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Cat scratch disease: Evidence for a bacterial etiology

A retrospective analysis using the Warthin-Starry stain

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Summary. 97 cases of acute necrotizing granulomatous lymphadenitis (so called "reticulozytärabszedierende Lymphadenitis") were examined retrospectively with the Warthin-Starry stain, in order to detect bacteria in patients with cat-scratch disease (CSD). 27 patients with CSD were found and among those, bacteria were demonstrated in 4. The organisms were pleomorphic rods of about 3 micron length and occurred in clumps within foci of fresh necrosis.

We found the organisms in a much lower percentage than has been described in the literature. The reasons may be that many of our patients had antimicrobial treatment prior to the biopsy and that step-sectioning of the specimen may be necessary in order to detect fresh lesions. Our findings seem to confirm the observation that CSD may have a bacterial aetiology.

Key words: Necrotizing granulomatous lymphadenitis – Cat-scratch disease – Bacterial Aetiology – Warthin-Starry-Stain

Introduction

In 1950, Debré et al. first reported cases of suppurative lymphadenitis occurring after a cat scratch or bite and named that syndrome cat-scratch disease (CSD). Today, after 35 years of research and hundreds of reports, its aetiologic agent is still undetermined.

Petzetakis (1937) reported on a virus found in lymph nodes of patients with a disease similar to CSD. The finding of a haemagglutinin in pus from two cases of CSD led to the isolation of a virus antigenically related to the herpes simplex virus but lacking its typical virulence and cytopathogenic properties (Dodd et al. 1950). Kalter et al. (1969) demonstrated herpes-like particles with the electron microscope in lymph nodes of patients with CSD.

Mollaret et al. (1950) observed inclusion bodies in lymph node sections of patients with CSD and considered them to be the aetiologic agent. Others were unable to confirm these findings (Kalter 1961), while Hedinger (1952) considered the inclusion bodies to be mast cell granules. A relationship to the psittacosis-lymphogranuloma venereum group of viruses was also postulated on the basis of complement fixation (Kalter 1961; Armstrong et al. 1956) and immunofluorescence tests (Emmons et al. 1976). Boyd and Craig (1961) reported the isolation of photochromogenic mycobacteria from lymph nodes of 8 patients with CSD.

Interest in the cause of CSD was stimulated anew with the demonstration of a bacterial agent by Wear et al. (1983). With the Warthin-Starry (W-S) silver impregnation (Luna 1968), small, pleomorphic and gram negative bacilli were seen in the walls of capillaries and in foci of necrosis. Their size ranged from 0.3 microns to 1.0 microns by 0.6 to 3.0 microns. This finding, first established in one patient, was later repeated in 29 of 34 patients. Gerber et al. (1985) then cultured a highly pleomorphic, gram positive bacterium from a lymph node of 1 of 3 patients with CSD. The authors believed that this was the same bacterium which had been seen by Wear et al. (1983). Biochemical and physiological analyses suggested that it might be a member of the genus Rothia. On this background we decided to retrospectively test lymph node sections of patients with CSD with the W-S silver impregnation in order to see whether or not we could reproduce the observation of Wear et al. (1983).

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Material and methods

Cases with a histological diagnosis of acute necrotizing granulomatous lymphadenitis (so called "retikulozytär-abszedierende Lymphadenitis" [RAL]) were drawn from the histopathological files of the Institute of Pathology, University of Zurich and of the Institute of Pathology, Kantonsspital Winterthur[1] for the years 1970 through 1984.

The histological term of RAL encompasses the following diagnoses: (1) CSD, (2) lymphadenitis pseudotuberculosa, (3) tularemia and (4) lymphogranuloma venereum (LGV) (Lennert 1961).

157 cases of RAL were found. Out of these, 60 were excluded from the study either because the morphology was considered atypical on review, or mostly because paraffin blocks and/or patients records were missing. Information on the fixatives used at the time of examination was lacking. Of these remaining 97 cases new paraffin sections were cut and stained with hematoxylin-eosin (H.E.) and with the Warthin-Starry silver impregnation for spirochetes and Donovan-bodies. The stain was carried out exactly as described in the Manual of Histologic Staining Methods of the AFIP (Third edition, 1968, L.G. Luna, editor) (Luna 1968). Additional step-sections were prepared and stained with H.E. and with the W-S silver impregnation for a subgroup of 27 cases classified as CSD.

Cat-scratch disease was diagnosed by the findings of: (1) clinical lymphadenitis with characteristic histopathology, (2) the prescence of two of the following additional criteria: (a) history of animal contact, (b) inoculation site or lesion, (c) serological exclusion of other etiologies.

The size of the bacilli found in one of our own sections and of those in control sections provided by D.J. Wear, MD[2], was measured.

Clinical and follow-up informations on patients were obtained from the hospital records and a questionnaire mailed to private physicians.

Results

According to the criteria mentioned above, we classified our cases of RAL as follows (Table 1). 14 cases (31%) were considered as CSD, 13 (29%) as compatible with CSD, 15 (34%) as lymphadenitis pseudotuberculosa, 2 (4%) as probably LGV and 1 (2%) as typhus abdominalis. No case of tularemia occured.

There remained a group with no etiologic diagnosis, because no further clinical investigations to establish a definitive diagnosis had been performed after the histological diagnosis of RAL was obtained.

Table 1. Aetiology in 97 cases of acute necrotizing granulomatous lymphadenitis ("Retikulozytär-abszedierende Lymphadenitis")

Cat-scratch-disease (CSD)	14	(31%)
Compatible with CSD	13	(29%)
Lymphadenitis pseudotuberculosa	15	(34%)
Lymphogranuloma venerum	2	(4%)
Tularaemia	0	
Others (Typhus abdominalis)	1	(2%)
	45	(100%)
Total cases with evidence for aetiology	45	(46%)
Cases without evidence for aetiology	52	(54%)
Total	97	(100%)

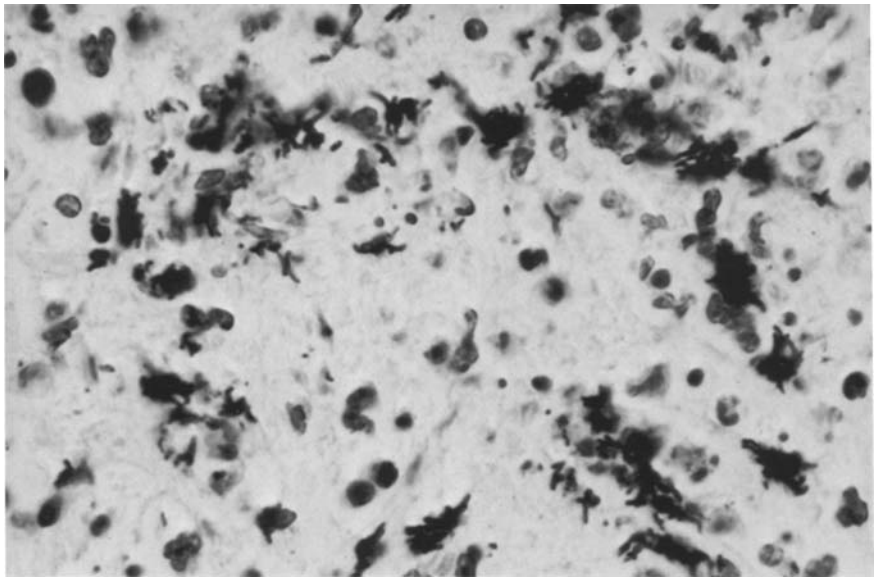

Fig. 1. Within an area of fresh necrosis, clumps of polymorphic bacilli are seen intermingled with single organisms. Warthin-Starry, 1,000 ×

[1] We are indebted to Prof. B. Egloff for permission to use the cases of the Institute of Pathology, Kantonsspital Winterthur

[2] We would like to thank Douglas J. Wear, Col, MC, USA, Chief Geographic Pathology Division, Armed Forces Institute of Pathology, Washington D.C. who kindly provided us with control sections for the W-S stain and supported this work with critical suggestions and additional information

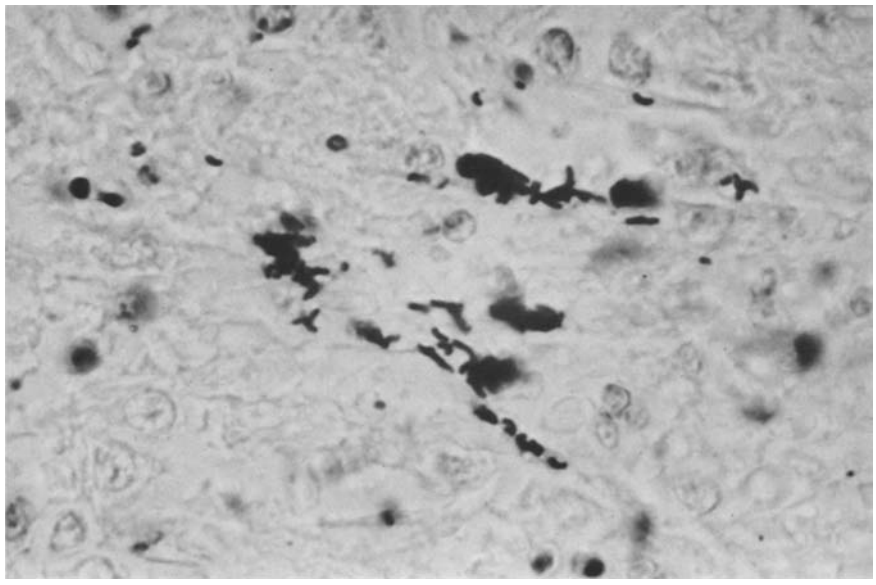

Fig. 2. More detailed photograph of small groups and single organism reveals their rod-like form and their length of about 3 microns. Warthin-Starry, 1,250×

Bacilli were demonstrated in the lymph node sections of 4 of 27 patients with CSD. They occurred in clumps, in chains, in microcolonies or as single organisms and were located in the walls of capillaries and in foci of fresh necrosis. The organisms ranged in size from 3.0 ± 0.7 microns in length and 0.6 ± 0.1 microns in diameter. (Figs. 1 and 2). These bacteria were not acid-fast and were not found in any of the 70 cases considered as non CSD. Morphologically they were identical with those described by Wear et al. (1983).

Discussion

Using the identical W-S silver stain as Wear et al. (1983), we found bacteria in 4 of 27 patients (15%) with CSD. In the other 70 cases of RAL, among which there must have been additional CSD patients, no organisms were detected.

Thus, our findings qualitatively confirm the observation of Wear et al. (1983) and of others, who in the meantime also reproduced their study (Gerber et al. 1985; Kitchell et al. 1985; Korbi and Toccanier 1985). Quantitatively, there is a considerable difference in that we found bacilli only in 15% of our cases, compared with 85%, (29/34) in Wears original study (1983) or about 62% (250/400) in recent material (Wear 1985).

In three other studies the positivity rate was 1 of 3 (Gerber et al. 1985) 4 of 7 (Kitchell et al. 1985) and 38 of 57 cases (Korbi and Toccanier 1985) respectively.

These findings and discrepancies raise a number of questions.

First of all, are the structures we found in 4 cases really bacteria? Our organisms had about the same size, localisation and morphology as those described by Wear et al. (1983) and those cultivated by Gerber et al. (1985). They were also considered as bacteria by an experienced microbiologist.[3]

Why did we find the organisms in such a small proportion of our cases?

Several factors may influence the chance of finding the bacteria.

Fixation by mercuric fixatives destroys the Warthin-Starry stain and the chance of finding the organism varies with the histologic stage of the lesion. It is lowest in caseating granulomas and highest if there is necrosis only (Wear 1985). The age of the paraffin block with loss of staining capacity is probably not decisive, since it was possible to get positive staining in tissue which had been stored in paraffin for 40 years (Wear 1985). In our first case, the bacteria were identified on the first section and in the other 3 only after we cut 3 step-sections of all 27 cases of CSD. Possibly, an inhomogeneous distribution of the bacteria could be the explanation and therefore further step-sections might be needed to find the organisms in younger lesions. Many of our patients had received antibiotics, mostly penicillin, co-trimoxazole or tetracyclin in an attempt to manage the lymphadenitis conservatively. The organisms isolated by Gerber et al.

[3] We are grateful to Prof. v. Graevenitz, Director, Institute of Microbiology, University of Zurich, for review of the slides and critical discussion of the findings

(1985) were sensitive to penicillin, erythromycin, co-trimoxazole, cephalothin and clindamycin. Thus, this treatment prior to biopsy may have partially destroyed the organism and could have influenced the results of our study considerably.

In 1985, Gerber et al. announced the successful cultivation of a gram positive to gram variable bacterium from a lymph node of a patient with CSD. They identified it as a possible member of the genus Rothia. Rothia dentocariosa is commonly found in the human oral cavity and has been implicated in the cause of periodontal disease. Injected in mice either subcutaneously or intraperitoneally, it produces ulcerating lesions. Microscopic studies of these lesions showed multiple granulomas with microabcesses consistent with the histology of CSD.

These interesting results stand alone. They contrast with negative experience with larger case series in which cultivation was attempted with various procedures and media (Hadfield et al. 1985) – among them a yeast extract agar which was successfully used by Gerber et al. (1985). Therefore, the findings of Gerber et al. must presently be interpreted with caution until repeated and confirmed by others.

In conclusion our study seems to confirm the findings of Wear et al. (1983) and supports the hypothesis of a bacterial aetiology for CSD. Definitive proof of the aetiology of CSD may be at hand if the findings of Gerber et al. (1985) can be reliabely reproduced in controlled prospective studies. Until a culture system is established we would advise pathologists to stain every lymph node of patients with cat-scratch disease with the Warthin-Starry silver impregnation to confirm the prescence of bacteria.

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