observed with a Hitachi H-300 electron microscope.

Clinical histories were obtained from the application forms for pathological diagnosis.

Results
The cases of ARHL were classified into three stages according to the following criteria.
Stage 1: The presence of small histiocytic granulomas or early micro-abscesses throughout the node. Some narrow micro-abscesses may also be present in the marginal zone of the lymph node (Fig. 1). Follicular hyperplasia and paracortical hyperplasia are also seen.
Stage 2: The presence of definite round micro-abscesses randomly distributed throughout the node, but not extending to form "stellate abscesses".
Stage 3: The presence of one or more large, irregular abscess, a so-called “stellate abscess”. (Features of the earlier stages may also be seen in this stage.)

Of the cases, 11 were classified as stage 1, nine as stage 2 and 12 as stage 3.

In the specimens from the cases of HNL, irregular focal lesions containing many transformed lymphocytes and histiocytes and a small amount of nuclear debris were seen. The border of the lesions was clear, but no palisade structure of histiocytes around the lesions was seen. Few neutrophils were found in the lesions on staining by the AS-D chloroacetate method.

Specimens from the cases of mesenteric lymphadenitis showed marked sinus histiocytosis and follicular hyperplasia. In addition, scattered small