Ubiquitination of pathogen-containing vacuoles promotes host defense to Chlamydia trachomatis and Toxoplasma gondii

Jörn Coers1,2,* and Arun K Haldar1
1Department of Molecular Genetics and Microbiology; Durham, NC USA; 2Department of Immunology; Duke University Medical Center; Durham, NC USA

Many intracellular bacterial and protozoan pathogens reside within host cell vacuoles customized by the microbial invaders to fit their needs. Within such pathogen-containing vacuoles (PVs) microbes procure nutrients and simultaneously hide from cytosolic host defense systems. Among the many PV-resident human pathogens are the bacterium Chlamydia trachomatis and the protozoan Toxoplasma gondii. Immune responses directed against their PVs are poorly characterized. We reported that activation of host cells with IFNγ triggers the attachment of polyubiquitin chains to Toxoplasma- and Chlamydia-containing vacuoles and thereby marks PVs for destruction. In murine cells PV ubiquitination is dependent on IFNγ-inducible Immunity Related GTPases (IRGs). Human cells also decorate PVs with ubiquitin upon IFNγ priming; however, the molecular machinery promoting PV ubiquitination in human cells remains unknown and is likely to be distinct from the IRG-dependent pathway we described in murine cells. Thus, IFNγ-inducible PV ubiquitination constitutes a critical event in cell-autonomous immunity to C. trachomatis and T. gondii in mice and humans, but the molecular machinery underlying PV ubiquitination is expected to be multifaceted and possibly host species-specific.

C. trachomatis and T. gondii are among the most prevalent human pathogens; C. trachomatis is the causative agent of the most common sexually transmitted bacterial infection in the Western world and the leading cause of preventable blindness worldwide.1 T. gondii infection is also exceptionally common. Seroprevalence of anti-T. gondii immunoglobulins varies substantially across the world but is typically in the range of 30 – 80% for a given human population.2 While most T. gondii infections remain asymptomatic, the parasite can induce serious illness in immunocompromised individuals and is able to cross the placenta causing spontaneous abortions.3

Both microbes are obligate intracellular pathogens highly adapted to a life inside tailor-made vacuoles known as C. trachomatis inclusions or T. gondii parasitophorous vacuoles, respectively.1,3 Both pathogens share a similar intracellular lifestyle and are susceptible to the same IFNγ-induced cell-autonomous immune responses.4-6 In IFNγ-primed murine cells members of the Immunity Related GTPase (IRG) protein family translocate to PV membranes surrounding C. trachomatis or T. gondii and subsequently induce the vesiculation and ultimate rupture of IRG-decorated PV membranes.7,11

The mechanism by which IRGs promote PV destruction is poorly characterized. In a recent publication we demonstrated that IFNγ priming of mouse fibroblasts or mouse macrophages prompts IRG-dependent ubiquitination of C. trachomatis and T. gondii PVs, a process that appears to precede PV disintegration.12 Ubiquitin is a small protein of 76 amino acids that can be covalently attached to protein substrates as a monomer or as lysine-linked polymers.13 We showed that K48- and K63-linked polyubiquitin chains are associated with C. trachomatis and T. gondii PVs in IFNγ-primed murine cells. We identified the ubiquitin E3 ligase TRAF6 as one mediator of PV ubiquitination. However, PV ubiquitination is only partly defective in...
TRAF6-deficient cells suggesting the involvement of additional E3 ligases. In support of this hypothesis we found that not only TRAF6 but also the E3 ligase Trim21 is recruited to PVs. The identification of the entire repertoire of PV-associated E3 ligases in future studies will be critical in order to understand how the host cell labels PVs with a variable ubiquitin code triggering potentially cell type- or pathogen-specific immune responses.

Ubiquitination of intracellular microbes has emerged as a focal point of cell-autonomous immunity to a variety of intracellular pathogens across many different host species. Accordingly, it comes as no surprise that IFNγ-primed human cells also tag T. gondii PVs with ubiquitin (see Fig. 1 and also Selleck et al.). Although both murine and human cells apply ubiquitin-centered mechanisms to battle T. gondii infections, it is currently unknown whether any components of the machinery involved in T. gondii PV ubiquitination are conserved between mice and humans.

Our studies demonstrated that PV ubiquitination can lead to the destabilization of PVs. Specifically, we found that the adaptor protein p62 binds to ubiquitinated C. trachomatis inclusions and together with TRAF6 promotes the destruction of these PVs and their bacterial occupants. We further demonstrated that p62 escorts members of the Guanylate Binding Protein (GBP) family to ubiquitinated PVs. GBP family is functionally linked to a plethora of innate immune responses that include inflammasome activation, antimicrobial autophagy (xenophagy) and host-mediated PV lysis. Because of the reported functional link between GBPs and PV destruction, it seems feasible that TRAF6 and p62 promote PV lysis through GBP recruitment. However, we have so far failed to confirm a direct role for GBPs in PV lysis. Therefore, the precise mechanism by which ubiquitination triggers vacuolar lysis requires further examination.

The association of intracellular microbes with ubiquitin plays an important role in the capture of pathogens inside autophagosomes or autophagosome-like vacuoles (ALVs). Selleck et al reported that ubiquitinated T. gondii PV's...
in human Hela cells become encapsulated inside LC3-decorated multilamellar vacuoles.\textsuperscript{16} To determine whether the capture of \textit{T. gondii} inside multilamellar ALVs is the predominant or potentially only fate of ubiquitinated \textit{T. gondii} PVs in human cells, additional cell types will need to be examined. Similarly, future studies should address whether ubiquitination-dependent PV lysis also takes place in human cells and whether loss of vacuolar integrity is linked to the capture of PVs inside multilamellar ALVs.

Three independent studies demonstrated very recently that the attachment inside multilamellar ALVs.

Accordingly, virulent strains of \textit{T. gondii} have evolved strategies to interfere with IFN\textgamma-inducible PV ubiquitination pathways in both murine and human hosts. Although the mechanisms for bacterial evasion of this host defense pathway remain unexplored, we can expect several bacterial pathogens to deploy either distinct or convergent strategies to block IFN\textgamma-inducible PV ubiquitination pathways. Defining these pathways on a molecular level and identifying the microbial evasion mechanisms may reveal novel microbial targets for the development of new drugs to treat bacterial and protozoan infections.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

References

1. Belland R, Ojcius DM, Byrne GL. Chlamydia. Nat Rev Microbiol (2004); 2:530-531; PMID:15248311; http://dx.doi.org/10.1038/nrmicro931
2. Pappas G, Roussos N, Falagas ME. Toxoplasmosis. Int J Parasitol (2009); 39:1385-1394; PMID:19435092; http://dx.doi.org/10.1016/j.ijpara.2009.04.003
3. Bohodur JC. Toxoplasma gondii: 25 years and 25 major advances for the field. Int J Parasitol (2009); 39:935-946; PMID:19650140
4. Coers J. Starnbach MN, Howard JC. Modeling infectious disease in mice: co-adaptation and the role of host-specific IFNgamma responses. PLoS Pathog (2009); 5:e1000333; PMID:19478881; http://dx.doi.org/10.1371/journal.ppat.1000333
5. Huhn JP, Feng CG, Sher A, Howard JC. The immunity-related GTPases in mammals: a fast-evolving cell-autonomous resistance system against intracellular pathogens. Mammm Genome (2011); 22:43-54; PMID:21052678; http://dx.doi.org/10.1007/s00335-010-9293-3
6. Dubaene W, MacKenzie CR. IFN-gamma-activated inducible nitric oxide synthase activity in human cells is an antiparasite and an antibacterial effector mechanism. Adv Exp Med Biol (1999); 467:517-524; PMID:10721095; http://dx.doi.org/10.1007/978-1-4615-4709-9_64
7. Coers J, Bernstein-Hanley I, Grondsy D, Parvanova I, Howard JC, Taylor GA, Dietrich WF, Starnbach MN. Chlamydia muridarum evades growth restriction by the IFN-gamma-inducible host resistance factor Irgb10. J Immunol (2008); 180:6237-6245; PMID:18424746; http://dx.doi.org/10.4049/jimmunol.180.9.6237
8. Haldar AK, Saka HA, Piro AS, Dunn JD, Henry SC. Taylor GA, Frickel EM, Valdivia RH, Coers J, IRG and GBP host defense: how cell-autonomous immunity protects bacteria with autophagy systems. Current biology : CB (2011); 21:R540-545; PMID:21775774; http://dx.doi.org/10.1016/j.cub.2012.06.001
9. Coers J, Bernstein-Hanley I, Grotsky D, Parvanova I, Howard JC, Taylor GA, Dietrich WF, Starnbach MN. Chlamydia muridarum evades growth restriction by the IFN-gamma-inducible host resistance factor Irgb10. J Immunol (2008); 180:6237-6245; PMID:18424746; http://dx.doi.org/10.4049/jimmunol.180.9.6237
10. Martens S, Parvanova I, Zerrahn J, Griffiths G, Schell G, Reichmann G, Howard JC. Disruption of Toxoplasma gondii parasitophorous vacuoles by the mouse p47-resistance GTPases. PLoS Pathog (2005); 1:e24; PMID:16363084; http://dx.doi.org/10.1371/journal.ppat.0010024
11. Zhao YO, Khaminets A, Huhn JP, Howard JC. Disruption of the Toxoplasma gondii parasitophorous vacuole by IFN\gamma-inducible immunity-related GTPases (IRG proteins) triggers necrotic cell death. PLoS Pathog (2009); 5:e1000288; PMID:19197351; http://dx.doi.org/10.1371/journal.ppat.1000288
12. Haldar AL, Zelmann Broz D, Akira S, Yamamoto M. p62 Plays a specific role in interferon-gamma-induced presentation of the AIM2 inflammasome by RAW 264.7 cells. Infect Immun (2015); 83:203-207; PMID:26416908; http://dx.doi.org/10.1128/IAI.01457-07
13. Shenoy AR, Wellington DA, Kumar P, Das R, Tiwari S, Mackie-McKee J. A family of IFN-gamma-inducible 65-kD GTPases promotes anti-microbial immunity in mammals. Nature (2011); 476:467-470; PMID:21677416; http://dx.doi.org/10.1038/nature10315
14. Shenoy AR, Wellington DA, Kumar P, Das R, Tiwari S, Mackie-McKee J. A family of IFN-gamma-inducible 65-kD GTPases promotes anti-microbial immunity in mammals. Nature (2011); 476:467-470; PMID:21677416; http://dx.doi.org/10.1038/nature10315
15. Neufeld E, Dick MS, Dreier RF, Schirmann K, Renz J, Reinhart M, Sisler M, Sauter H, Hagele S, Sauter T, Akira S, Yamamoto M. p62 Plays a specific role in interferon-gamma-induced presentation of the AIM2 inflammasome by RAW 264.7 cells. Infect Immun (2015); 83:203-207; PMID:26416908; http://dx.doi.org/10.1128/IAI.01457-07
16. Debarboulo KJ, Schirrmann K, Renz J, Reinhart M, Sisler M, Sauter T, Akira S, Yamamoto M. p62 Plays a specific role in interferon-gamma-induced presentation of the AIM2 inflammasome by RAW 264.7 cells. Infect Immun (2015); 83:203-207; PMID:26416908; http://dx.doi.org/10.1128/IAI.01457-07
17. Bekpen C, Huhn JP, Rohde C, Parvanova I, Guehrlein L, Dunn DM, Glowalla E, Lepitin M, Howard JC. The interferon-inducible p47 (IRG) GTPases in vertebrates: loss of the cell autonomous resistance mechanism in the human lineage. Genome Biol (2005); 6:R92; PMID:16277747; http://dx.doi.org/10.1186/gb-2005-6-11-r92
18. Rupper AC, Cardelli JA. Induction of guanylate bind-}