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A NEW AVENUE TO INVESTIGATE: THE AUTOPHAGIC PROCESS. FROM CROHN'S DISEASE TO *CHLAMYDIA*

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SUMMARY

The finding that a variant (T300A) of the autophagy-related 16-like 1 *(*ATG16L1*) gene is associated with Crohn's disease suggests that the inability to eliminate intestinal intracellular microbes via (macro)autophagy may be involved in the pathogenesis of this disease. The variant induces an autophagy-associated defect in Paneth cells, specialized cells in the crypts of Lieberkühn within the small intestine that secrete defensins and other antimicrobial peptides. Moreover, other loci,* IRGM *and* LRRK2 *involved in autophagy and implicated in clearance of intracellular bacteria have been found to be associated with Crohn's disease. These unexpected findings have changed the focus of research in Crohn's disease and have stimulated an in-depth study of the complex process of autophagy. Autophagy is regulated by many genes and is emerging as a central player in the immunologic control of intracellular bacteria.* Chlamydia trachomatis *is able to inhibit apoptosis and the production of nuclear factor* κ *B (NF*κ*B) in order to survive in the host. Extensive studies on association of genes regulating the inflammatory response in experimental models and in humans as revised in other sections of this supplement have failed to explain the long-term complications of* C. trachomatis *infection. The advances in the molecular pathways of* Chlamydia *infection and their effects on the Golgi apparatus and other*

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cytoplasmic organelles suggests that defects in autophagic genes may predispose the host to chronic infection and be responsible for the long-term complications. A new genomic approach of the complete autophagic pathway may reveal new insight to understand the presence of a complication in affected individuals, even if at present there is no evidence that C. trachomatis *is affected by this pathway.*

INTRODUCTION

Chronic inflammatory diseases called T helper type 1 (Th1)/Th17-mediated diseases in mammals and in invertebrates such as corals are influenced by a symbiotic relationship with a metabolically active population of bacteria. Host genetic susceptibility in the form of a defective mucosal barrier function and/or abnormal bacterial killing due to a defective autophagic process can lead to enhanced exposure to bacteria. Defective immunoregulation leads to lack of appropriate immunosuppression. This can lead to a disease state that has been classified as "barrier disease" resulting from the lack of integration of symbiotic microorganisms, pathogens and the mucosal immune system. In a way, these new concepts are the modern interpretation of the concept of a common mucosal immune system that was put forward 30 years ago. The observations were based on the origins of immunoglobulin-containing cells in intestinal, respiratory, mammary and genital tissues (1-3). This field has evolved in the direction of the development of vaccines and, over the years, it has become apparent that microbiota in the gut contribute to shaping the mucosal immune system. Since there is evidence that in the gut immunoglobulin (Ig) A regulates microbial composition, a requirement for the initiation and maintenance of the fitness of our immune system, it is becoming apparent that gut-associated lymphoid tissues play a major role in shaping the repertoire of gut microbiota (4). This interaction of the microflora and the gastrointestinal tract of the host reaches as far as the genetic machinery contributing to health, disease and evolution. In man it seems that genetic polymorphisms affecting the immune system and the relatively recent changes in lifestyle in industrialized societies may have turned the genetic variability controlling the balance of beneficial versus aggressive bacteria into disease-causing mutations (5).

AUTOPHAGY

Autophagy, the cellular homeostatic process by which eukaryotic cells transport damaged and unnecessary cytoplasmic constituents to lysosomes for degradation, has been first described as a response to starvation, but it is now clear that it plays a major role in many cellular processes and has been implicated in several diseases. Several studies reveal the role of autophagy in innate and adaptive immunity. Autophagy is an example of a cellular process that is not restricted to innate immunity.

The first link between autophagy and the innate immune system was shown by the elimination of intracellular pathogens from cells via the autophagy pathway which involves the host response to bacteria and serves to limit viral replication, e.g., by engulfment and destruction, and is implicated in antigen processing and presentation. Other viruses hijack the autophagocytic process for replication or even encode mechanisms to evade autophagy (6). For example, induction of autophagy seems to decrease the viability of intracellular mycobacteria and to eliminate *Listeria monocytogenes* upon its escape from the phagosome into the cytosol (7, 8). Furthermore, severe and invasive human diseases caused by *Streptococcus pyogenes* might be induced by the attenuation of autophagic activity (9).

In addition, the involvement of autophagy in adaptive immunity has been established by studies demonstrating that this pathway is not limited to its role in direct elimination of invading pathogens.

Cells expressing major histocompatibility complex (MHC) class II proteins use the autophagy pathway in processing endogenously synthesized viral or self-antigens into autophagosomes antigens for presentation to CD4+ T cells (10, 11). Schmid et al. demonstrated that MHC class II positive antigen-presenting cells (APCs), such as B-cell lines, monocytes, dendritic cells and epithelial cell lines with interferon (IFN)-γ-inducible MHC class II expression, display significant levels of steady-state macroautophagy (12). Therefore, autophagy has a role in T-cell homeostasis by controlling T-cell lifespan once they exit the thymus and functions as an effector of Th1–Th2 polarization in defense against intracellular pathogens (13, 14).

Knockout mice models demonstrate that the autophagy gene *Atg5* plays multiple roles in lymphocyte development and function, thereby suggesting that autophagy may not only play an essential role in T-cell homeostasis but also in T-cell development and central tolerance (13, 15, 16).

THE AUTOPHAGIC PROCESS IN CROHN'S DISEASE

Crohn's disease (CD) is a complex chronic inflammatory autoimmune condition of the gastrointestinal tract, often affecting the eyes, the skin and the joints.

Polymorphisms in genes that regulate autophagy have been recently discovered in CD, namely, the *autophagy-related 16-like 1* gene (*ATG16L1*) and the *immunity-related GTPase family, M* locus (*IRGM*)(17-22) and also a common 20-kb deletion in the promoter region of *IRGM*, which induces an altered expression pattern in the modulation of cellular autophagy of internalized bacteria (23). A recent meta-analysis confirmed that the *ATG16L1* rs2241880 (encoding a threonine to alanine substitution "T300A"), and noncoding *IRGM* rs13361189 and rs4958847 polymorphisms are important low-penetrant markers for CD susceptibility and indicate that these variants may also play a role in ulcerative colitis, the other major inflammatory bowel disease (24). Furthermore, the autophagy-associated gene encoding leucine-rich repeat kinase 2 (*LRRK2*) has been implicated in CD susceptibility (25). It could be shown that the signal by rs11175593 (within 40 kbp from *LRKK2*) in the genome-wide association study of Barrett et al. was not attributable to mucin family gene *MUC19* tagging variants within 360 kb at the same locus (25, 26).

The *ATG16L1* coding variant has been demonstrated to impair the capture of internalized *Salmonella* within autophagosomes of human intestinal epithelial cells (27). In addition, CD patients homozygous for the *ATG16L1* risk allele display Paneth cell granule abnormalities (28), while *ATG16L1*-deficient macrophages following lipopolysaccharide stimulation produce high amounts of inflammatory cytokines interleukin (IL)-1β and IL-18 (29).

MOLECULAR EVENTS OF AUTOPHAGY AND ITS POSSIBLE LINK TO *CHLAMYDIA*

Autophagy, a lysosomal degradation pathway implicated in the degradation of intracellular bacteria, is mediated by evolutionarily conserved genes (called *atg* genes) (30). In the case of *Chlamydia*, the invading bacterium is not being targeted for lysosomal degradation and establishes itself in an intracellular replicative niche, thus partially antagonizing the host autophagic process. In this niche *Chlamydia,* through a type III secretion system, allows the entry of proteins into the cytoplasm. One such protein is the protease *Chla*Dub1 which prevents release of nuclear factor κ B (NFκB). The *C. trachomatis* intracytoplasmic inclusions appear to be devoid of any marker of endocytic traffic, but induce Golgi apparatus fragmentation and react with endoplasmic reticulum and the host cytoskeleton (31). In addition, *Chlamydia* activates type I IFN-regulated pathways during infection (32).

Thus far the intracellular replicative niche of *Chlamydia*-infected cells has not been linked to the autophagic process. However, since chlamydial inclusion bodies are juxtaposed to LC3-positive structures, it has been postulated that Chlamydiae utilize nutrients derived from autophagy to promote intracellular growth (33). Modulation of host cell apoptosis or autophagy by intracellular bacterial pathogens plays an important role in pathogenesis, but little is known in this regard in relation to *Chlamydia*.

Similarly to the genetic findings in CD, we can postulate that a genomic screening of the apoptotic and autophagy pathways of the host may provide insight into the persistence and pathogenesis of the long-term complications of *Chlamydia* infections, such as pelvic inflammatory disease and infertility, and the possible role in coronary artery disease, reactive arthritis and Alzheimer's disease.

CHLAMYDIAE ESCAPE THE HOST RESPONSE

The lessons that we can draw from diseases such as Crohn's disease and diseases where *Chlamydia* is involved, together with the approaches followed to define the basic defects, is that these diseases may serve as a lead to understand the physiologic processes involved in the infectious process and the pathology induced by chlamydiae. This in turn will suggest new areas of therapeutic targeting.

Chlamydiae are obligate intracellular pathogens that cause a number of diseases both in humans and in animals. *C. trachomatis* is a primarily human pathogen mainly associated with sexually transmitted diseases, pulmonary infection and blinding trachoma. The disease conditions caused by *C. trachomatis* vary from asymptomatic infections to severe pathology, causing significant morbidity worldwide. The first line of defense against chlamydiae is the innate immune response. Chlamydiae manipulate signaling pathways to hinder the activation of innate immune responses that are detrimental to bacterial, or host survival by the inhibition of apoptosis and the manipulation of NFκB-mediated signaling.

Although autophagy can serve to protect cells, it also may contribute to cell damage. Most invading pathogens are first engulfed into phagosomes, which

then convert into autophagosomes and finally fuse with lysosomes, where the pathogens are destroyed by lysosomal enzymes (34). However, some of the invading pathogens escape the host defense mechanism by blocking or altering the maturation of the phagosome. Even organisms that manage to escape phagosomes can still be taken up by autophagosomes and can subsequently be decomposed (9). *C. trachomatis* is one of these pathogens that have developed strategies to circumvent this defense mechanism, and seems to either inhibit or resist autophagy (35).

Furthermore, cytokines and chemokines produced during the innate response recruit inflammatory cells and T cells that are needed for adaptive immunity. The T-cell-dependent adaptive immune response and subsequent *Chlamydia* clearance is predominated by IL-12- and IFN-γ-dependent mechanisms. IFN-γ plays a central role in innate immunity against intracellular pathogens, for example, by inducing autophagy. In human cells, IFN-γ can effectively suppress growth of *C. trachomatis*. Among the genes highly induced by IFN-γ are the immunity-related GTPases (IRGs). Al-Zeer et al. have shown that IFN-γ stimulates association of different IRG proteins (Irga6, Irgb6, Irgd, Irgm2 and Irgm3) with inclusions harboring *C. trachomatis* (36). Interestingly, Irga6 was found to be responsible for immune resistance to *C. trachomatis*. Other IRGs could have cooperative interactions. Cells lacking Irga6 were highly permissive to *C. trachomatis* infection, although other IRGs were recruited in response to IFN-γ (36).

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DISCLOSURE

The authors have nothing to disclose.

REFERENCES

1. McDermott, M.R., Bienenstock, J. *Evidence for a common mucosal immunologic system. I. Migration of B immunoblasts into intestinal, respiratory, and genital tissues*. J Immunol 1979, 122(5): 1892-8.
2. Tomasi, T.B. *Mucosal immune system. A general review*. Ann Otol Rhinol Laryngol 1976, 85(2 Suppl 25 Pt 2): 87-9.
3. Tomasi, T.B., Jr., Larson, L., Challacombe, S., McNabb, P. *Mucosal immunity: The origin and migration patterns of cells in the secretory system.* J Allergy Clin Immunol 1980, 65(1): 12-9.
4. Suzuki, K., Ha, S.A., Tsuji, M., Fagarasan, S. *Intestinal IgA synthesis: A primitive form of adaptive immunity that regulates microbial communities in the gut*. Semin Immunol 2007, 19(2): 127-35.
5. Rosenstiel, P., Till, A., Schreiber, S. *NOD-like receptors and human diseases.* Microbes Infect 2007, 9(5): 648-57.
6. Kirkegaard, K., Taylor, M.P., Jackson, W.T. *Cellular autophagy: Surrender, avoidance and subversion by microorganisms.* Nat Rev Microbiol 2004, 2(4): 301-14.
7. Gutierrez, M.G., Master, S.S., Singh, S.B., Taylor, G.A., Colombo, M.I., Deretic, V. *Autophagy is a defense mechanism inhibiting BCG and Mycobacterium tuberculosis survival in infected macrophages*. Cell 2004, 119(6): 753-66.
8. Rich, K.A., Burkett, C., Webster, P. *Cytoplasmic bacteria can be targets for autophagy*. Cell Microbiol 2003, 5(7): 455-68.
9. Nakagawa, I., Amano, A., Mizushima, N. et al. *Autophagy defends cells against invading group A Streptococcus.* Science 2004, 306(5698): 1037-40.
10. Eskelinen, E.L., Deretic, V., Neufeld, T., Levine, B., Cuervo, A.M. *4th International Symposium on Autophagy: Exploiting the frontiers of autophagy research*. Autophagy 2007, 3(2): 166-73.
11. Schmid, D., Munz, C. *Innate and adaptive immunity through autophagy*. Immunity 2007, 27(1): 11-21.
12. Schmid, D., Pypaert, M., Munz, C. *Antigen-loading compartments for major histocompatibility complex class II molecules continuously receive input from autophagosomes*. Immunity 2007, 26(1): 79-92.
13. Pua, H.H., Dzhagalov, I., Chuck, M., Mizushima, N., He, Y.W. *A critical role for the autophagy gene Atg5 in T cell survival and proliferation*. J Exp Med 2007, 204(1): 25-31.
14. Harris, J., De Haro, S.A., Master, S.S., Keane, J., Roberts, E.A., Delgado, M., Deretic, V. *T helper 2 cytokines inhibit autophagic control of intracellular Mycobacterium tuberculosis*. Immunity 2007, 27(3): 505-17.
15. Mizushima, N., Yamamoto, A., Matsui, M., Yoshimori, T., Ohsumi, Y. *In vivo analysis of autophagy in response to nutrient starvation using transgenic mice expressing a fluorescent autophagosome marker.* Mol Biol Cell 2004, 15(3): 1101-11.
16. Nedjic, J., Aichinger, M., Emmerich, J., Mizushima, N., Klein, L. *Autophagy in thymic epithelium shapes the T-cell repertoire and is essential for tolerance*. Nature 2008, 455(7211): 396-400.
17. Wellcome Trust Case Control Consortium. *Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls*. Nature 2007, 447(7145): 661-78.

18. Hampe, J., Franke, A., Rosenstiel, P. et al. *A genome-wide association scan of nonsynonymous SNPs identifies a susceptibility variant for Crohn disease in ATG16L1*. Nat Genet 2007, 39(2): 207-11.
19. Rioux, J.D., Xavier, R.J., Taylor, K.D. et al. *Genome-wide association study identifies new susceptibility loci for Crohn disease and implicates autophagy in disease pathogenesis*. Nat Genet 2007, 39(5): 596-604.
20. Prescott, N.J., Fisher, S.A., Franke, A. et al. *A nonsynonymous SNP in ATG16L1 predisposes to ileal Crohn's disease and is independent of CARD15 and IBD5*. Gastroenterology 2007, 132(5): 1665-71.
21. Parkes, M., Barrett, J.C., Prescott, N.J. et al. *Sequence variants in the autophagy gene IRGM and multiple other replicating loci contribute to Crohn's disease susceptibility*. Nat Genet 2007, 39(7): 830-2.
22. Annese, V. *Digging out Crohn's disease genes*. Inflamm Bowel Dis 2009, 15(9): 1436-7.
23. McCarroll S.A., Huett A., Kuballa P. et al. *Deletion polymorphism upstream of IRGM associated with altered IRGM expression and Crohn's disease*. Nat Genet 2008, 40(9): 1107-12.
24. Palomino-Morales, R.J., Oliver, J., Gomez-Garcia, M., Lopez-Nevot, M.A., Rodrigo L., Nieto, A., Alizadeh, B.Z., Martin, J. *Association of ATG16L1 and IRGM genes polymorphisms with inflammatory bowel disease: A meta-analysis approach*. Genes Immun 2009, 10(4): 356-64.
25. Barrett, J.C., Hansoul, S., Nicolae, D.L. et al. *Genome-wide association defines more than 30 distinct susceptibility loci for Crohn's disease*. Nat Genet 2008, 40(8): 955-62.
26. Phillips, A.M., Nimmo, E.R., Limbergen, J.V., Drummond, H.E., Smith, L., Satsangi, J. *Detailed haplotype-tagging study of germline variation of MUC19 in inflammatory bowel disease*. Inflamm Bowel Dis 2009 (in press).
27. Kuballa, P., Huett, A., Rioux, J.D., Daly, M.J., Xavier, R.J. *Impaired autophagy of an intracellular pathogen induced by a Crohn's disease associated ATG16L1 variant*. PLoS One 2008, 3(10): e3391.
28. Cadwell, K., Liu, J.Y., Brown, S.L. et al. *A key role for autophagy and the autophagy gene Atg16l1 in mouse and human intestinal Paneth cells*. Nature 2008, 456(7219): 259-63.
29. Saitoh, T., Fujita, N., Jang, M.H. et al. *Loss of the autophagy protein Atg16L1 enhances endotoxin-induced IL-1beta production*. Nature 2008, 456(7219): 264-8.
30. Levine, B., Deretic, V. *Unveiling the roles of autophagy in innate and adaptive immunity.* Nat Rev Immunol 2007, 7(10): 767-77.
31. Heuer, D., Lipinski, A.R., Machuy, N. et al. *Chlamydia causes fragmentation of the Golgi compartment to ensure reproduction*. Nature 2009, 457(7230): 731-5.
32. Nagarajan, U.M., Prantner, D., Sikes, J.D., Andrews, C.W., Jr., Goodwin, A.M., Nagarajan, S., Darville, T. *Type I interferon signaling exacerbates Chlamydia muridarum genital infection in a murine model.* Infect Immun 2008, 76(10): 4642-8.
33. Al-Younes, H.M., Brinkmann, V., Meyer, T.F. *Interaction of Chlamydia trachomatis serovar L2 with the host autophagic pathway*. Infect Immun 2004, 72(8): 4751-62.
34. Shintani, T., Klionsky, D.J. *Autophagy in health and disease: A double-edged sword*. Science 2004, 306(5698): 990-5.
35. Pachikara, N., Zhang, H., Pan, Z., Jin, S., Fan, H. *Productive Chlamydia trachomatis lymphogranuloma venereum 434 infection in cells with augmented or inactivated autophagic activities.* FEMS Microbiol Lett 2009, 292(2): 240-9.
36. Al-Zeer, M.A., Al-Younes, H.M., Braun, P.R., Zerrahn, J., Meyer, T.F. *IFN-gamma-inducible Irga6 mediates host resistance against Chlamydia trachomatis via autophagy.* PLoS One 2009, 4(2): e4588.