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Disseminated cat-scratch disease: detection of *Rochalimaea henselae* in affected tissue

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Abstract An immunocompetent 9-year-old boy with disseminated catscratch disease involving spleen, cervical and abdominal lymph nodes, skull, and one clavicle is reported. Antibodies to *Rochalimaea quintana* and *R. henselae* were detected, at increasing, then decreasing concentration. DNA extracted from the biopsied skull lesion was amplified by polymerase chain reaction and hybridized with species-specific oligonucleotides proving the presence of *R. henselae* in affected tissue. Our findings suggest that *R. henselea* plays a pathogenic role in cat-scratch disease.

Key words Cat-scratch disease *Rochalimaea henselae* *Rochalimaea quintana* · Serology Diagnosis

Abbreviations *CSD* cat-scratch disease · *PCR* polymerase chain reaction · *RH1 Rochalimaea henselae* 1 (oligonucleotide) *RQ1 Rochalimaea quintana* 1 (oligonucleotide)

Introduction

Cat-scratch disease (CSD) manifests itself as a self-limited lymphadenitis after an inoculation injury in most cases. Injury is usually inflicted by a cat [2]. The detection of pleomorphic bacilli in typical granulomatous lesions has long suggested a bacterial aetiology [16]. In 1988, English et al. [5] reported the isolation of a bacterium, now termed *Afipia felis* [1], from affected lymph nodes of patients with CSD. Most recently, in a independent study, the majority of patients with clinically suspected CSD were shown to have significant antibody titres to *Rochalimaea henselae* [14], a newly identified bacterium [13]. We report an immunocompetent 9-year-old boy with a unusual, disseminated form of CSD and high antibody titres to *R. henselae* and to *R. quintana*, two bacterial species associated with bacillary angiomatosis in immunocompromised adults [6, 13, 17] and children [11]. Polymerase chain reaction (PCR) amplification followed by specific DNA hybridization proved the presence of *R. henselae* in the boy's affected tissue.

Case report

A previously healthy 9-year-old boy was referred to our hospital because of a 6-day history with recurrent fever of up to 40°C, abdominal cramps, and arthralgia (Fig. 1). On admission, the boy was afebrile and in good general condition. Physical examination was normal except for a reticulate maculopapular rash on the trunk and ankles, enlarged cervical and right-sided submandibular lymph nodes, and a crusted 10 mm skin lesion on the left of his nose. Laboratory work-up disclosed an elevated ESR. Leucocyte count and differential and platelet count were normal. Abdominal ultrasound showed multiple small hypo-echoic lesions in the moderately enlarged spleen, enlarged abdominal lymph nodes, and minimal ascites, ^{99m}Technetium bone scintigraphy was normal. The boy was observed for 1 week. The rash was transient and recurred daily, fever recurred twice, and lymph node enlargement persisted. Cultures from blood drawn on admission remained sterile. No bacter-

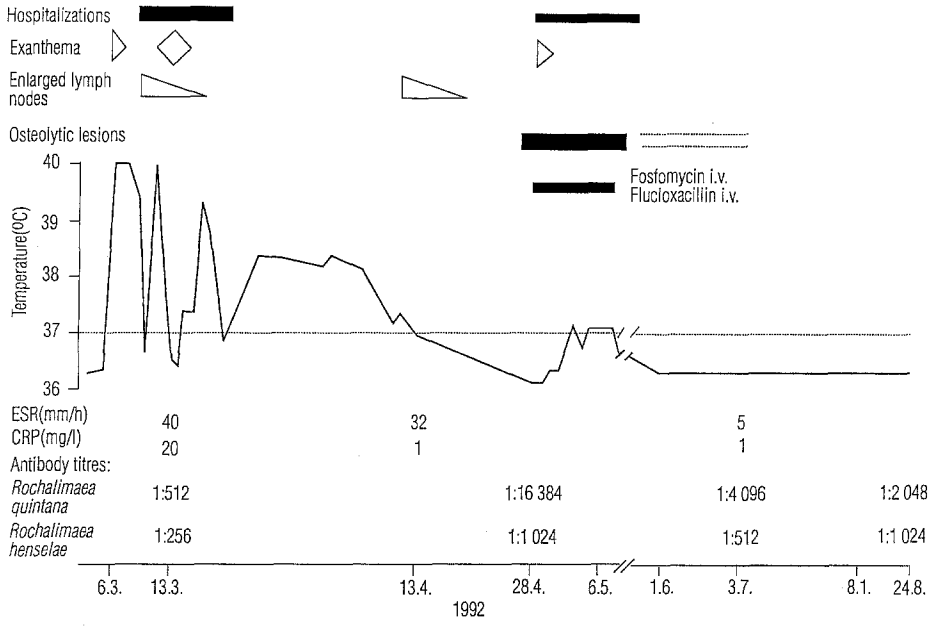

Fig. 1 Synopsis of symptoms and findings in a 9-year-old boy with disseminated CSD (*CRP* C-reactive protein)

ial or viral pathogens grew in cultures of a pharyngeal swab, urine, or stool. The patient was discharged without diagnosis.

After 6 weeks, the boy was readmitted because of headaches, back pain, a tender erythematous swelling of the right sternoclavicular joint, and a fluctuating swelling over the right parieto-occipital region of the skull. X-rays showed osteolytic lesions in the right parietal area of the skull (Fig. 2) and in the medial aspect of the right clavicle. A repeat ^{99m}Technetium bone scintigraphy now showed moderate hyperactivity in the skull and the right clavicle. CT demonstrated the cranial lesion in greater detail and disclosed a subperiosteal abscess (Fig. 2). Leucocyte count, ESR, C-reactive protein, transaminases, and creatinine were normal. No antibodies to streptolysin-0, *Brucella*, *Yersinia*, *Borrelia burgdorferi*, Epstein-Barr virus, cytomegalovirus, or human immunodeficiency virus were detected. Routine analysis of CSF and a bone marrow aspirate were normal. Immunological work-up disclosed no antibody dificiency and normal granulocyte and lymphocyte function.

Biopsy specimens were obtained from the lesions of the skull and the clavicle. Gram and Ziehl-Neelsen stainings were negative. The patient was started on a 10-day course of intravenous flucloxacillin (100 mg/kg/day) and fosfomycin (200 mg/kg/day). He recovered clinically.

Histological analysis showed granulomatous necrotizing inflammation with multinucleated giant cells and a marked proliferation of connective tissue (Fig. 3). No bacteria, mycobacteria, fungi, or viruses grew in cultures. Targeted history revealed that the patient had been exposed to cats and had been scratched several times. Therefore, Warthin-Starry staining of biopsy specimens was done and showed argyrophilic pleomorphic bacilli characteristic of CSD (Fig. 3, inset).

Fourteen months later, the boy was doing well, the osteolytic lesions had regressed, abdominal lymph nodes were judged normal, and no lesions were seen in the spleen.

Materials and methods

Serum samples from the patient, the patient's mother (the father and the brother were not available), and three healthy individuals as well as three different batches of commercial intravenous immunoglobulin (Sandoglobulin) were tested in a blinded fashion for the presence of antibodies to *R. henselae* and *R. quintana*.

Antibodies to *R. henselae* and *R. quintana* were determined by immunofluorescence as previously described [14]. *Rochalimaea henselae*, Houston-1 isolate [13], and *R. quintana*, RLO-90-263 isolate [17], were used as sources of *Rochalimaea* antigen. Antigen was prepared by co-cultivation with Vero cell monolayers as previously described [14]. *Rochalimaea* and Vero cells were inactivated by gamma irradiation prior to spotting on microscope slides and acetone fixation. Twofold dilutions of antisera (beginning at a dilution of 1:32) were made in 5% skim milk in phosphate-buffered saline (pH 7.6) with merthiolate as preservative. Titres ≤ 32 were regarded as negative.

DNA was extracted from the formalin-fixed, paraffin-embedded biopsy specimens from the lesions of the skull and the clavicle as previously described [18]. The extracted DNA was used as template in a PCR assay. Primers derived from the *htr*A locus of *R. henselae* (GenBank accession number L20127) were used for amplification; primers CAT1 and CAT3 define a 153-base pair fragment of that gene [Anderson et al. in preparation]. Template DNA was amplified for 35 cycles at 94°C for 1 min, 52°C for 2 min, and 70°C for 1.5 min. The resulting PCR products were resolved on a 3.0% NuSieve agarose gel.

PCR products were alkaline denatured and spotted onto a nylon membrane. The resulting membrane filter was hybridized to digoxigenin-labeled oligonucleotide probes for *R. henselae* (RH1) and *R. quintana* (RQ1) as described elsewhere, which are species-specific and react with either *R. henselae* RH1) or *R. quintana* (RQ1) [Anderson et al., in preparation].

Results

Serological studies revealed no antibodies to *Rochalimaea* species in serum samples from the patient's mother, in the healthy controls and in commercial immunoglobulin. In contrast, markedly elevated titres to *R. quintana* and *R. henselae* were detected in all serum samples of the patient (Fig. 1), first with increasing then with decreasing titres.

In molecular hybridization studies, the template DNA extracted from the biopsied osteolytic lesion yielded a

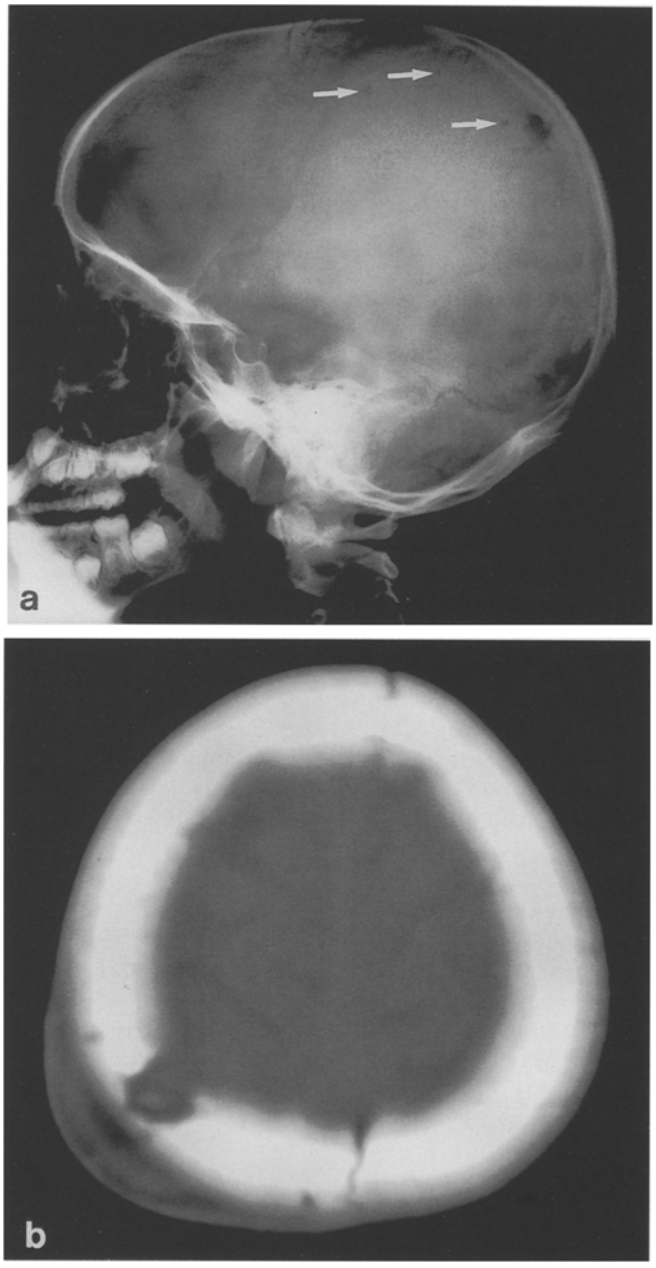

Fig. 2 **a** Lateral view of skull. One large and several small, "satellite" (*arrows*) osteolytic lesions; note lack of sclerotic margins. **b** Axial comupted tomography of the skull. Note extensive right parietal osteolysis and subperiosteal abscess

153-base pair fragment characteristic of *R. henselae* and *R. quintana* [Anderson et al., in preparation]. A control sample without DNA failed to yield the same size fragment. Upon hybridization with species-specific probes RH1 and RQ1, the PCR product hybridized only with RH1, indicating that *R. henselae* was present in the lesion sample.

Discussion

After exclusion of other causes of a lymphadenopathy, CSD remained the likely diagnosis in the reported case, given the triad of exposure to cats, a scratch of primary lesion of the skin, and a typical histopathology with various patterns of predominantly granulomatous necrosis. Demonstration of silverstaining bacilli in the affected tissue confirmed the diagnosis [5]. The detection of DNA species-specific for *R. henselae* proved the presence of *R. henselae* in this lesion.

The multi-organ involvement in our patient is unusual. In approximately 10% of affected children, complications such as Parinaud oculoglandular syndrome, encephalopathy/encephalitis, radiculopathy, pneumonitis, and skin rashes are observed [2, 3, 8]. Only a few cases have been reported with splenic abscesses and osteolytic lesions [2, 7, 10, 15]. Concomitant multiple involvement of both, spleen and bones, in a single patient with CSD has not been reported. No underlying immunodeficiency was found in our patient.

A bacterium, *A. felis* [1], has been grown from affected tissue of only few patients with CSD, However, recently most individuals with clinically suspected CSD were found to have antibodies to *R. henselae* [14] and, even more recently, this bacterium was isolated from two adults with adenitis suggesting CSD [4]. Thus, the pathogenic role of *A. felis* in CSD may need re-evaluation [12]. In the present case, cultures for *A. felis* [5] and *Rochalimaea* species [4, 6] were not attempted. However, high serum antibody titres were detected to *R. quintana* and *R. henselae*. More importantly, analysis of his affected tissue by amplification of extracted DNA used as template in a PCR assay and subsequent hybridization to species-specific oligonucleotides proved the presence of *R. henselae* in the CSD lesions.

Both *R. quintana*, the agent of trench fever, and *R. henselae* have been isolated from patients with cutaneous bacillary angiomatosis [6], a disorder with argyophilic bacilli in the lesions that are related to cat-scratch injuries. The spectrum of disease caused by *R. henselae* further includes bacteraemia in immunodeficient and immunocompetent patients, bacillary peliosis hepatitis, splenitis, and adenitis [4, 6, 9, 13, 17]. Molecular studies have shown that neither *R. quintana* nor *R. henselae* are closely related to *A. felis* [6, 13, 17]. Moreover, there was no detectable cross-reactivity between human antibodies to *Rochalimaea* species and antibodies to *A. felis* [14], but definitively between human antibodies to some isolates of *R. quintana* and to *R. henselae* [Regnery et al., in preparation].

Initial studies indicated that the indirect immunofluorescent antibody test for human sera was species-specific for infection with either *R. henselae* or *R. quintana* [14]. This original study utilized the species prototype isolates

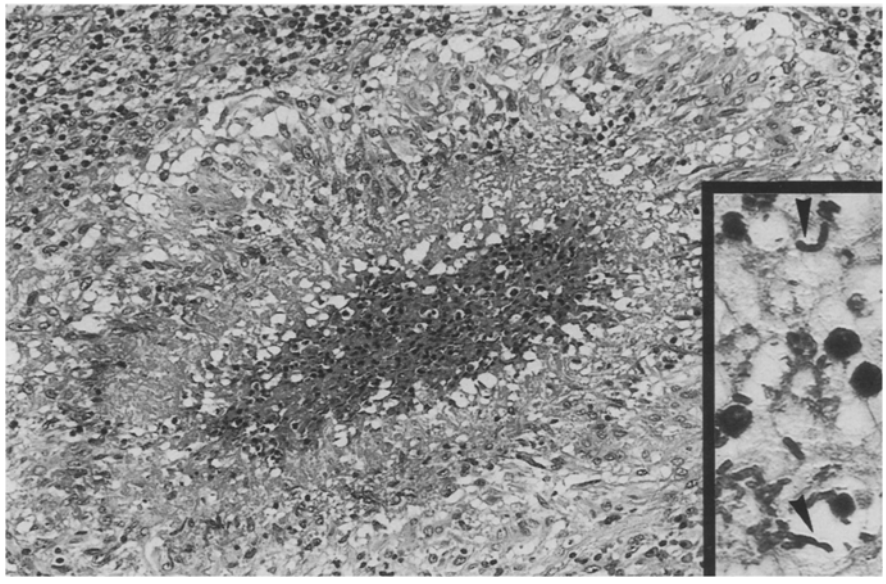

Fig. 3 Necritizing granuloma with central micro-abscecc. Palisading epithelioid cells are located at the periphery. (Haematoxylin-eosin stain; final magnification.) Inset: Extracellular pleomorphic bacilli (*arrow-heads*). Warthin-Starry stain; final magnification)

of *R. quintana* (Fuller isolate) and *R. henselae* (Houston-1 isolate [13]). Convalescent-phase sera, derived from persons with infections with either the Fuller isolate of *R. quintana* or the Houston-1 isolate of *R. henselae*, were used for the present analysis; these sera had minimal crossreactivity with the heterologous species' antigens [14]. However, it is now apparent that substantial serological crossreactivity exists between the antigens of several isolates of *R. quintana*, other than the Fuller isolate, and *R. henselae* convalescent-phase antisera. The Fuller isolate of *R. quintana* appears to lack an important genus-specific epitope(s) and thus may be an inadequate source of immunodiagnostic antigen (data not shown). A more recent, well-characterized *R. quintana* isolate (RLO-90-268 [17]) was used as an alternative source of antigen for the present study. Our patient showed high levels of antibody to the antigens of both *Rochalimaea* species tested. It is important to recognize that this serological test for *Rochalimaea*-associated disease is genus-specific, not species-specific as was originally thought. Therefore, conclusions as to which species of *Rochalimaea* must be held responsible for an infection, based on relative serological titres alone, are not warranted.

So far, the association between *R. henselae* and CSD has been suggested only for individuals living in North America [4, 14, 19]. Our findings underscore the possible pathogenic role of *R. henselae* in CSD and indicate that this pathogen is involved in CSD also in Europe. Furthermore, serological tests for *Rochalimaea* species might be a useful diagnostic tool in suspected CSD, rather than the skin test [2], for which the antigen is not easily available and not standardized.

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