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Ultrastructural study of granulomatous tissue in tonsillar malakoplakia

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Summary. The ultrastructural study carried out in a case of tonsillar malakoplakia confirmed that granulomatous lesions consisted mainly of macrophages containing peculiar calcified inclusions (Michaelis-Gutman bodies) considered pathognomonic for the disease.

Moreover macrophages frequently contained ingested Gramnegative bacilli and presented aspects of mitochondrial degeneration and autophagy. These latter features were probably the consequence of bacterial infection rather than the expression of primary cellular defects, as the clinical evolution of this case of malakoplakia did not support the existence of generalized macrophage alterations.

Key words: Malakoplakia – Michaelis Gutmann bodies – Tonsil

Introduction

Malakoplakia is a rare inflammatory disease, generally associated with bacterial infection, which affects different organs and apparatus with the presence of granulomatous lesions. The latter typically consist of macrophages containing large calcified inclusions (Michaelis-Gutmann bodies) which are thought to arise from the defective digestion of ingested bacteria (For review, see Mc Clure, 1983; Le Bourgeois and Parlier, 1984).

In this paper we describe a relatively uncommon case of tonsillar and pharyngeal malakoplakia in which histological and ultrastructural study of the lesions allowed for identification of intracellular Gram-negative bacteria.

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Materials and methods

Malakoplakia was found in a 74-year-old man presented to the otorhinolaryngology service with a several year history of recurrent laryngitis.

Physical examination of the patient revealed the presence of four roundish plaques ranging in diameter from 2 to 5 mm, one of which was located on the upper part of the right tonsil, while the other ones were scattered in the glossoepiglottic fold and on lingual tonsil. All the plaques were excised and submitted to light and electron microspy examination.

For light microscopy, the tissues were fixed in 10% buffered formalin, embedded in paraffin and stained with hematoxilin-eosin, PAS (with and without diastase predigestion), von Kossa's method for calcium and Perl's stain for iron.

For electron microscopy, tissues were fixed in 3% glutaraldehyde in 0.1M cacodylate buffer, ph 7.2, postfixed in 1% osmium tetroxide and embedded in epoxy resin. Semithin sections (1 pm) were stained with toluidine blue. Ultrathin sections were conterstained with uranyl acetate and lead citrate before electron microscope examination.

The patient, not submitted to medical therapy after surgery, did not present signs of infection in a follow-up period of two years.

Results

Light microscopy

In paraffin and semithin sections, the plaques appeared to be lined by stratified squamous epithelium similar to that of the mucous membrane of the pharynx (Fig. 1), but heavily invaded by bacteria and leucocytes (Fig. 2).

Granulation tissue consisted of irregular aggregates of macrophages separated by thin layers of extracellular matrix (Fig. 1, 3). The cells contained an ovoid,

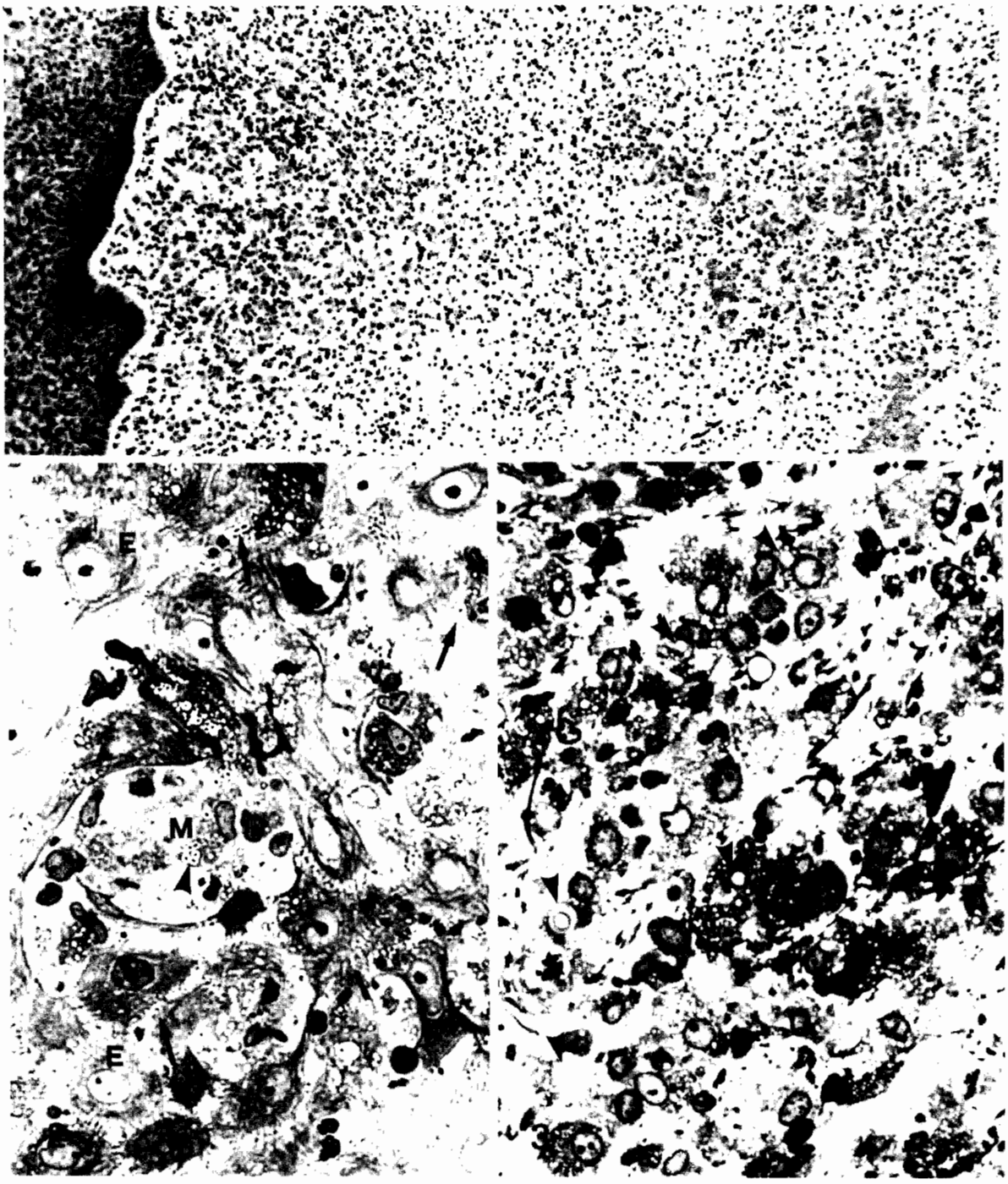

Fig. 1. Photomicrograph of a paraffin section showing aggregates of macrophages beneath tonsillar epithelium surface (E). Hematoxylin-eosin. ×100

Fig. 2. Semithin section tangential to surface epithelium showing the presence of intercellular (arrows) and intracellular (arrowheads) bacteria. M. macrophage. ×640

Fig. 3. Semithin section showing macrophages containing Michaelis-Gutmann bodies (arrowheads). ×640

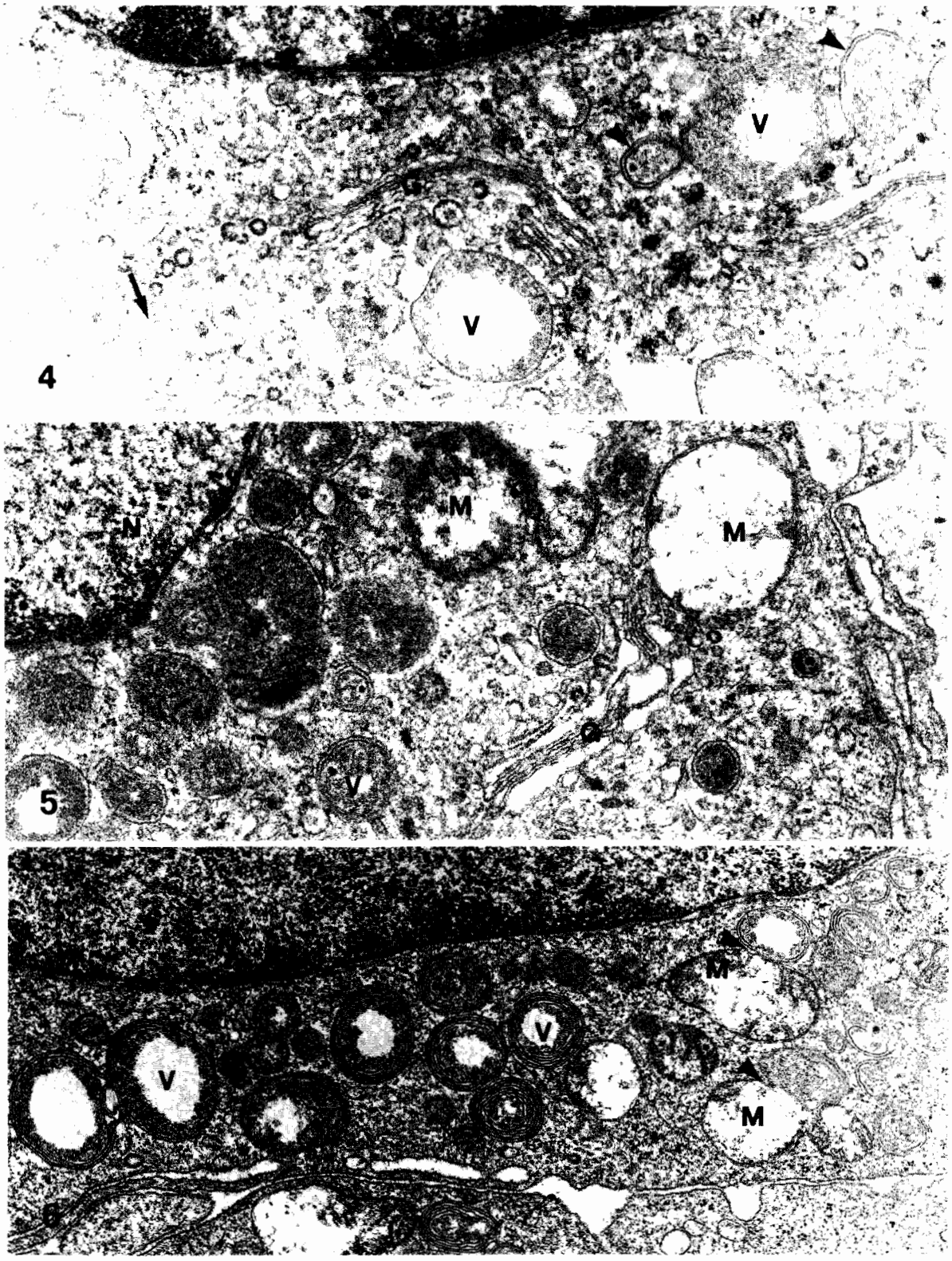

Fig. 4. Golgi region of a macrophage showing flattened cisternae (arrows) and vacuoles (V) with simple or double membrane envelopes (arrowheads). ×32,000

Fig. 5. Macrophage containing degenerating mitochondria (M) and vacuoles (V) in proximity of a Golgi apparatus (G). N:nucleus. ×17,500

Fig. 6. Macrophage containing numerous vacuoles (V), wrapped by single or multiple membrane envelopes, and degenerating mitochondria (M) which seem to fuse with vacuoles (arrowheads). N:nucleus. ×19,500

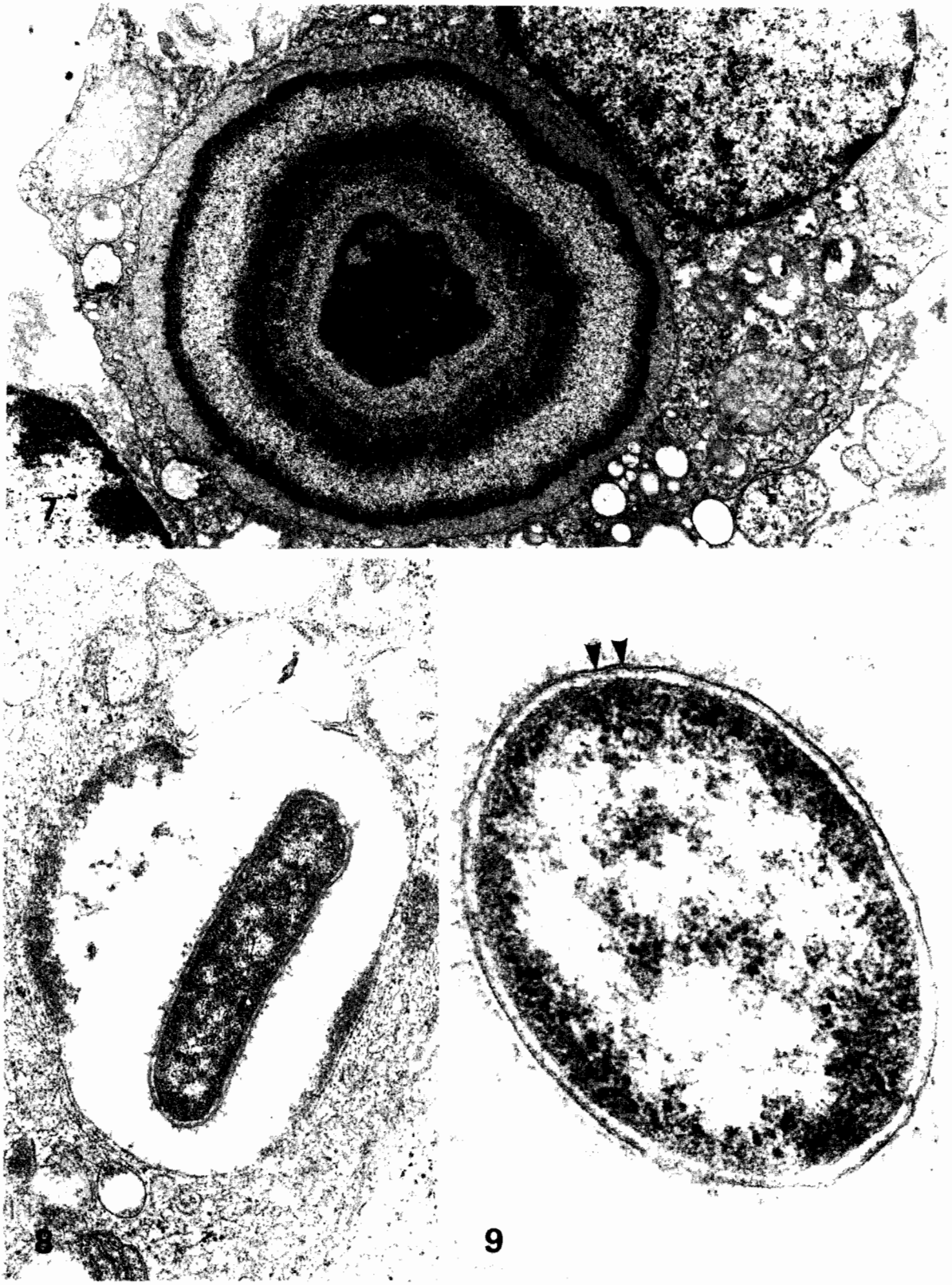

Fig. 7. Macrophage containing a Michaelis-Gutmann body. ×12,500

Fig. 8. Macrophage containing an ingested bacterium. ×32,000

Fig. 9. Detail at higher magnification of a well-preserved intracellular bacterium. Arrowheads indicate outer and inner membranes. ×92,000

eccentrically placed nucleus bearing one or two nucleoli, and abundant foamy cytoplasm, rich in round granulations of different sizes (Fig. 3). The largest cytoplasmic inclusions were formed by concentrical laminations and displayed the typical structure of Michaellis-Gutmann bodies (Fig. 3). These latter ones were positive to PAS, von Kossa's and Perl's methods. Intracellular bacteria were easily recognizable mainly in the most superficial macrophages (Fig. 2).

Granulocytes, lymphocytes and plasma cells were also present interspersed among the macrophages.

Electron microscopy

At the ultrastructural level, the macrophage cytoplasm was characterized by the presence of rough and smooth endoplasmic reticulum, free ribosomes, well-developed Golgi complexes (Fig. 4), bundles of filaments and coated vesicles. Mitochondria frequently showed dilatation with loss of cristae and accumulation of microgranular substance (Figs. 5, 6).

However, the most remarkable feature was the high number of primary and secondary lysosomes and of residual bodies characterized by a pronounced plemorphism. Vacuoles wrapped by single or multiple membrane envelopes, and containing finely granular material adhering to the inner aspect of their limiting membrane, were widely represented. The formation of these vacuoles seemed to be initiated from wrapped, flattened sacs of smooth endoplasmic reticulum segregating portions of cytoplasm (Fig. 4). Moreover aspects suggesting fusion of vacuoles with degenerating mitochondria were frequently seen (Fig. 6). Many macrophages contained Michaelis-Gutmann bodies consisting of alternating electron-opaque and electron-lucent concentric layers, whose variable density was due to the different concentration of microcrystalline structures (Fig. 7). Moreover, phagosomes containing bacterial fragments or apparently intact bacteria were frequently observed (Figs. 8, 9). In the latter case, microorganisms were separated from the limiting membrane of the phagosome by an electron-transparent halo (Fig. 8).

Bacteria were rod-shaped (Fig. 8) and were surrounded by a plasma membrane and by an envelope containing an outer membrane (Fig. 9) typical of Gram-negative bacilli.

Discussion

To our knowledge, only two cases of malakoplakia involving oropharyngeal region have been so far reported (Destombes et al., 1975; Kalfayan and Seager, 1982).

The morphological characteristics of granulomatous lesions just described, including the presence of intracellular bacteria, are largely comparable to those previously reported in other cases of malakoplakia and considered pathognomonic for the disease (McClure, 1983). In addition, we have observed that the majority of intracellular membranous whorls, which are typical of malakoplakik macrophages and have been generally considered remnants of bacterial digestion (Sencer et al., 1979), are actually autophagic vacuoles. The nature of such structures is demonstrated by the fact that, as autophagic vacuoles (Glaumann et al., 1981), they form progressively from preexisting cytoplasmic membranes wrapping portions of cytoplasm. Although the presence of autophagic vacuoles in macrophages is common and is related to membrane recycling, the accumulations of such organelles in malakoplakik macrophages and the presence of mitochondrial degeneration indicate a situation of cell sufferance. It is impossible to say whether these morphological alterations are the consequence of primary defects of cell biochemistry or are produced by bacterial infection. It is known, in fact, that autophagy is induced by various experimental or pathological conditions and can be related either to intrinsic metabolic alterations or to various exogenous factors (Glaumann et al., 1981).

Although the pathogenesis of the malakoplakia is still unclear, it is generally thought that the disease is related to an impairment of the macrophage bactericidal activity caused by defective function of cytoskeleton preventing phagosome lysosome fusion (Abdou et al., 1977; Mc Clure, 1981). However, the asymptomatic course and the prompt recovery observed in this case of malakoplakia does not support the existence of generalized defects of macrophages. It is more probable that temporary decrease of patient's immune defense could have affected pathogen-macrophage interplay in favour of the former. On the other hand, it is known that bacteria can resist phagocytosis with several mechanisms, the most common of which is the inhibition of phagosome-lysosome fusion (Ryter and Chastellier, 1983). It is worth remembering that electron-transparent haloes surrounding intracellular bacteria similar to those observed in this case of malakoplakia have been previously described in pathogenic strains of Mycobacteria and related to the presence of a capsule inhibiting phagocytosis (Ryter and Chastellier, 1983). The effect of the toxic substances produced by bacteria during their prolonged intracellular survival could account for the morphological alterations observed in macrophages.

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