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Shigella flexneri induces Cell Death by Damage to and Autophagy of Host Cell Mitochondria: An Electron-Microscopic Study of Infected Human Monocyte Derived Macrophages

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Shigella flexneri is a Gram negative bacterium that causes acute inflammatory disease of the colon. Virulent *Shigella* can escape the host phagocytes to replicate in epithelial cells, ultimately resulting in tissue destruction. In a recent study we reported that virulent *Shigella* induced a necrotic death in infected human monocyte-derived macrophages (HMDM), which was associated with a damage of the host-cell mitochondria [1]. The current study focused on the morphological alterations induced by *Shigella* in infected HMDM.

Monocytes were isolated from the peripheral blood of healthy donors and cultured for a minimum of 7 days before infection as described [1]. The cells were fixed and processed for conventional transmission electron microscopy after infection with virulent or avirulent bacteria. For groups infected with bacteria, cells with intracellular bacterial phagosome(s) were randomly selected for examination and photography.

HMDM infected with virulent *Shigella* were larger in size than uninfected cells and their plasma membrane showed numerous processes. Their cytoplasm was filled with tabulated or vasiculated intracellular membranes and a large amount of lipid bodies. Striking alterations were found in mitochondria. Compared with uninfected HMDM or HMDM infected with avirulent Shigella, the mitochondria in cells infected with virulent *Shigella* showed a condensed matrix and ballooned cristae (Fig 1), indicative of a mitochondrial damage. Mitochondria in virulently infected cells were frequently transformed into multilamellar structures by forming whorl-shaped cristae (Fig 1c and Fig 2a) or ring-shaped bodies with a lumen (Fig 2b). This mitochondrial transformation was confirmed by serial sections. In necrotic cells infected for 2 hrs, the mitochondria were extremely swollen and some ruptured (Fig 3). Occasionally, the intracellular virulent bacteria were found in phagosomes surrounded by multilamellar membranes composed of ring-shaped mitochondria and ER (Fig 4).

These results support our observation that *Shigella* kills HMDM by damaging their mitochondria [1]. Our data also suggest that the multilamellar structures, resembling autophagosomes [2, 3], were frequently derived from mitochondria damaged by virulent *Shigella*.

References

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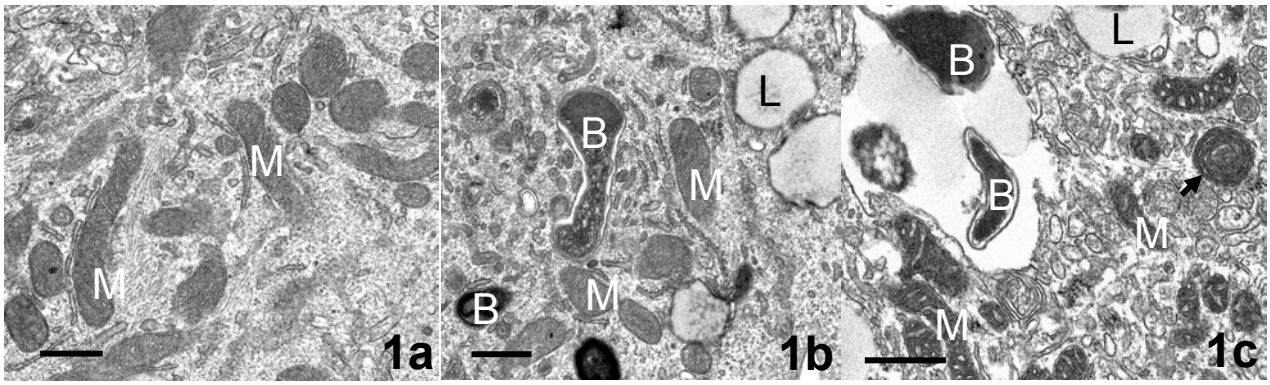

Fig 1. TEM micrographs of uninfected (1a), avirulent infected (1b), and virulent *Shigella* infected (1c) HMDM. Mitochondria (M) were distinctively darkened with condensed matrix and ballooned cristae in cells infected for 2 hrs with virulent (1c), but not with avirulent *Shigella* (1b). A multilamellar structure formed by a mitochondrion with whorl-shaped cristae was also seen (arrow in 1c). Bacteria, B and lipid body, L. Bars=0.5μm.

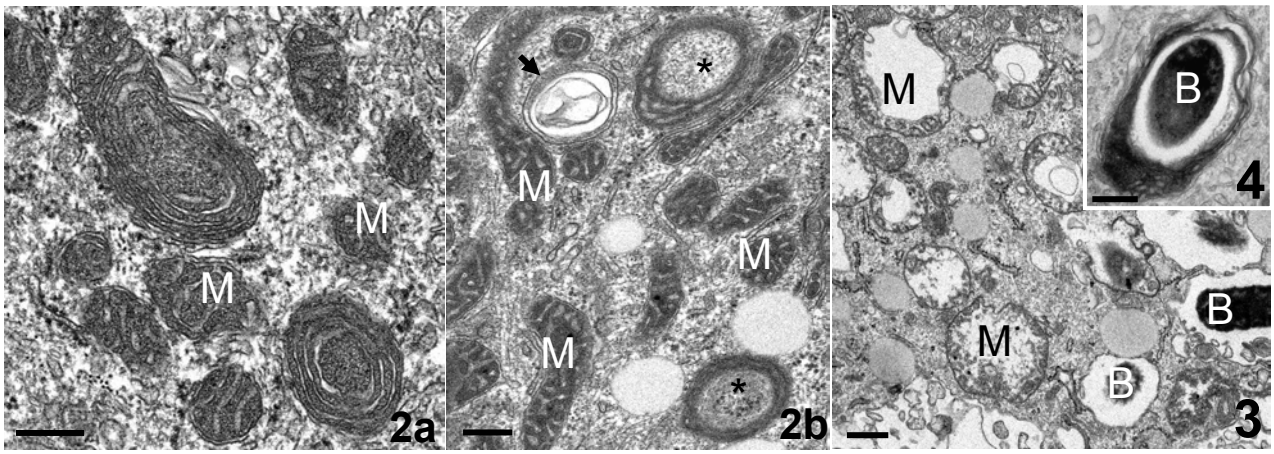

Fig 2. Mitochondria transformed into multilamellar structures in HMDM infected with virulent *Shigella.* Multilamellar structures were formed by mitochondria (M) with whorl-shaped cristae (2a) or ring-shaped mitochondria with a lumen containing cytoplasm (* in 2b). A myelinoid body was also seen (arrow in 2b). Bars=0.2 μm.

Fig 3. Mitochondria were grossly swollen in HMDM infected with virulent Shigella for 2 hrs. The mitochondria (M) were ballooned with cavitations of the matrix, flatten or lose of cristae. A number of intracellular bacteria (B) were detected nearby. The cells showed a dashed plasma membrane profile (not shown) and a remarkable translucent appearance. Bar=0.5 μm.

Fig 4. A ring-shaped mitochondrion (M) together with ER formed a phagosome with multilamellar structure to wrap a virulent bacterium (B) within a cell infected for 2 hrs. The cristae in the mitochondrion were still clearly recognizable. Bar=0.5 μm.

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