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Research article

Cytoprotective effect of autophagy on phagocytosis of apoptotic cells by macrophages

Pei Zhou [a], Yu-zhen Tan [a], Hai-jie Wang [a,*], Ting Li [a], Tao He [a], Ying Yu [b], Jian Zhang [b], Dan Zhang [a]

[a] Department of Anatomy, Histology and Embryology, Shanghai Medical School of Fudan University, Shanghai 200032, China
[b] Key Laboratory of Food Safety Research, Institute for Nutritional Sciences, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai 200032, China

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ABSTRACT

Clearance of the apoptotic cells by phagocytes plays pivotal roles in maintenance of tissue homeostasis, promotion of immunological tolerance and anti-inflammatory response. Recent studies show that autophagy is involved in phagocytosis of the apoptotic cells. However, contribution of autophagy to phagocytosis of the apoptotic cells by macrophages is not clearly defined. Here, we assessed cytoprotective effect of autophagy on clearance of the apoptotic cells. Apoptosis of murine splenic lymphocytes and human T-cell leukemia cells was induced with cyclophosphamide. After engulfment of the apoptotic cells, expression of Belin-1 and LC3 in macrophages was upregulated, the number of MDC-positive vesicles, LC3-positive autophagosomes and autophagic ultrastructures increased significantly. Autophagosome was fused with phagosome containing fragments of the nuclei or other debris of the apoptotic cells to form amphisome. Some cells in macrophages phagocytosing the apoptotic cells became apoptotic. After autophagy of macrophages was inhibited with 3-MA, viability and survival of macrophages reduced, phagocytosis of the apoptotic cells by macrophages deceased significantly. These results demonstrate that autophagy plays an important role in promoting clearance of the apoptotic cells by protecting macrophages from apoptosis during phagocytosis as well as degrading the contents of phagosomes via amphisome formation.

1. Introduction

Apoptosis, which is also called programmed cell death, is a common physiological or pathological phenomenon occurring throughout life as part of development, homeostasis and pathogenic processes. In human body, almost 500 billion cells die each day. For example, during the maturation of T-lymphocytes in the human immune system, more than 95% of T-lymphocytes undergo apoptosis. Numerous tumor cells become apoptotic after irradiation and chemotherapy. Morphological changes of the apoptotic cells include cell shrinkage, membrane budding, and nuclear condensation. Then the cell breaks up into membrane-bound apoptotic bodies containing organelles or even nuclear fragments [1,2].

Apoptotic cells are rapidly (within hours) cleared by professional phagocytes such as macrophages and immature dendritic cells, or by neighbouring nonprofessional phagocytes. Resident macrophages are located at various tissues widely such as alveolar macrophages, peritoneal macrophages, macrophages of spleen and lymph node, Kupffer cells in liver and microglias in the nervous system. In inflammatory diseases, circulating monocytes transmigrate into the local tissue and differentiate into macrophages. Although macrophage subsets are phenotypically and functionally different, all of them play a crucial role in clearing apoptotic cells. Rapid recognition and clearance of dying cells by macrophages play pivotal roles in development, maintenance of tissue homeostasis, promotion of immunological tolerance and anti-inflammatory response. The impaired clearance of apoptotic cells causes the release of their cellular components, which can act as self-antigens and may induce several different diseases that involve infection, inflammation, autoimmunity and cancer [3–5]. Conversely, under certain conditions, such as the killing of tumor cells by specific cell-death inducers, the recognition of apoptotic tumor cells can promote an immunogenic response and antitumour immunity [6]. However, fate of macrophages after phagocytosis of the apoptotic cells is unknown.

Clearance of the apoptotic cells by the phagocytes may be divided into four steps. The “find-me” step occurs when the apoptotic cells release soluble chemoattractants that promote chemotaxis of phagocytes via corresponding receptors on the phagocyte. The “eat-me” step is characterized by the appearance of ligands on

* Corresponding author.
E-mail address: hjwang@shmu.edu.cn (H.-j. Wang).

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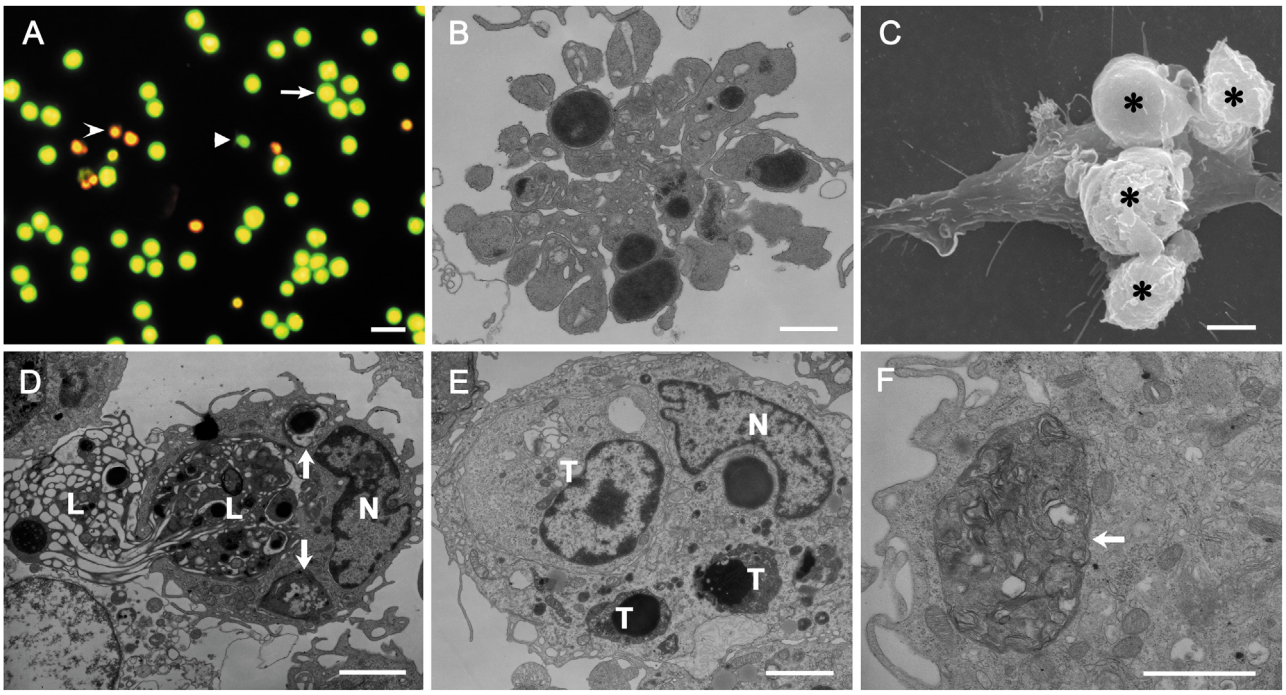

Fig. 1. Engulfment of the apoptotic lymphocytes or Jurkat T cells by macrophages. (A) Lymphocytes stained with EB/AO solution. Triangle, arrow and arrowhead show viable, early apoptotic and late apoptotic cells respectively. Bar = 10 μm. (B) Transmission electron image of a broken apoptotic lymphocyte. The cell breaks up into apoptotic bodies containing organelles or the condensed nuclear fragments. (C) Scanning electron image of a macrophage phagocytosing the apoptotic lymphocytes (asterisks). (D) A macrophage engulfing a apoptotic lymphocyte (L) containing the condensed nuclear fragments. Arrows show phagosomes containing the apoptotic bodies of lymphocytes. (E) A macrophage phagocytosing three apoptotic Jurkat T cells (T). N, nucleus of macrophage in D and E. (F) A macrophage phagocytosing an apoptotic body (arrow) of Jurkat T cells.

the surface of the dying cell that mark it as a target to be engulfed by phagocytes expressing appropriate recognition receptors. The "engulfment" step presents cytoskeletal rearrangement and formation of the phagocytic cup around the target and subsequent internalization. In "processing" step, the cell corpse undergoes degradation within the phagocyte through the phagolysosomal pathway [4]. Over the past few years, the "find-me" and "eat-me" signaling pathways in engulfment of apoptotic cells and how they regulate cell clearance are investigated intensively [6,7]. Interestingly, recent studies reveal that autophagy is involved in clearance of apoptotic cells. Autophagy is a cellular pathway for protein and organelle degradation, with connections to human physiology and disease [8,9]. Autophagy may be divided into macroautophagy, microautophagy and chaperone-mediated autophagy based on the pathways by which cargos are delivered into lysosomes. Macroautophagy (hereafter referred to as autophagy) proceeds through several phases, including formation of a autophagosome precursor (also called phagophore), autophagosome maturation and cargo sequestration, and autophagosome – lysosome fusion. In the final stage, autophagosomal contents are degraded by lysosomal acid hydrolases and the contents of the autolysosome are released for metabolic recycling [10]. It is now well established that endocytosis and autophagy share regulation of lysosome degradation [11,12]. Recently, more and more attention focuses on functional convergence of autophagy and phagocytosis. Signallings of Toll-like receptor [13] and TIM-4 glycoprotein [14] link pathways of autophagy and phagocytosis. Autophagy genes or proteins are involved in mediating engulfment of apoptotic cells [15–18]. Removal of cell corpses during embryonic development requires autophagy machinery [15,17–19]. Enhancement of autophagy promotes clearance of the apoptotic cells by macrophages [14,20,21]. However, contribution of autophagy to clearance of the apoptotic cells is not as yet clearly defined.

This investigation was designed to examine changes of autophagic activities in macrophages phagocytosing the apoptotic lymphocytes and tumor cells and to evaluate effects of autophagy on clearance of the apoptotic cells by macrophages. Here we demonstrated that expression of autophagy proteins Beclin-1 and LC3 (microtubule-associated protein 1 light chain 3) are upregulated, and the autophagic structures and amphisomes increase after macrophages engulf the apoptotic cells. Engulfment of the apoptotic cells may induce apoptosis of macrophages. This study suggests that autophagy promotes clearance of the apoptotic cells by macrophages via protecting the cells from apoptosis and amphisome formation.

2. Materials and methods

2.1. Isolation of macrophages

Sprague-Dawley (SD) rats (male, 6–8 wk of age) were injected intraperitoneally with 10 ml of 4% thioglycollate (Sigma-Aldrich, St. Louis, MO) 4 d previously. The elicited peritoneal macrophages were harvested by lavage in 10 ml of ice-cold Hanks' balanced salt solution (HBSS). The cells were suspended in Roswell Park Memorial Institute' medium (RPMI) 1640 (Life Technologies, Carlsbad, CA) supplemented with 10% fetal bovine serum (FBS; Gibco Invitrogen, Grand Island, NY), 100 U/ml penicillin and 100 μg/ml streptomycin. After incubation for 2 h at 37 °C in 5% CO_2/95% air, nonadherent cells were removed by aspiration in ice-cold HBSS without Ca^{2+} and Mg^{2+}. The cells isolated with this procedure were >95 macrophages by nonspecific esterase (Moto Chemicals, Tokyo) staining, phagocytosis of acetylated low density lipoprotein (Dil-Ac-LDL, Biomedical Technologies, MA) and ED1 (Serotec, Oxford) immunostaining [22].

2.2. Induction of apoptotic cell death

The spleen was removed from male SD rat aseptically and put in 0.01 M PBS. After mincing, the splenic tissue was passed through stainless mesh (mesh size, 74 μm). The filtered cell pellets were