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Author

Jensen, Kirk DC

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Spotlight

Antigen Presentation of Vacuolated Apicomplexans – Two Gateways to a Vaccine Antigen

Kirk D.C. Jensen[1,*]

For parasites that sequester themselves within a vacuole, new rules governing antigen presentation are coming into focus. Components of the host's autophagy machinery and the parasite's membranous nanotubular network within the parasitophorous vacuole play a major role in determining antigenicity of *Toxoplasma* proteins. As such, both parasite and vaccinologist may exploit these pathways to regulate the ever important CD8 T cell response to apicomplexan parasites.

T cell mediated immune responses are required for host immunity to most parasitic pathogens. In particular, T cells of the CD8 lineage are critical components for immunity to *Toxoplasma gondii* and the prevention of liver stage *Plasmodium* infection following vaccination. Antigen-specific CD8 T cells recognize unique 8- to 10-mer peptides presented by major histocompatibility complex class 1 (MHC1) molecules expressed on the surface of nearly all somatic cells. The major source of peptides that enter the MHC1 antigen presentation pathway is derived from cytosolic proteins degraded by the host's proteasome (Figure 1). Cytosolic pathogens provide a continual flow of proteins into this pathway, while phagocytosed antigens enter the cytosol through a process termed 'cross-presentation' in specialized immune cells (e.g., dendritic cells). Apicomplexan parasites, however, sequester themselves in a non-fusogenic parasitophorous vacuole (PV) mostly devoid of host proteins. How does the immune system gain access to vacuolar antigens that are not within conventional endocytic compartments of the cell? By analyzing CD8 T cell activation to *Toxoplasma* infected cells, recent work from the Blanchard [1] and Yamamoto [2] labs suggests two ways to acquire antigen from a parasitophorous vacuole.

Acquiring Antigen from the Parasitophorous Vacuole Membrane

Pervious work on MHC1 presentation of *Toxoplasma* antigens demonstrated that secreted or surface proteins of the parasite are superior in their ability to stimulate CD8 T cells [3]. Heat killed or invasion blocked tachyzoites fail to elicit CD8 T cell activation, ruling against phagocytosis and cross-presentation as a dominant mode of MHC1 antigen presentation during *Toxoplasma* infection [4]. Although dense granules and rhoptry proteins are secreted and can enter the host cytosol, the dense granule secretory pathway is better able to stimulate CD8 T cells [5]. This suggests the PV is a suitable platform for the MHC1 antigen presentation machinery, but what components of this machinery are needed is unclear. Previous work by Blanchard and colleagues identified an immuno-protective epitope derived from the C-terminus of the dense granule GRA6 [6]. GRA6 has three locations within the PV: inside the vacuole lumen, tightly associated with the intravacuolar network of membranous tubules (IVN) and integrated in the PV membrane (PVM). Lopez *et al*. [1] asked whether location of GRA6 within the PV influences MHC1 antigen presentation. GRA6 was highly enriched in the PVM of Δ*gra2* parasites, which have defects in the biogenesis and structure of the IVN. Importantly, increased GRA6 association with the PVM correlated with enhanced GRA6-specific CD8 T cell activation to Δ*gra2* parasite strains. While true for GRA6, this was not the case for another immune targeted protein, TGD057. Lopez *et al*. [1] demonstrated TGD057 is a luminal dense granule protein, however, tampering with the IVN structure had no influence on the CD8 T cell response to TGD057. The identity of the host protease that trims the C-terminus of GRA6 is not known, but it is predicted to be part of the antigen presentation machinery that targets membrane associated proteins. The identity of this protease will undoubtedly aid our understanding of how PVM-associated proteins become selected for antigen presentation.

Acquiring Vacuolar Luminal Antigen via Selective Autophagy

For vacuolar luminal antigens not integrated in the PVM, a second mechanism for antigen presentation is needed. By analyzing a *Toxoplasma* strain that secretes the model antigen ovalbumin (OVA) into the PV lumen [3], Lee *et al*. [2] demonstrated that OVA-specific CD8 T cell activation is highly dependent upon a mechanism akin to 'selective autophagy'. The authors showed that CD8 T cell activation required ATG7 (E1-), ATG3 (E2-), ATG16L1 (E3-ubiquitin ligases) and the P62 sequestosome-1, but not the classical autophagy initiator ATG14. Interestingly, P62 co-localized with the OVA antigen and through its ubiquitin associated (UBA) domain recognized ubiquitinated products that appear on the PVM in IFNγ stimulated host cells (Figure 1). In the absence of P62, OVA-specific CD8 T cell activation *in vitro* was nearly abolished. The ubiquitinated substrate on the PVM is unknown, but its presence was dependent upon the aforementioned autophagy machinery and the regulatory immunity related GTPases Irgm1 and Irgm3, all of which are required for PV destruction in mouse cells. Similar findings were reported by the Coers laboratory, with the additional observation that P62 recruited several E3 ligases and guanylate binding proteins (GPBs) to the PVM [7]. However, $P62^{-/-}$ murine cells had only a minor defect in parasite killing [2,7], suggesting this pathway plays a more prominent role in acquiring vacuolar antigens than in PV destruction *per se*. Whether

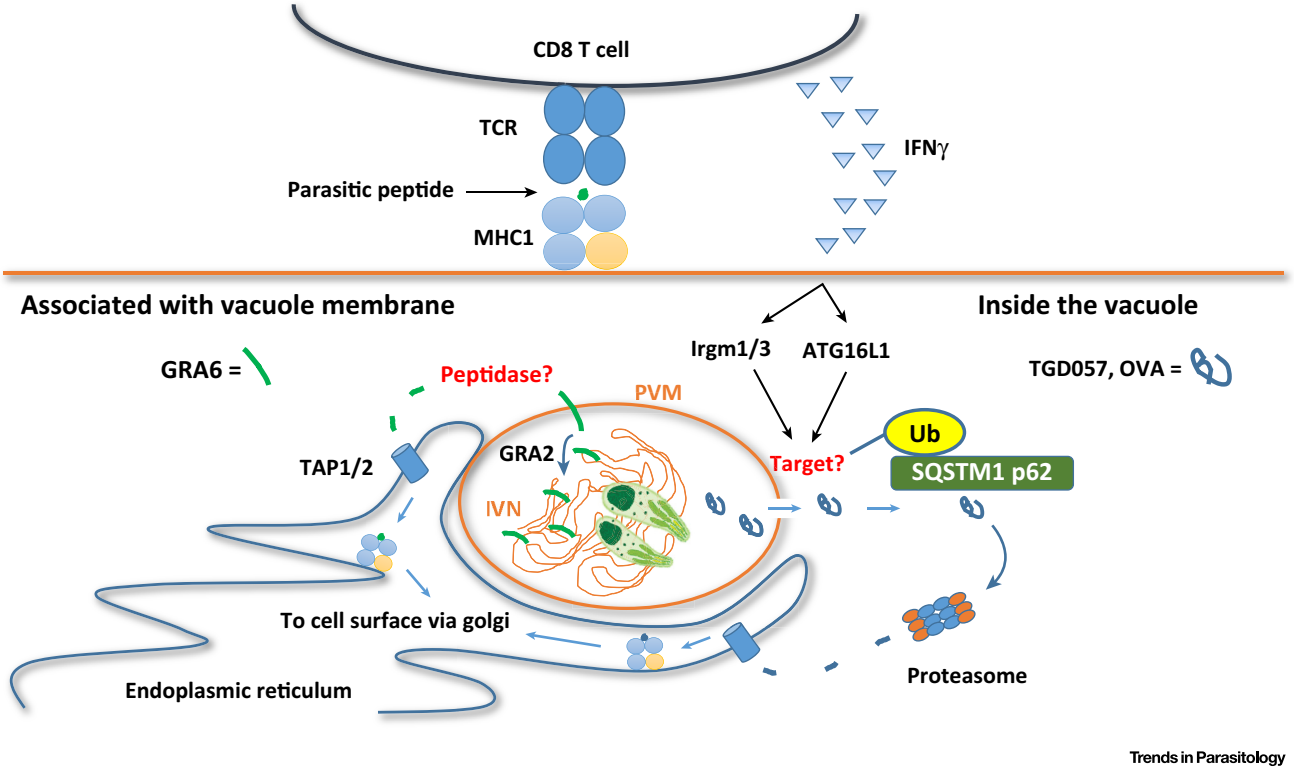

Figure 1. Two Ways for *Toxoplasma* Proteins to Enter the Major Histocompatibility Complex Class 1 Antigen Presentation Pathway. Left: The *Toxoplasma* dense granule GRA6 is integrated within the parasitophorous vacuole membrane (PVM) and the intravacuolar network of membranous tubules (IVN). In order for GRA6 to enter the major histocompatibility complex class 1 (MHC1) antigen presentation pathway an unknown protease cleaves GRA6 freeing a C-terminus GRA6 peptide into the host cytosol. The peptide enters the lumen of the endoplasmic reticulum through the TAP1/2 translocon where they are loaded into empty MHC1 molecules. The antigen-MHC1 complex is exported to the surface of infected cells and are recognized by the T cell receptor (TCR) of CD8 T cells. The dense granule GRA2 is required for IVN integrity which favors location of GRA6 to the IVN. In the absence of GRA2 however, Lopez *et al*. [1] showed that GRA6 preferentially localizes to the PVM and readily enters the MHC1 antigen presentation pathway. Right: Parasite proteins that reside in the lumen of the parasitophorous vacuole (PV), like the secreted model antigen OVA or the dense granule TDG057, enter the antigen presentation pathway with the help of IFNγ. Lee *et al*. [2] showed that components of the autophagy machinery (ATG16L1) and the immunity related GTPases system (Irgm1 and Irgm3) leads to a situation where the PVM becomes decorated with poly-ubiquitin chains and is recognized by the P62 sequestosome (SQSTM1). These events are downstream of IFNγ activation. Presumably, P62 binds to and targets the vacuolar antigen to the proteasome, thus directing PV luminal antigens into the MHC1 antigen presentation pathway. The nature of the ubiquitinated target on the PVM and how the antigen crosses the PVM is unknown.

the PVM is momentarily 'torn open' allowing vacuolar luminal antigen to escape, or whether the P62-ubiquitin system 'pulls' proteins through the PVM is unknown. Analysis of *Toxoplasma* strains that are resistant to IRG-mediated PV destruction, but permissible to P62-mediated antigen presentation may clarify this issue and touch upon the complicated issue of protein transport across the PVM.

Acquiring the Right Vaccine Antigen

Do antigen-specific human CD8 T cells require selective autophagy to respond to apicomplexan parasites? Following *Plasmodium* infection in a human liver cell line, sporozoite PVMs become decorated with ubiquitin and P62 [8]. Likewise, *Toxoplasma* PVs are coated with ubiquitin and P62 in human HeLa cells stimulated with IFNγ [9]. *Plasmodium* sporozoite vaccination, the gold standard for malaria vaccines, elicits a broad spectrum CD8 T cell response against multiple liver stage antigens [10], but the identities of these antigens are largely unknown. If the CD8 T cell response requires selective autophagy, then a proteomic analysis of PVM-associated or ubiquitin-P62 associated proteins may yield an enriched source of novel and protective liver stage sporozoite antigens sought after in malaria vaccines. Second, *Toxoplasma* virulence factors that intersect the murine IRG system should manipulate MHC1 antigen presentation through the PV-ubiquitin-P62 pathway, for example through ROP18 [7]. Regulation of IVN biogenesis and structure may also influence antigen presentation of PVM associated antigens, for example through polymorphisms in GRA2. If we probe further, our understanding of antigen presentation of vacuolated pathogens may someday guide us to a vaccine for apicomplexan parasites.

[1]School of Natural Sciences, University of California, Merced, 5200 North Lake Road, Merced, CA 95343, USA

*Correspondence: kjensen5@ucmerced.edu (K.D.C. Jensen).

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References
1. Lopez, J. *et al.* (2015) Intravacuolar membranes regulate CD8 T cell recognition of membrane-bound *Toxoplasma gondii* protective antigen. *Cell Rep.* 13, 2273–2286
2. Lee, Y. *et al.* (2015) p62 plays a specific role in interferon-gamma-induced presentation of a toxoplasma vacuolar antigen. *Cell Rep.* 13, 223–233
3. Gregg, B. *et al.* (2011) Subcellular antigen location influences T-cell activation during acute infection with *Toxoplasma gondii*. *PLoS ONE* 6, e22936
4. Dupont, C.D. *et al.* (2014) Parasite fate and involvement of infected cells in the induction of CD4+ and CD8+ T cell responses to *Toxoplasma gondii*. *PLoS Pathog.* 10, e1004047
5. Grover, H.S. *et al.* (2014) Impact of regulated secretion on antiparasitic CD8 T cell responses. *Cell Rep.* 7, 1716–1728
6. Feliu, V. *et al.* (2013) Location of the CD8 T cell epitope within the antigenic precursor determines immunogenicity and protection against the *Toxoplasma gondii* parasite. *PLoS Pathog.* 9, e1003449
7. Haldar, A.K. *et al.* (2015) Ubiquitin systems mark pathogen-containing vacuoles as targets for host defense by guanylate binding proteins. *Proc. Natl. Acad. Sci. U.S.A.* 112, E5628–E5637
8. Prado, M. *et al.* (2015) Long-term live imaging reveals cytosolic immune responses of host hepatocytes against *Plasmodium* infection and parasite escape mechanisms. *Autophagy* 11, 1561–1579
9. Selleck, E.M. *et al.* (2015) A noncanonical autophagy pathway restricts *Toxoplasma gondii* growth in a strain-specific manner in IFN-gamma-activated human cells. *MBio* 6, e01157-15
10. Butler, N.S. *et al.* (2011) Superior antimalarial immunity after vaccination with late liver stage-arresting genetically attenuated parasites. *Cell Host Microbe* 9, 451–462

Spotlight

Red Blood Cell Spectrin Skeleton in the Spotlight

Catherine Braun-Breton[1,*] and Manouk Abkarian[2]

Das *et al.* recently reported a role for the major merozoite surface protein MSP1 in malarial parasite egress from the red blood cell (RBC). On the basis of these new data and physical considerations, we propose an updated model for the main steps of this essential process for parasite proliferation.

Malaria mortality essentially results from the intra-erythrocytic development of *Plasmodium falciparum* parasites. This part of the parasite life cycle starts with the active penetration of a merozoite into a red blood cell (RBC) and the concomitant formation of a specific compartment called the parasitophorous vacuole (PV) in which the parasite grows and multiplies; around 48 h later, the sequential rupture of the PV and RBC membranes releases up to 32 new infectious merozoites in the blood stream. Around the time of the PV membrane rupture, the most abundant merozoite surface protein, MSP1, is cleaved by the parasite serine-protease SUB1, converting MSP1 into four polypeptides that remain as a complex at the merozoite surface. One of these processing products, the glycosylphosphatidylinisotol (GPI)-anchored MSP1-42 protein, further processed into MSP1-19 and MSP1-33 by the serine-protease SUB2 upon invasion, was implicated in invasion [1], although its precise function in this process remains unclear.

Using elegant genetic approaches to generate MSP1 mutants refractory to SUB1 processing, Das *et al.* [2] reported for the first time an unexpected role of MSP1 in merozoite egress. Because preventing the processing of MSP1 by SUB1 is deleterious for the parasite survival, Das *et al.* generated a mutant displaying a delayed processing of MSP1 by mutating two out of the three SUB1 cleavage sites (referred to as 38/42 cleavage) separating the MSP1-42 polypeptide from its 80 kDa precursor. In these parasites, no growth defect was detected but the delayed production of MSP1-42 mirrored a delayed egress of the merozoites. Moreover, the Cre recombinase-mediated and rapamycin-dependant excision of the *msp*1 gene 3′ segment, produced merozoites that conditionally expressed a secreted processed form of MSP1 and accordingly showed a dramatic egress defect.

To further characterize the potential role of MSP1-42 in egress, Das *et al.* [2] used recombinant versions of full length MSP1 and monitored their processing by recombinant SUB1. This *in vitro* processing generated the expected four fragments of MSP1 that, such as *in vivo*, remained associated as a complex. Das *et al.* showed that this processing also induced conformational changes of MSP1 depending on the 38/42 cleavage and the ability of MSP1 to bind spectrin using inside-out erythrocyte ghosts, isolated erythrocyte skeleton and purified erythrocyte spectrin.

Taken together these data suggested that, while both the surface-anchored and soluble truncated forms of MSP1 have the ability to bind to the erythrocyte spectrin network following their processing by SUB1, only wild-type MSP1 at the merozoite surface induces efficient merozoite egress.

Das *et al.* [2] proposed a model where upon PV rupture and MSP1 processing by SUB1, the merozoite binds to the host cell cytoskeleton, via a MSP1–spectrin interaction, and induces RBC membrane rupture. However, as presented in Figure 1, efficient merozoite egress from RBCs depends on the succession of three major events: around 10 min before egress, osmotic swelling of the infected RBC is observed [3]. That generates RBC membrane tension and marks the beginning of a cascade of events leading to the rupture of the PV membrane, followed around 1 minute later by the opening and nucleation of an osmotic pore in the RBC membrane allowing the release of a limited number of merozoites; then, due to its parasite-induced destabilization, the RBC membrane curls and buckles, and this results in the wide angular dispersion of the remaining merozoites [4]. This late step is essential for efficient parasite proliferation since mutant parasites affected in this particular step (and thus releasing only one to two merozoites per intra-erythrocytic cycle) exhibited *in vitro* an increase of parasitaemia two to two and a half times lower than that of the wild-type parasites