Communicative & Integrative Biology 8:6, e1115163; November/December 2015; Published with license by Taylor & Francis Group, LLC

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

Jörn Coers[1,2,*] and Arun K Haldar[1]

[1]Department of Molecular Genetics and Microbiology; Durham, NC USA; [2]Department 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.

Keywords: autophagy, Chlamydia, guanylate binding proteins, immunity related GTPases, interferon, pathogen-containing vacuoles, Toxoplasma, ubiquitin, vacuolar lysis

*Correspondence to: Jörn Coers; Email: jorn.coers@duke.edu

Submitted: 10/12/2015

Revised: 10/26/2015

Accepted: 10/26/2015

http://dx.doi.org/10.1080/19420889.2015.1115163

C. trachomatis and *T. gondii* are among the most prevalent human pathogen; *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

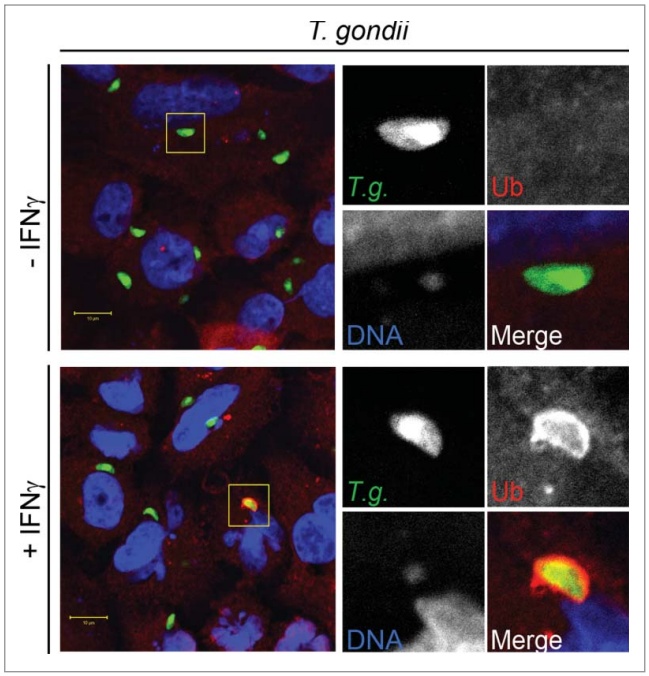

Figure 1. IFNγ-primed human cells decorate *T. gondii* PVs with ubiquitin. Human alveolar epithelial A549 cells were primed with IFNγ (200 U/mL) overnight or left untreated and subsequently infected with the avirulent GFP-expressing type II *T. gondii* strain Pru A7 (*T.g.*). At 1 hour post-infection cells were fixed and stained for DNA with Hoechst and for ubiquitin (Ub).

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.[12] 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.[14,15] 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.[16]). 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 (**Fig. 2**). Some fundamental differences in the underlying molecular mechanisms of PV ubiquitination appear likely considering that human cells lack a subset of the IRG proteins that we have shown to be critical for PV ubiquitination in mice.[12,17]

Our studies demonstrated that PV ubiquitination can lead to the destabilization of PVs.[12] 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.[12] GBPs are host resistance proteins functionally linked to a plethora of innate immune responses that include inflammasome activation, antimicrobial autophagy (xenophagy) and host-mediated PV lysis.[18-25] Because of the reported functional link between GBPs and PV destruction,[21] 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.[20] 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).[14] Selleck et al reported that ubiquitinated *T. gondii* PVs

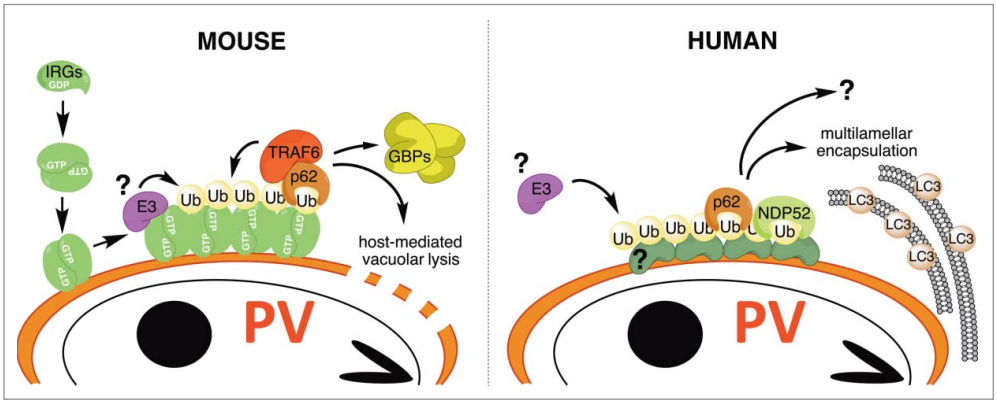

Figure 2. IFNγ-induced PV ubiquitination in mice and humans. In mice a subset of IFNγ-inducible IRGs (so-called GKS proteins) detect PV membranes through a missing-self principle.[8,27] PV-bound IRGs facilitate the translocation of TRAF6 and other ubiquitin E3 ligases to PVs resulting in the decoration of PVs with ubiquitin. GKS proteins themselves are likely among the ubiquitinated PV-resident proteins, as GKS proteins in the GTP-bound active state are known to acquire K63-linked polyubiquitin chains.[28] PV ubiquitination triggers PV lysis and the recruitment of IFNγ-inducible GBPs. In human cells IFNγ priming also leads to the decoration of *T. gondii* PVs but the underlying mechanism and the ubiquitinated substrates are unknown.[16] Parasites inside ubiquitin-associated *T. gondii* PVs become encased within multilamellar autophagsome-like structures and cease replication.[16]

in human Hela cells become encapsulated inside LC3-decorated multilamellar vacuoles.[16] To determine whether the capture of *T. gondii* inside multilamellar ALVs is the predominant or potentially only fate of ubiquitinated *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 of ubiquitin to *T. gondii* or *C. trachomatis* PVs is advantageous to the host.[12,16,26] Accordingly, virulent strains of *T. gondii* have evolved strategies to interfere with IFNγ-inducible PV ubiquitination pathways in both murine and human hosts.[12,16,26] 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 IFNγ-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.

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