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Links between Autophagy, Innate Immunity, Inflammation and Crohn's Disease

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Autophagy · Innate immunity · Crohn's disease

Abstract
Autophagy is a fundamental biological process that endows eukaryotic cells with the ability to autodigest portions of their own cytoplasm. Autophagy plays roles in aging, development, neurodegeneration, cancer and immunity. The immunological role of autophagy was first recognized for the ability of autophagy to sanitize the cellular interior by killing intracellular microbes and, indirectly, by the adaptations that successful intracellular pathogens have evolved to protect themselves from autophagy. Since then, the repertoire of autophagy functions in immunity has been vastly expanded to include numerous intersections of regulatory and effector nature with innate and adaptive immunity. Autophagy acts both as an effector and a regulator of pattern recognition receptors, it supports MHC II presentation of cytosolic (self and microbial) antigens, it shapes central tolerance via thymic selection of the T cell repertoire, is an effector of Th1/Th2 polarization, affects homeostasis of T, B, and specialized immune cells such as Paneth cells, and – when defective – can be a contributing factor to chronic inflammatory conditions in human populations such as Crohn's disease.

Introduction

The study of the immunological roles of autophagy has become a rapidly growing field taking on the role of a frontier in contemporary immunological research. Autophagy is the evolutionarily conserved, ubiquitous biological process of cleaning the eukaryotic cell's interior. During autophagy, large portions of the cytoplasm that can be as big as whole organelles (e.g. mitochondria) are captured by isolation membranes (phagophores) and sequestered into autophagosomes for degradation within the specialized lytic organelles termed autolysosomes [1–6]. The genes *(Atg)* involved in this pathway [7] have been identified in species from yeast to humans. Autophagy affects a wide range of immunological processes: (1) innate and adaptive immunity against intracellular pathogens, including bacteria (e.g. *Mycobacterium tuberculosis*), protozoa and viruses [3, 4, 8–18; see also Deretic V. (ed): Autophagy in Immunity and Infection: a Novel Immune Effector. Weinheim, Wiley-VCH, 2006]; (2) antigen presentation [18–21]; (3) homeostasis of immune cells [22–24], and (4) inflammatory disorders, such as Crohn's disease [25–29]. More broadly, autophagy affects cell death and survival, and is implicated in many human health and disease states such as cancer, aging and longevity [30–32]. We particularly stress the utility of autophagy as a cell-autonomous defense against intracellular pathogens. When induced, autophagy can eliminate notorious intracellular microbes [8, 9, 33]. In contrast to

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the notorious resistance of successful intracellular pathogens to a number of other microbicidal effectors, autophagy can efficiently eliminate intracellular microorganisms [8]. These observations have been confirmed by several groups in different contexts, including a report showing that autolysosomes contain ubiquitin fragments that act as mycobactericidal peptides [33], a study linking TLR stimulation and autophagy [34], a screen for novel autophagy inducers [35] and a recent study [36] showing that the previously reported ATP stimulation of P2X7 receptor leading to an elimination of intracellular mycobacteria [37] does so through autophagy [36]. Furthermore, a paper has just been published in *Nature Medicine* showing that autophagy induction can improve the vaccine potency of bacillus Calmette-Guérin (BCG) [38]. Only recently did mechanistic studies of autophagy become possible. The first book [Deretic V. (ed): Autophagosome and Phagosome. Totowa, Humana Press, 2008] that compiles autophagy methods has just recently been published [39]. The present toolbox for autophagy studies is limited by having very few pharmacological agonists and antagonists, boiling down to 2: rapamycin, an inducer, and 3-methyladenine, an inhibitor. However, rapamycin has been used clinically in the treatment of chronic conditions and transplantation and thus holds promise for application in Crohn's disease.

Autophagy as a Mechanism for Cleaning up Cellular Interiors

Autophagy impacts every cell in the human body and plays a role in a broad range of health and disease states. Autophagy is a fundamental cytoplasmic homeostasis process enabling individual cells to clean up, in a highly regulated fashion, their own cytoplasm by sequestering portions of the cytoplasm and degrading the captured constituents [1–6]. This primordial function is evolutionarily preserved in all eukaryotes, from yeast to human. The main morphological feature of autophagy is a membrane that wraps around portions of the cytoplasm earmarked to be sequestered, forming a double-membrane organelle termed autophagosome. The formation of autophagosomes is heralded by the appearance of punctate structures in the cytoplasm representing newly formed phagophores and autophagosomes. The captured material, once corralled into an autophagosome, is degraded upon autophagosomal fusion with lysosomal organelles. Autophagy has many physiological roles and is often employed to remove damaged or surplus organelles. It is also used by cells to turn over long-lived proteins and other macromolecules, either to get rid of protein aggregates or to supply nutrients for essential anabolic needs under conditions of nutrient deprivation or growth factor withdrawal. The broad spectrum of autophagy functions is hard-wired into a wide range of health-related issues, including cancer, neurodegeneration, aging and infections [32, 40]. The most recent additions to the list of processes affected by autophagy are the control of intracellular pathogens [8, 10–12, 15–17, 40–42] and inflammatory conditions such as Crohn's disease [25–29].

Autophagy in Immunity

Autophagy plays a role in a wide spectrum of immunological processes [40, 43] with the list of immunological autophagy categories (dubbed 'immunophagy' [3, 4]) rapidly growing [44].

(1) *Autophagy in direct elimination of microbes:* Autophagy is an innate immunity mechanism for the cell-autonomous elimination of intracellular microbes [8, 10–12, 15–17, 40, 42]. In the context of infectious disease, autophagy – when induced by physiological (starvation), pharmacological (rapamycin) or immunological (IFN-γ) means – can eliminate a number of important intracellular pathogens, including prominently *M. tuberculosis* [8, 9, 33]. Attesting to the role of autophagy in eliminating microbes, many successful pathogens had to evolve ways to deal with or inhibit it [17, 45] as a part of their repertoire of anti-immune defenses.

(2) *Autophagy as an effector of PRR/TLR signaling:* Autophagy is an effector of TLR signaling [34, 46, 47], and can enhance or interfere with innate antiviral responses regulated by TLR7 [48] and RIG-I [49].

(3) *Autophagy as an effector of Th1/Th2 polarization:* Autophagy, in its role as an antimicrobial defense mechanism, is controlled by Th1/Th2 polarization [50]. The Th1 cytokines such as IFN-γ [8, 51], and TNF-α [52] also acting downstream of CD40 ligation [16], stimulate autophagy, whereas the Th2 cytokines IL-4 and IL-13 inhibit autophagy [50, 53–55].

(4) *Autophagy in immune cell homeostasis:* Autophagy controls T and B cell development, survival, and proliferation [23, 24, 56].

(5) *Autophagy, MHC II presentation and thymic selection:* Autophagy contributes to MHC-II-restricted endogenous (cytosolic) antigen presentation [18, 19, 57, 58] with a role in thymic selection, allergy and autoimmune diseases [59].

(6) *Autophagy and vaccines:* Recent studies have shown that this newly discovered power of autophagy (MHC II presentation of cytoplasmic antigens) can be used for vaccine betterment as in the case of the influenza virus antigens [19, 60] and the widely used tuberculosis vaccine BCG [38].

(7) *Autophagy in inflammation:* Autophagy has recently been implicated in predisposition to Crohn's disease, a prevalent inflammatory bowel disease [61–63]. This latest breakthrough, made possible by the powerful genome-wide association screenings, has uncovered the role of autophagy in Crohn's disease, clearly demonstrating the role of autophagy in innate immunity in human populations [25–27, 29, 64], and a need to target this process for the treatment of a broad range of infectious and inflammatory diseases.

Innate Immunity, Autophagy, ATG16L1 and IRGM in Crohn's Disease

Genetic predisposition to Crohn's disease has been linked to regulators of innate immunity, of which Nod proteins (specifically Nod2) [65, 66] and most recently autophagy factors, including ATG16L1 and IRGM [25, 27, 67], are now some of the most prominent examples [26]. The specific role of Nod2 in Crohn's disease pathophysiology provides an obvious link with innate immunity and inflammation. Nod2 has been extensively studied and a considerable body of literature exists on this topic, including a very recent review [68]. The latest breakthroughs made possible by the powerful genome-wide association screenings have uncovered its role in at least 2 additional immunity pathways [26]: (1) IL-12 and IL-23 (IL23R-Arg381Gln) driving the Th17 differentiation of Th1 cells, with the Th17 phenotype often associated with organ-specific autoimmunity and inflammation, and (2) autophagy, a fundamental cellular homeostatic process involved in innate immunity against intracellular pathogens [3, 4, 40] and in endogenous antigen presentation [19, 43].

In contrast to the extensively studied Nod2 pathway, almost nothing is known about the role of IRGM in Crohn's disease, due to the only very recent recognition of its linkage to Crohn's disease [25–27]. Interestingly, *IRGM* is the only human gene representative of an otherwise prolific class of innate immunity effectors in vertebrates, called immunity-related GTPases (IRG) [69], also known as p47 GTPases [70]. In the mouse, there are 24 IRG genes and many of them have been initially recognized by their role in the defense against a variety of intracellular bacterial and protozoan pathogens. Intriguingly, humans and chimpanzees have only one IRG, *IRGM* [69], and now this gene has turned out to be a Crohn's disease predisposition locus [25–27].

Since the initial reports of an association of ATG16L1 and IRGM polymorphisms with Crohn's disease [25–29, 67], a growing number of replicating studies have confirmed this genetic link in general and in several specific populations [28, 71–79]. Although several risk loci are common to ulcerative colitis and Crohn's disease, the autophagy genes ATG16L1 and IRGM – along with NOD2 – appear to be specific to Crohn's disease [80] with some indications of the specificity of IRGM association with ileal disease in some populations [81]. A study including 2,731 Dutch and Belgian patients (1,656 with Crohn's disease and 1,075 with ulcerative colitis) and 1,086 controls showed association of ATG16L1 (rs2241880) and IRGM (rs4958847) specifically with Crohn's disease [79]. Single-nucleotide polymorphisms in the IRGM gene (rs1000113 and rs4958847) have confirmed that IRGM is a susceptibility locus specifically for Crohn's disease, either of adult or childhood onset, in Italian populations possibly being associated with fistulizing disease [79].

Function of ATG16L1 and IRGM

Functional information regarding the role of autophagy in humans in the context of Crohn's disease is still lacking. Some information has been gleaned from studies of ATG16L1 in vitro with cell lines or in vivo in mice, with the 3 published studies pointing to different, albeit potentially congruent, effects: (1) reduced capacity of the ATG16L1*300A allele to control intracellular enteric pathogens when examined in a human epithelial cell [82]; (2) susceptibility to dextran sulphate sodium-induced acute colitis in mice lacking *Atg16L1* in hematopoietic cells and increase in IL-1β signaling with possible proinflammatory action [83], and (3) direct or indirect effects on Paneth cells in the intestinal crypts of ATG16L1-hypomorphic mice [84]. The role of IRGM cannot be properly investigated in mice, as the mouse has 24 IRGM-like genes, while humans have only 1 (IRGM); albeit 1, *Irgm1*, of the 3 putative murine orthologs shows effects on hematopoietic stem cell proliferation and T cell survival [85, 86]. Some information on the direct function of the human IRGM in antibacterial defenses has been known even before autophagy loci have been linked with Crohn's disease [9]. Future work will be needed to establish the scope and extent of the role of autophagy in Crohn's disease.

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