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Ultrastructural Evidence of the Evolutional Process in Malakoplakia

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Abstract

Context: Malakoplakia can be caused by incomplete digestion of *Escherichia coli* by lysosomes, leading to recurrent urinary tract infections and consequential mass-forming events that mimic tumors.

Objectives: By using ultrastructural findings, we aimed to specify the process of phagolysosome to evoke malakoplakia.

Design: We observed a series of processes to form a peculiar Michaelis-Gutmann (MG) body in three patients with malakoplakia and compared with xanthogranulomatous pyelonephritis.

Results: The ultrastructural findings were realigned according to the sequence of events as pre-phagosomal, phagosomal, and post-phagosomal stages. For the mature MG body, numerous lysosomal aggregates targeting pathogens and subsequent incomplete digestion are prerequisite factors for the pre-phagosomal stage. Scattered lamellated residue is late evidence of the pre-phagosomal stage. Phagosomes can be formed by the fusion of multiple pathogens and multiple lysosomes. We utilized transmission and scanning electron microscopy to speculate on the process of phagolysosomal formation.

Conclusion: The recognition of *E. coli* captured by phagosomes or partially damaged by lysosomal attack within the cell was recorded for the first time. Furthermore, SEM observation was performed on human tissue.

Key Words: malakoplakia, Michaelis-Gutmann body, E-coli, xanthogranulomatous pyelonephritis, Transmission electron microscope, Scanning electron microscope
Introduction

Approximately 500 cases of malakoplakia have been reported in the literature; these predominantly involved genitourinary tracts including the urinary bladder, kidney, ureter, or prostate, but also outside the urinary tract such as gastrointestinal tract, central nervous system, female genital tract, and tongue (Curran, 1987; Dobyan et al., 1993; Ballesteros Sampol, 2001; Yousef, 2009). Its pathogenesis is caused by incomplete digestion of *Escherichia coli* by lysosomes, regardless of phagocytosis, likely caused by defective lysosomal function (Lou and Teplitz, 1974; Raposo et al., 2002). Pathologically, many large, lamellated mineralized phagosomes, known as Michaelis–Gutmann (MG) bodies, aggregate within von Hansenmann histiocytes (Curran, 1987; Dobyan et al., 1993; Ballesteros Sampol, 2001; Yousef, 2009). Due to mineralized calcospheroid bodies, *von Kossa* staining and ultrastructural analysis of phagosomes are useful in cases of vague histologic findings. Recently, many studies of malakoplakia have focused on the protean nature of its clinical presentation as a cancer mimicker or event associated with cancer (Bhaijee et al., 2012; Lee et al., 2015; Medlicott et al., 2016). Ultrastructural descriptions of malakoplakia are rare (Sencer et al., 1979; Esparza et al., 1989; Lusco et al., 2016). We clarified six evolutionary processes of MG-body formation via transmission electron microscopy (TEM) and scanning electron microscopy (SEM). We used the TEM and SEM analyses to obtain more convincing ultrastructural evidence of the differences between classical xanthogranulomatous (XG) pyelonephritis and typical malakoplakia, in particular evaluating malakoplakia in human tissues.

Materials and methods

A total of three cases of malakoplakia, two cases of XG pyelonephritis, two cases of XG cholecystitis, and one case of XG salpingitis were included in this study. Two of three cases of malakoplakia (a 65-year-old woman with affected urinary bladder; a 72-year-old man with affected prostate gland) revealed numerous calcospheroid bodies and mineralized lamellated globules. Another malakoplakia
patient (a 75-year-old man with affected prostate glands) exhibited only a few MG bodies. XG inflammatory disease was characterized by foamy histiocytes, rather than slightly acidophilic or amphophilic cytoplasm as seen in malakoplakia. XG patients exhibited a variable mixture of inflammatory cells and fibrosis.

**Special Stain**

For special histochemistry, periodic acid–Schiff (PAS), *von Kossa*, and iron staining were applied. For immunohistochemistry, lysozyme staining was performed using a routine automatic *Ventana* system.

**Scanning Electron Microscopy**

Paraffin-removed slides were fixed for 2hrs in *Karnovsky*’s fixative (2% glutaraldehyde, 2% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4) and washed twice for 30 min in 0.1 M PB. Slides were fixed with 1% OsO₄ for 1hr and dehydrated in an ascending gradual series of ethanol (50-100%) and dried with a Critical Point Dryer (LEICA EM CPD300). Slides were coated with platinum by ion sputter (LEICA EM ACE600) and observed with a field emission scanning electron microscope (MERLIN, ZEISS).

**Transmission Electron microscopy**

**Samples** removed from paraffin were fixed for 2hours in Karnovsky's fixative (2% Glutaraldehyde, 2% Paraformaldehyde in 0.1M phosphate buffer, pH 7.4) and **washed** in 0.1M phosphate buffer. They were postfixed with 1% OsO₄ for 2hr and dehydrated in ascending gradual series (50 ~ 100%) of ethanol and infiltrated with propylene oxide. Then, mix and infiltrate Epon mixture(Polysciences) and propylene oxide by 1:1, them with epon mixture and polymerize them at 65°C oven(TD-700, DOSAKA, Japan). The block is cut using the ultramicrome (LEICA EM UC-7, Leica Microsystems, Austria) and observed with a *Transmission Electron Microscopy*(JEM-1011, JEOL, Japan) at the acceleration voltage of 80kV.
Results

Histologically, the mass was entirely composed of packed histiocytes with minor elements of neutrophils and plasma cells. Sheets of plump ovoid histiocytes showed amphophilic to slightly eosinophilic plump cytoplasm with many spheroid bodies within the cytoplasm. (Fig 1) Common findings were intracellular spheroid bodies of variable sizes, often larger than the nuclei of histiocytes, and occasional calcified lamellation, and MG bodies. (Fig 1, inset) They were strongly positive for PAS, Von Kossa, and lysozyme staining (Fig 2). Lysozyme was strongly and diffusely stained in small granules and large spheroid bodies. The lysozyme-positive spheroid bodies had double-membrane bound globular appearances. Von Kossa strongly stained only the mineralized MG bodies at a relatively lower frequency than did lysozyme. Iron was not stained in our collected samples.

Ultrastructurally, intracellular organelles consisted predominantly of lysosomes and phagosomes with a paucity of mitochondria or ER. Occasionally, coliform bacteria (1-2 μm in length and 0.1-1 μm in diameter) were identified. Well-preserved *E. coli* with no flagella were also identified. Lysosomes approaching and fusing with *E. coli* were observed. The double-membranes of lysosomes were easily identifiable. The walls of some *E. coli* were partially ruptured and degraded by lysosomal attack. Degenerated bacteria consisted of rudimentary whorled membranous structures similar to fingerprint-like myelin figures. These structures eventually formed large phagosomes. (Fig 3)

Mature phagosomes are frequently seen in histiocytic cytoplasm. The number of phagosomes varies, but there can be more than 5-6 phagosomes per cytoplasm of von Hansemann histiocytes. Condensed lysosomes with engulfed bacteria form giant phagosomes. Inner electron-dense bodies are characteristically needle-shaped crystalloid structures with thick outer electron-lucent membranes (approximately 5-15 μm in length). Later crystalloid structures have smoothened contours and apparent targetoid appearances and are nearly equivalent in size to the adjacent nuclei. The process of mineralization can be visualized as multiple steps by EM, including partial crystallization, aggregation to the inner zone, compaction of the inner mass, and distinct lamellation.
Histiocytes within a cutting slice of a tissue on a glass slide are easily observed by SEM. Numerous intact *E. coli* were seen in captured form within the cavity of large phagolysosomes. The surface of *E. coli* was ragged and warty due to lysosomal degradation *(Fig 5)*. Large phagosomes were easily observed in the adjacent perinuclear cytosol, which is often conglomerated and larger than the nucleus. *(Fig 6)*

XG inflammation consisted of histiocyte aggregates, but revealed several different findings than the malakoplakia tissues, including abundant foamy cytoplasm and variable stages of inflammation and fibrosis. On EM, aggregates of ellipsoidal histiocytes with foamy cytoplasm were packed with variable sizes of fat vesicles. There was no evidence of any bacteria, myelin figures, or MG bodies.

The complete lack of MG bodies within XG tissues is a dramatic difference between XG and malakoplakia. We observed foamy histiocytes on TEM and SEM. *(Fig 7)*

**Discussion**

Malakoplakia can involve multiple organs, but is often focused in the genitourinary tract. In the kidney, experimental studies have shown strong similarities between megalocytic interstitial nephritis and malakoplakia, except for the presence of MG bodies. Therefore, the former has been regarded as a pre-diagnostic spectrum of malakoplakia *(Smith, 1965; Garrett and McClure, 1982)*. In experimentally induced malakoplakia, MG bodies are commonly observed together in plump histiocytes that eventually form a mass 35 days after injection of O antigen in mouse kidney *(Csapo et al., 1975)*. Ultrastructurally, phagosomes were initially present after post-injection day 3, Von Kossa and PAS staining were positive on post-injection day 8, and fully brown mineralization was seen on post-injection day 35.

Since the earliest pathologic diagnostic criterion of malakoplakia may be the ultrastructural identification of bacilli or their remnants amidst very large phagolysosomal inclusions, we thoroughly examined malakoplakia samples using TEM and SEM. Therefore, we speculate the full evolutionary
order of MG-body formation as: pre-phagosomal, phagosomal, and post-phagosomal stages. Each stage includes a couple of processes in more detail according to EM findings, as follows (Fig 4E):

I. Pre-phagosomal stage
Step 1) Bacterial predominant stage (1-2 μm). Unlike normal engulfing histiocytes, intact rod-bacilli were identified, alone or together with lysosomal aggregates.
Step 2) Bacterial captured stage means double-membrane lysosomes directly target the bacterial wall and partially destroy the bacteria.
Step 3) Bacterial degradation stage can be defined as the degenerated cell wall forms finger-printed lamellar bodies.

II. Phagosomal stage
Step 4) Phagolysosome stage (mostly 3-5μm or possibly more than 5μm). Fused lysosomes destroy bacteria and form large, integrated phagolysosomes. Simultaneous formation can leave several phagosomes in a single histiocyte.

III. Post-phagosomal stage
Step 5) Crystallization stage. Ca/P/Iron deposition occurs in the inner zone beginning with needle-like crystallization. Relatively, the outer zone is less mineralized and electron-lucent.
Step 6) Complete mineralization stage. MG-body formation is completed at this stage by repeated deposition through concentric lamellation. The mature MG body eventually forms a distinct round-oval inner electron-dense mass and outer electron-lucent zone.

Here, we identified many intact Gram-negative bacteria (E-coli), either floating in extracellular space or captured by intracytoplasmic lysosomes. The first pre-phagosomal stage is the state of infection by numerous rod bacteria, predominately *E. coli* species. *E. coli* typically measures 1-2 μm in length
with no flagella. Its wall is smooth, intact, and electron-dense when identified alone, but some are partially ruptured or diffusely damaged by lysosomal attack. A “rule of thumb” based upon generations of light and electron microscopic measurements of the dimensions of an *E. coli* cell is that the dimensions are approximately 1 μm × 2 μm with a volume of approximately 1 μm³ (1 fL). The shape can be approximated as a spherocylinder (cylinder with hemispherical caps). Given the quoted diameter and length we can compute a more refined estimate for the volume of approximately 1.3 μm³ (5π/12, precisely). (R., 2015) Double-membrane lysosomes directly target bacterial walls and cause destruction, leading to fingerprinted lamellar bodies.

Identification of the involvement of *E. coli* in malakoplakia was reported using TEM as a pathognomonic clue (Smith, 1965; McClurg *et al.*, 1973; Wang *et al.*, 2012). However, the process of degradation by lysosomes before the phagosomal stage in human tissue is a new observation using TEM. Furthermore, this is the first report of SEM assessment of malakoplakia.

The second phagosomal stage is the fused phase of lysosomes and endoplasm engulfing the bacteria. Phagolysosomes measure 5-15 μm in diameter. The number of phagosomes is also variable, but is typically 5-6 per cell. The shape, size, and electron density are all variable, from the small, irregular, electron-lucent phagosome to the large, spheroidal, electron-dense phagosome. Early phagosomes can show rudimentary rod-shaped bacteria.

The third post-phagosomal stage begins with crystallization by Ca/P/Iron deposition and complete mineralization with repeated lamellar formation. These forms are manifested during the typical evolution of MG bodies that can be easily recognized via light microscopy. MG bodies are a bacteria-independent product caused by clearance-troubled histiocytic indigestive phagosomes. Crystalline structures are occasionally found just before the pre-calcification/mineralization stage. These forms are termed “pre-calcification formations” because they possess an organic crystalline structure. These formations may act as nucleation centers for further incorporation of organic and inorganic materials. Deposition of fine crystalline material in the outer region may occur as the structure becomes saturated with inorganic elements, thus completing formation of the MG body.
Quantitative x-ray microanalysis of MG bodies demonstrated the presence of phosphorus, calcium, and iron, with average concentrations of 2.1%, 2.6%, and 0.7% by weight, respectively (Sencer et al., 1979). Eventually, mineralized concreted bodies (MG bodies) were occasionally observed but were easily peeled-off in the process of ultrastructural procedure.

Conventional XG inflammation showed tightly packed histiocytes with foamy cytoplasm, but lacking MG bodies. Common findings in XG and malakoplakia are repeated genitourinary tract infection and a sheet of aggregated histiocytes forming a mass predominantly involving the genitourinary tract as a kind of non-neoplastic pseudotumor. However, XG and malakoplakia are decisively different whether microcalciospheroid bodies are observed or not (Mering et al., 1973; Esparza et al., 1989; Li and Parwani, 2011). Unlike malakoplakia, granulomatous inflammation with fibrosis in variable proportions can be identified. Ultrastructural comparison of two mimickers has been reported since Kelly et al. made the first report (Kelly and Murad, 1981). We confirmed that foamy histiocytes in xanthogranulomatous pyelonephritis (XGPN) are only packed with lipid droplets. There is no evidence of bacteria or phagolysosomes in the process of MG-body formation. However, one report that identified bacteria was documented to be XGPN; however, they were in neutrophils or extracellular spaces, but not in histiocytes (Khayl-Mawad et al., 1982). XGPN and malakoplakia are different in terms of pathogenesis and morphogenesis based on different organelles in the histiocytes and abundant lipids in the former and abundant lysosomes in the latter.

Though the pathogenesis of malakoplakia has been examined, the evolutionary stages of MG bodies have been seldom described. Thus, we examined the full process of MG-body formation in malakoplakia at the ultrastructural level using both SEM and TEM. We described the series of phagolysosomal formation by virtue of both TEM and SEM. Furthermore, intact E. coli within phagosomes was observed by SEM.
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REFERENCES


FIGURE LEGENDS

**Fig 1.** Light microscopic findings. Sheet of plump histiocytes having pinkish cytoplasm with many spheroid bodies within the cytoplasm. The magnified inset shows calcified spheroid bodies of variable size, often larger than the nuclei of histiocytes, so called Michaelis-Gutmann bodies that are pathognomonic in malakoplakia. (x200)

**Fig 2.** Immunohistochemical reaction in Michaelis-Gutmann body. A: Lysozyme is strongly and diffusely positive in small granules and large spheroid bodies. Lysozyme-positive spheroid bodies have a double-membrane bound globular appearance. B: Von Kossa strongly stained only the mineralized Michaelis-Gutmann bodies at a relatively lower frequency than lysozyme. (x200)

**Fig 3.** A. Neutrophil engulfing two rod bacteria (*E. coli*). (x6,000) B. Histiocytes engulf many rod-shaped bacteria. Most bacteria have intact walls and are approximately 1-2 μm in length. (x12,000) C. Well-preserved *E. coli* with no flagella that measure 400 nm in diameter and 1.5 μm in length. (x25,000) D. Lysosomes (green arrowhead) approach and fuse with *E. coli*. The double-membrane of lysosomes are well visualized. The wall of *E. coli* begins with partial rupture and degradation (red arrow). (x25,000) E. Many lysosomes aggregate and surround bacteria. Bacteria are captured by lysosomes and eventually form phagosomes. Green arrowheads indicate scalloped bacterial walls. (x25,000) F. Finger-print-like myelin figures are noted in phagosomes. (x25,000)

**Fig 4.** Mineralization in Michaelis-Gutmann (MG) bodies. A: Pre-mineralized phagosomal stage. Condensed lysosomes engulf bacteria to form giant phagosomes. Fairly well-preserved bacteria show partially destroyed walls. (x15,000) B: Mature forms of phagosomes (arrowheads) in each different stage are frequently seen in the histiocytic cytoplasm. (x6,000) C. Numerous phagosomes and MG bodies in one histiocyte. Phagosomes in various stages are observed. Pre-mineralized phagosomes (red asterisk), crystallized mineralization (purple asterisk), and lamellated mineralized mature MG body (yellow arrow) are displayed in a single cytosol. (x3,500) D: Inner electron-dense body contains characteristically needle-shaped crystalloid structures with thick, electron-lucent outer membranes, approximately 3-5 μm in length. (x25,000) E: Schematic diagram of MG body formation. a engulfing stage b lysosomal attack c phagosome formation with partial degradation d crystallization of phagosome inner mass e mature phagosome evolves Michaelis-Gutmann body.

**Fig 5 A:** Engulfing *E. coli* as visualized via SEM. On thin-sectioned slides, a cutting slice of a histiocyte is observed, in which intact *E. coli* was captured in the cavity of a large phagosome. (x3,000) B: Rod-shaped bacteria were captured and are intact without signs of degradation. (x5,000) C: Closer view of *E. coli* with a length of approximately 2 μm. (x10,000) D: *E. coli* surfaces are ragged and warty, likely caused by lysosomal degradation. (x20,000)
**Fig 6.** A: A large phagosome (red circle) is seen in perinuclear cytosol and is larger than the nucleus. (x2,500) B: Front view of a different slice of phagosome reveals multiple phagosomal loci in a single histiocyte. Aggregates of lysosomes are noticed. (x5,000) C: Conglomerated phagosome is visible. (x5,000)

**Fig 7.** Xanthogranulomatous pyelonephritis. A. Aggregates of ellipsoidal histiocytes with foamy cytoplasm. (x1,000) B. The majority of the cytoplasm is packed with variable-sized fat vesicles. The red arrow indicates a large lipid droplet equivalent in size to the nucleus. The nuclear contour is not indented. (x6,000) C. Some organelles show cistern of vesicles. (x6,000) D. Tightly packed microvesicles of lipid droplets. There is no evidence of bacteria, myelin figures, or Michaelis-Gutmann bodies. (x6,000) E. Perinuclear lipid droplets compress the nuclear border, resulting in an indented contour similar to lipoblasts. (x5,000)