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Histological and Ultrastructural Study of One Case of Oral Bacillary Angiomatosis in HIV Disease and Review of the Literature

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Bacillary angiomatosis (BA) is a new clinicopathological entity defined as a pseudo-neoplastic capillary proliferation secondary to an opportunistic infection by one of two *Rochalimaea* sp.: *R. quintana* or *R. henselae*. Although BA is a recently recognised entity, numerous cases have been reported. Most of the patients affected are reported to have low absolute CD_4 lymphocyte counts associated with AIDS. Yet, very few oral cases associated or not with cutaneous lesions have been reported or simply identified. Histopathological and ultrastructural features of one case of oral BA with gingival and palatal lesions are presented. Clinical aspects of oral BA do not hold pathognomonic features and the lesions may resemble either a reactive lesion of the gingiva, pyogenic granuloma or Kaposi's sarcoma. The lesion is characteristically composed of circumscribed lobular capillary proliferations and the presence of granular amphophilic material on haematoxylin and eosin sections surrounded by neutrophils and neutrophilic debris is a clue to diagnosis. Demonstration of bacilli in the interstitium by the Warthin–Starry silver method or, better, by electron microscopy is diagnostic. BA may contribute to the death of the patient but erythromycin has proved to be very effective treatment.
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INTRODUCTION

EPITHELIOID ANGIOMATOSIS, bacillary epithelioid angiomatosis, or bacillary angiomatosis (BA) is a bacterial infection that produces a pseudoneoplastic capillary proliferation. Initially reported as occurring in the skin and lymph nodes of patients with AIDS [1, 2], BA was later described in immunosuppressed patients [3, 4] and in an immunocompetent individual, although then with no propensity for spreading [5]. Although attempts to culture a potential bacterial agent remained unsuccessful for a long period of time, the association of BA with bacilli was quickly postulated on histological and ultrastructural grounds [1, 6] and as a consequence of the rapid regression of the process after treatment with antibiotics [1, 6–10]. Oral BA lesions have seldom been described precisely [11] and infrequently identified and reported [2, 12, 13]. Therefore, the aim of the present work was to study the histological and ultrastructural features of one case of oral bacillary angiomatosis, observed twice 6 months apart early in its development, and to review the literature related to this recently characterised disease.

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REVIEW OF LITERATURE

Cutaneous features

The most common clinical feature of BA is a cutaneous, painless blood-red angiomatous papule, or nodule. Some larger lesions are almost pedunculated, but no propensity to form macules, patches, or plaques is observed [14, 15]. Lesions may be solitary, few and scattered, zosteriform, or systematised. Cutaneous lesions are of two types—superficial and deep. The superficial type is usually red, purple, or skin coloured, dome-shaped or slightly pedunculated. The surface may be smooth, or ulcerated with crusting. Most of the time, superficial lesions are surrounded by an epidermal collarette. The deep type is often large and rounded, skin-coloured, hyperpigmented or dusky. The consistency of individual lesions is usually somewhat rubbery to firm [2, 9, 10, 14, 16–18]. BA is usually readily distinguished from other diseases the exception being from verruga peruana [14, 18].

Systemic disease in BA

Various visceral manifestations (splenic and hepatic peliosis) have been reported. Widespread visceral involvement is not exceptional and in some cases BA has contributed to the death of the patient [2, 19]. Symptomatic osteolytic lesions and bone marrow involvement associated with cutaneous BA have also been described [20–28]. Concurrent development of BA and Kaposi's sarcoma has been observed [10, 16, 29]. Conversely, lymph node and soft tissue involvement have been reported in HIV-positive's AIDS, and immunosuppressed

patients without the presence of skin lesions [4, 18, 30, 31]. Finally, it has been postulated that peliosis hepatis, and persistent fever and bacteremia in immunocompromised persons are diseases caused by the same agent(s) as cutaneous BA [32–37].

Oral lesions

The first case reported with oral lesions was a 32-year-old man. Numerous 0.1–1 cm diameter red papules and nodules developed on the skin of his head, trunk, and extremities. Conjunctival, nasal, and oral (no further details given) mucosa lesions were associated. HIV antibody was initially negative but a test 2 weeks later was positive [2]. Another case was that of a 32-year-old, HIV-positive man presenting numerous red-purple to dark-red papules and nodules, 0.5–4 cm in diameter, on the skin and on anal, conjunctival, nasal, and palatal mucosa [12]. The third case was a 36-year-old man, with a T4/T8 ratio of 0.3 and cutaneous BA involving the upper part of the body, with bluish to purple lesions on the oral mucosa [13]. Nonetheless, in 1991, the first case of oral BA without concomitant skin involvement was reported [11]. The patient was a 47-year-old man, HIV 1 and 2-positive who presented with a 4-day history of a bluish-purple, ulcerated, and raised lesion of the palate. Two further flat lesions were noted on the gingiva palatal to the upper incisors. Erythromycin 1 g/day halted the progression of the disease, but after discontinuation other large lesions appeared on the gingiva. Histological features of the biopsied lesions appeared identical to previous described cutaneous lesions. However, the authors found that it was not possible to unequivocally identify bacilli.

Histopathology

Lesions are characteristically composed of circumscribed lobular capillary proliferations. The lobules, like those of pyogenic granuloma/lobular capillary haemangioma, are rounded aggregates of capillaries, usually organised so that more mature, ectatic vessels are situated centrally and less mature ones, with or without apparent lumina, are located peripherally. In superficial lesions, covering epithelium may present with or without thinning and ulceration, and there is often a marginal epithelial collarette which imparts even more similarity to pyogenic granuloma [5, 9, 14, 38]. According to the distribution of the components, two histological types have been identified, although they may represent the ends of a spectrum. The first type, superficial, is characterised by a loose and oedematous stroma with a mixed inflammatory infiltrate of lymphocytes, histiocytes, and scattered neutrophils. Only electron microscopy (EM) may show the presence of a number of organisms [14, 16, 18, 38]. The second type, deeper, is characterised by a denser stroma with the occurrence of a dense mixed inflammatory cell infiltrate with neutrophils and neutrophilic debris predominating. Granular amphophilic or purplish material surrounded by neutrophils is typical in haematoxylin and eosin (H and E) sections. In these areas, Warthin–Starry staining allows the demonstration of numerous extracellular clusters or clumps of bacilli and, less often, individual intracellular organisms. Variable results are seen with Giemsa and Brown–Hopps stains. Conventional special stains such as acid fast, PAS, and the Brown–Brenn modification of Gram's stain fail to demonstrate the bacilli [5, 6, 13, 14, 38–40]. The endothelial cells vary considerably in appearance from a slight to marked protuberance into vascular lumina. There is usually only mild nuclear atypia, but pleomorphic nuclei and mitoses are occasionally seen. These cells express factor-VIIIr antigen and are positive for Ulex europaeus with vessels in the centre of the lobules reacting more than peripheral ones, therefore resembling those of capillary haemangioma [11, 12, 38]. Endothelial cells give the impression of multilayering with deeper cells merging imperceptibly onto the interstitial population, where large cuboidal/polygonal cells containing abundant cytoplasm, often closely adherent, are present [41]. The close adhesion of the interstitial cells gives the lesions their epithelioid appearance. Many of them but not all are positive with both reagents. Cells present in the interstitium are also positive for α1-anti-chymotrypsin, lysozyme, and factor-XIIIa [2, 12, 42]. Enzyme histochemistry demonstrates patchy luminal staining, similar to that of small vessels, with positivity for 5′-nucleotidase, alkaline phosphatase, and ATPase [38]. Endothelial cell necrosis is a variable finding and may be observed in the centres of vascular lobules. Atypical endothelial cells and cells in mitosis are often present adjacent to the necrotic zones.

Electron microscopy

EM studies have shown that organisms are located extracellularly amidst collagen fibres, in areas where discontinuity of normal tissue and some cellular debris are present. The ultrastructural appearance of these organisms is consistent from case to case but does not enable specification [17]. Bacilli disclosed in BA, and in cases of bacteraemia with fever are pleomorphic and measure 0.2–0.5 μm by 1–3 μm by transmission electron microscopy [6], and 0.5–0.6 μm by 1–2 μm when studied by scanning electron microscopy [43]. They possess trilaminar walls [6].

Biochemistry

Before molecular biology studies could be conducted, Slater *et al.* [34] described a newly recognised fastidious Gram-negative bacillus causing prolonged fever with persistent bacteremia in immunocompromised or immunocompetent patients, as being most similar to *Rochalimaea quintana* (the agent of trench fever) on the basis of cellular fatty-acid content. Later, two BA strains studied for fatty-acid methyl esters (FAME) by gas chromatography were demonstrated as being characterised by the presence of significant amounts of 16 and 18 carbon saturated FAME as well as 18 carbon unsaturated FAME [44]. Finally, studies have shown that *R. henselae* contains C-18 in amounts averaging $\geqslant 22\%$ in contrast to *R. quintana* which contains only 16–18% of this cellular fatty acid [37].

Bacteriology and molecular biology

Because, initially, pathogens could not be grown, their identity remained controversial. In 1990, the use of broad-range 16S ribosomal DNA (rDNA) primers demonstrated that BA was caused by a previously uncharacterised rickettsia-like organism, designated strain BA-TF [33], closely related to *R. quintana* [45]. Other studies suggested that this organism might be the causative agent of two other diseases occurring in HIV-positive patients: bacillary peliosis hepatis and fever with bacteraemia [32, 34]. Regnery *et al.* [43] and Welch *et al.* [37] have proposed a new member of the family *Rickettsiaceae*

with the name of *Rochalimaea henselae* as an agent of the vascular proliferative lesions and prolonged fever with bacteraemia that may be encountered in immunocompromised or immunocompetent patients. Later, the 16S rRNA sequences of BA-TF and *R. henselae* were determined, their comparison suggesting that these sequences were derived from the same organism, or from strain variants thereof. Furthermore, it is now evidenced that BA-TF/*R. henselae* is distinct from *Afipia felis*, the cat scratch bacillus [43, 46]. Nonetheless, recently, Koehler *et al.* [20] have evidenced that either of two species of rochalimaea: *R. quintana* or *R. henselae*, can be isolated from cutaneous lesions or blood from patients with BA. Growth of the agent(s) of BA is difficult, and only recently two methods of culture have been proposed. Optimal growth occurs on BHIA and tripticase soy agar (TSA) medium supplemented with 5% sheep blood as well as HIA supplemented with 5% rabbit blood. Original colonies are observed after 9–15 days of incubation at 35°C in 5% CO_2 humidified atmosphere, or at 30°C in air. Cells are oxidase and catalase negative, or weakly catalase positive, non-reactive in carbohydrate utilisation tests, and urease negative [43]. Two alternative methods have been proposed, one is based on direct plating of homogenised tissue onto solid agar and the other relies on endothelial-cell monolayer culture [20].

Immunology

Cockerell *et al.* [47] have assessed the specificity of induced murine antibodies for antigens from *R. henselae* and the closely related species *R. quintana*. All of the *R. henselae*-induced immune sera reacted with all of the *R. henselae* strains tested and cross-reacted minimally with *R. quintana*. Demonstration of immunocytochemical reactivity of *R. henselae* in cutaneous BA, parenchymal bacillary peliosis of the liver and spleen, and in the blood of patients with persistent fever with bacteraemia was achieved on formalin-fixed, paraffin-embedded tissues with the use of a polyclonal antiserum raised in rabbits. The antiserum was not cross-reactive with *Afipia felis* [48].

Occurrence of viruses

In one study, reporting 2 cases of BA, Epstein–Barr virus (EBV) was demonstrated by *in situ* hybridisation in the nuclei of endothelial cells and occasional histiocytes. However, the virus was not seen in the other skin lesions present concomitantly and neither patient showed clinical evidence of active EBV infection [49]. Cytomegalovirus (CMV) occurrence in endothelial cells of BA has been described in two AIDS patients, nonetheless, it has been previously postulated that CMV may function as a stimulator of angiogenesis [50–52].

Management and prevention

Erythromycin, 500 mg orally four times daily for several weeks to 2 months, appears the treatment of choice for BA [1, 9, 12, 14–16, 53]. Successful treatments based on the use of doxycycline, gentamicin and ciprofloxacin have also been reported [4, 9, 26, 54]. However, recurrences after cessation of the therapy may occur [8, 12]. Antibiotics that inhibit cell wall synthesis, like penicillin, have been shown to fail to cure BA [9, 13, 32]. If untreated, the disease may be life threatening, although spontaneous regression of cutaneous BA in one HIV-positive patient has been observed [9]. Preliminary data suggest that the domestic cat can serve as a potential reservoir for *R. henselae*-associated disease in man [55] and a recent cat scratch or bite has been reported frequently. Nonetheless, the association between BA and exposure to cats should be interpreted with caution [56–57].

REPORT OF CASE

In February 1992, a 32-year-old man sought treatment by a private periodontist for a gingival mass located between the maxillary incisors and inducing discomfort rather than true pain. The lesion had been present for approximately 2–3 months and had been steadily increasing in size. On intraoral examination, the mass was located on the labial aspect of the interdental papilla of the upper central incisors, measured about 10 mm in diameter and extended about 8 mm forward in front of the gingiva. It presented as an elevated, rather broad-based proliferation with a tendency to extension over the adjacent teeth. The gingival enlargement was reddish-pink, not ulcerated, smooth, shiny, and the consistency was not really firm (Fig. 1). Bleeding occurred on slight probing. Probing depth was not taken, due to the overgrowth. Central incisors were separated by a diastema where the lesion was extending giving rise to a broad based tumefaction (15 mm × 7 mm) involving the areas of the incisive (palatine) papilla. The tumefaction was red–blue in colour and the surface was smooth and shiny (Fig. 2). A periapical radiograph did not reveal bone loss. Oral hygiene was poor and plaque deposits were present. Careful examination of the mouth revealed no further similar lesions. The patient did not appear to be in good health, but medical interrogation was not contributory since he was very reluctent to answer questions. At that time, oral hygiene instruction was given and the patient was provided with chlorhexidine mouth rinses, spiramycin (4 500 000 IU/day) and metronidazole (750 mg/day). One week later, although the oral hygiene was better, drastic improvement was not observed. Biopsy specimens were taken on both labial and palatine aspects of the lesion, fixed in Bouin's solution and transmitted with the provisional clinical diagnosis of a reactive lesion of the gingiva. Follow-up remained impossible, the patient not showing at the following appointments. However, 6 months later, the patient sought treatment at the ENT-Maxillofacial Surgery Department of

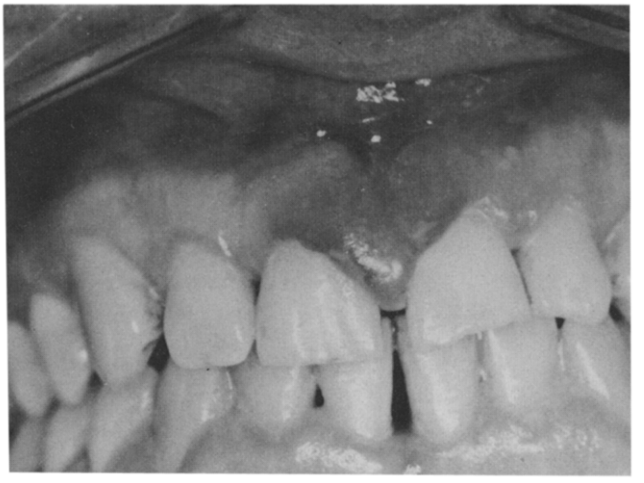

Fig. 1. Initial lesion: reddish–pink gingival overgrowth extending about 8 mm forward.

the University Hospital for pharyngeal pain associated with fever (39°C). Clinical examination revealed red–purple nodules of about 3–8 mm in diameter located in the area of the incisive (palatine) papilla, the right retromolar area and the pharyngeal mucosa. Cutaneous lesions were not present. At that time, it was found that the patient was a homosexual man, HIV1-positive since 1986 and had been hospitalised several times. He had been treated with zidovudine for several years. Retrospectively, the medical file revealed that his absolute CD_4 lymphocyte count was 30/mm^3 when he consulted the periodontist. Kaposi's sarcoma was suspected and two biopsy samples were taken in the retromolar area. Material was fixed in Bouin's solution and in glutaraldehyde for electron microscopy. After the diagnosis of BA on histopathological grounds, treatment with erythromycin in an oral dose of 3 g per day was initiated. Lesions healed after 15 days of treatment.

Material and methods

For routine histopathology, all samples were fixed in Bouin's solution and embedded in paraffin. Sections 5 μm thick were obtained, dewaxed in xylene and hydrated. Sections were then stained with haematoxylin and eosin, reticulin stain, and Gram, Grocott, Ziehl–Neelsen, Giemsa and Warthin–Starry stains. Other sections were processed for immunohistochemistry using standard technique. In brief, streptavidin–biotin–peroxidase complex (Dako) was applied in conjunction with a mouse monoclonal antibody raised against factor-VIIIr antigen/von Willebrand factor (Dako). Primary antibody was used at a dilution of 1:60 for 90 min at room temperature. The amino 3-ethyl 9-carbazole was used as a chromogen. Sections were counterstained with haematoxylin. Negative controls were produced by substituting the primary antibody with normal serum. Positive controls were sections of an epithelioid haemangioendothelioma. Samples from one of the biopsies taken from the retromolar area during the second stage of the disease were minced into 1 mm cubes and fixed in 2.5% phosphate buffer glutaraldehyde, post-fixed in OsO_4 in the same buffer, and processed for electron microscopy by routine methods. Two μm sections were stained with toluidine blue and observed under light microscopy. Thin sections were contrasted with uranyl acetate–lead citrate and were observed under a JEM 1200 EX (Jeol) transmission electron microscope at 80 kV.

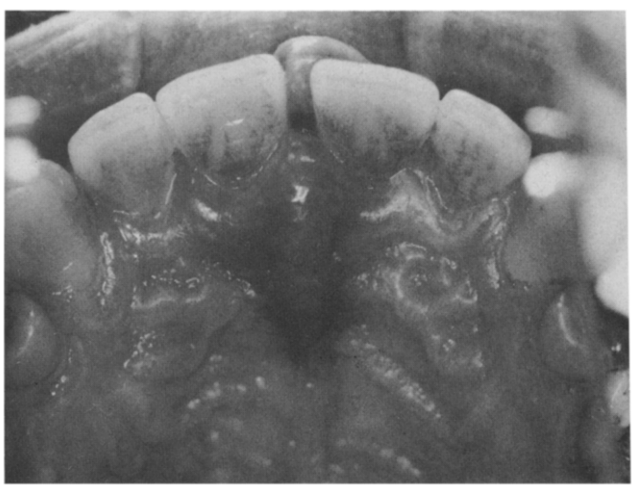

Fig. 2. Initial lesion: broad based red–blue tumefaction of the incisive (palatine) papilla.

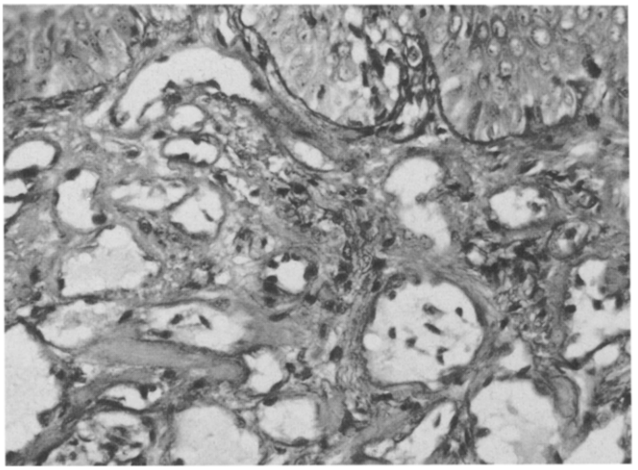

Fig. 3. Biopsy specimen of the second stage of the disease. Lobular proliferation of ectatic round vessels. Reticulin stain, initial magnification × 90.

Histopathology

Sections of both biopsies taken during the initial stage of the disease and stained with haematoxylin and eosin showed a moderately hyperacanthotic, parakeratinised squamous epithelium covering an abundant vascular proliferation. Proliferation, extending up to the epithelial basement membrane, was made of small, well defined and ectatic blood vessels. The vast majority of the vessels were thin walled and composed of a simple endothelium made of flat cells. Some other rarer vessels with a more organised wall and lined by cuboidal endothelial cells protruding in the lumina were present. Interstitium was limited in extent, oedematous, and extremely rare inflammatory cells were observed. Reticulin stain demonstrated fibres surrounding individual capillaries but a true lobular pattern was lacking. Extravasation of erythrocytes was observed but hemosiderin deposits were absent. All bacteriological stains such as Gram, Grocott, Ziehl–Neelsen, and Giemsa stains as well as the Warthin–Starry silver method failed to demonstrate bacilli within the lesion. Immunohistochemical staining with antibody to factor-VIIIr antigen was positive in the prominent endothelial cells of the rare well-defined capillary vessels but not in the other vessels nor in any cell of the interstitium. Diagnosis of atypical lobular capillary haemangioma was made. Sections of the biopsies taken during the second stage of the disease and stained with haematoxylin and eosin revealed the same basic aspect seen during the initial stage. However, with reticulin stain, the proliferation appeared more lobular and made predominantly of round vessels with ectatic luminae (Fig. 3). Well-organised vessels were more numerous than previously and prominent endothelial cells more frequent. Mitoses were exceptional. Interstitium was more developed but remained highly oedematous. Few foci of neutrophils and scattered mast cells were present. All bacteriological stains used except the Warthin–Starry stain remained negative. In the interstitium, the Warthin–Starry silver method revealed black deposits corresponding to small clumps of bacteria (Fig. 4). Single rods could be seen at the edges of the clumps. Again, antibody to factor-VIIIr antigen

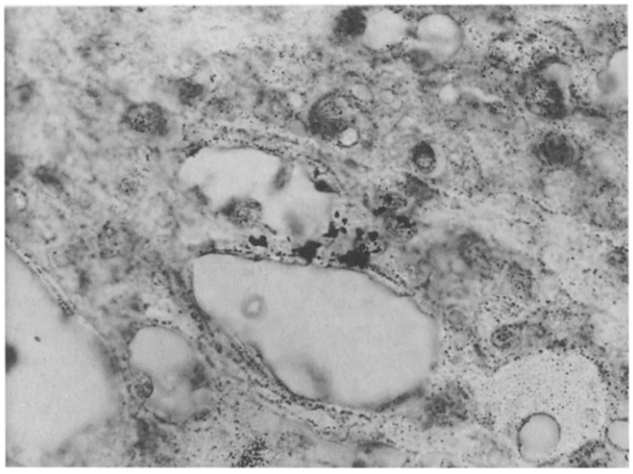

Fig. 4. Biopsy specimen of the second stage of the disease. Clumps of bacilli in the interstitium. Warthin–Starry stain, initial magnification ×225.

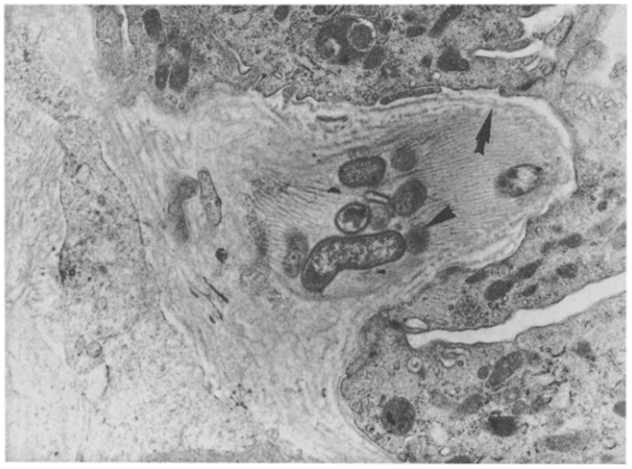

Fig. 5. Biopsy specimen of the second stage of the disease. Bacilli in close relation with endothelial cells. T.E.M., initial magnification ×10 000 (arrow = endothelial cells; arrow head bacilli).

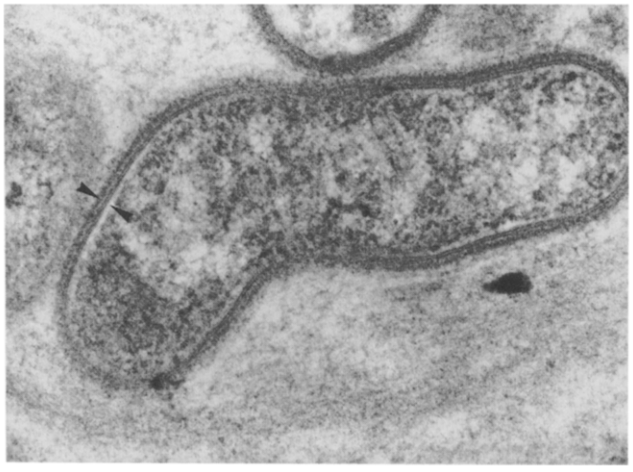

Fig. 6. Biopsy of the second stage of the disease. Characteristic trilaminar cell wall structure of the bacilli. T.E.M., initial magnification ×60 000 (arrow head = clearly visible trilaminar aspect).

was restricted to the prominent endothelial cells or to the more organised vessels and no labelling was observed in the interstitium. Electron microscopic observation showed more abundant bacilli diffusely present. In the interstitium, they occurred either as single organisms, arranged in chains or in collections. Some were in close contact with endothelial cells but remained located extracellularly (Fig. 5). Bacilli, 1.25–2.5 μm in length and 0.35 μm in diameter, presented with a characteristic trilaminar cell wall structure: two electron-dense layers separated by a less electron-dense layer (Fig. 6). The inner structure was granular with electron-dense areas. A diagnosis of bacillary angiomatosis was made.

DISCUSSION

BA is a pseudo-neoplastic capillary proliferation secondary to an opportunistic infection by one of two rochalimaea species: *R. quintana* or *R. henselae*, mostly seen in immunodeficient or in immunosuppressed patients [20, 33, 34, 37, 43, 44, 46]. Although BA is a recently recognised entity, numerous cases have been reported most often related to cutaneous disease but also to various internal involvements. Very few oral cases associated or not with cutaneous lesions have been reported or even identified [2, 11–13]. Early reports have repeatedly shown that the main challenge to the diagnosis of cutaneous BA is linked to its clinical resemblance to pyogenic granuloma when it is superficial and solitary, and to Kaposi's sarcoma when it is deep-seated [6, 10, 18, 38, 39, 49, 50]. The apparent rarity of oral disease in BA may be related to this resemblance although the possibility of rare involvement of the oral mucosa could be associated with the mode of contagion if cats do represent a potential reservoir for *R. henselae* [55], or to oral immunity. In the present case, both aspects of the lesion were not clinically identical. The vestibular component of the lesion, presenting as an exophytic proliferation, clinically resembled reactive lesions of the gingiva which present considerable overlap with regards to clinical features [58]. The palatine component was sessile, like a Kaposi's sarcoma as in the case reported by Speight *et al.* [11]. Therefore, it seems that oral BA has no pathognomonic features nor enough distinctive features to make a definitive diagnosis on clinical grounds alone. Nonetheless, although 1 case of BA has been reported in an immunocomponent individual [5], cases with oral involvement have been in patients with an absolute CD_4 lymphocyte count lower than 200/ml. Therefore, the occurrence of concurrent immunodeficiency or immunosuppression may help to suggest BA as a provisional/presumptive clinical diagnosis.

Cutaneous BA lesions are categorised as either superficial or deep dermal [14]. Due to the structure of the masticatory mucosa only the superficial type is likely to develop in the gingiva. As observed by others [12], the lesions in our patient lacked the prominent leucocytic inflammatory infiltrate, nuclear dust and granular amphophilic material, while several clusters of bacilli were shown by the Warthin–Starry silver method in sections corresponding to the biopsies of the second stage. The conjunction of lobular proliferation of small round vessels with plump endothelial cells protruding in the vascular lumina and occurrence in the interstitium of bacilli, demonstrated by the Warthin–Starry method or better by electron microscopy, is considered to be diagnostic [2, 5, 14–16, 29, 57, 59]. In the case reported here, no bacteria could be demonstrated in the biopsies of the initial lesion by the Warthin–Starry method, while several clumps were identified in the

second stage material. Furthermore, far more numerous bacilli were disclosed by ultrastructural observation than by the silver technique. This discrepancy may be related to a greater efficiency of ultrastructural observation. It may also be due to the antibiotic treatment prescribed, since BA has been observed to respond well to various antibiotic treatments apart from penicillin [1, 4, 5, 9, 12, 15, 16, 26, 53, 54]. Ultrastructurally, organisms were diffusely present in the interstitium. Although without a tendency to clump, abundant organisms were found extracellularly. Some were arranged in chains or in collections scattered between collagen fibrils. Others, single, were seen in close association with endothelial cells, characterised by the occurrence of Weibel–Palade bodies. Bacilli were characterised by a trilaminar wall with two electron-dense layers separated by a less electron dense layer, as in previous BA cases [1, 6, 26, 27, 31, 39, 51, 53]. However, bacilli with similar ultrastructural aspects have been described in a closely related disease, the verruga peruana of Carrion's disease [60]. Many of the large interstitial cells were observed to be factor-VIIIr antigen positive by LeBoit *et al.* [38]. In the case reported here, presenting with very limited interstitium, such positivity could not be found. Only endothelial cell protruding into the lumina or endothelial cells of the more organised vessels were stained. This observation is consistent with the results of Burgdorf *et al.* [61] evidencing that the endothelial cells of small normal cutaneous vessels give the strongest reaction while in pyogenic granuloma positivity is either patchy or of lesser intensity. The main histological differential diagnoses include Kaposi's sarcoma because of its frequent occurrence in AIDS patients, pyogenic granuloma/lobular capillary haemangioma [62] because it is rather common in the oral cavity [63, 64], and verruga peruana. The most salient histological difference between BA and Kaposi's sarcoma is the absence of fascicles of spindle cells in the former. BA and pyogenic granuloma/lobular capillary haemangioma share features like polyploid architecture, clusters of rounded vessels with ectatic luminae and smaller capillaries surrounding ectatic ones. Only the demonstration of bacilli in the interstitium by the Warthin–Starry method or better by electron microscopy can lead to the proper diagnosis. The most difficult differential diagnosis is certainly with verruga peruana, the second or eruptive phase of Carrion's disease (bartonellosis), since both conditions are vascular proliferations caused by bacilli and both bacilli have the same ultrastructural presentation. It has been postulated that bacteria in BA are always interstitial [38], whereas some are phagocytised and present in intracytoplasmic vacuoles in verruga peruana [60]. Furthermore, infections with *B. bacilliformis* are almost entirely restricted to Peru.

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