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Selective autophagy in host defense against mycobacterial infection

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Citation
Zhang, R. (2018, November 8). *Selective autophagy in host defense against mycobacterial infection*. Retrieved from https://hdl.handle.net/1887/66789

Version:	Not Applicable (or Unknown)
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Note: To cite this publication please use the final published version (if applicable).

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Title: Selective autophagy in host defense against mycobacterial infection
Issue Date: 2018-11-08

Chapter 5

Summary and discussion

The effective treatment of tuberculosis (TB) remains a significant challenge. Drug-resistant Mycobacterium tuberculosis (Mtb) strains and co-infection with HIV increase the problem of controlling TB. Thus, under the current situation, it is essential to develop effective treatment strategies for Mtb infections. A bottleneck in TB treatment is the long-term residence of bacteria inside organized structures of immune cells, called granulomas [1]. The traditional antibiotics cannot sufficiently penetrate into granulomas and therefore lengthly antibiotic treatment regimes are required to eradicate the infection. Poor patient compliance with such antibiotic therapy results in a rise of multidrug-resistant Mtb strains. Host-directed therapies can help to overcome the limitations of direct anti-bacterial therapies. However, the development of host-directed therapies requires a complete understanding of the interaction between the host and invading pathogens to identify host processes that can be targeted. A useful tool for such studies is the zebrafish model for TB. Zebrafish can be infected with *Mycobacterium marinum* (Mm), which is closely related to Mtb and causes similar disease characteristics. Importantly, the early life stages of the zebrafish (embryos and larvae) provide access to the earliest steps in the host-pathogen interaction that lead to the initiation of granulomas [2, 3].

Mtb is an intracellular pathogen which mainly resides inside immune cells, predominantly macrophages. Thus, increasing the cell's capability to kill Mtb is a valid approach to restrict TB disease progression. Autophagy is a lysosomal degradation process and substantial experimental evidence has demonstrated that autophagy is an important host immune defense mechanism against mycobacterial infection [4-6]. Work in our laboratory has shown that the autophagy modulator Dram1 is activated downstream of mycobacterial recognition by the TLR/MyD88/NFkB pathway to activate autophagy and restrict mycobacterial infection [5]. Overexpression of zebrafish *dram1* increased autophagic targeting of mycobacteria and resulted in lower bacterial burdens. The autophagic control of infection by Dram1 required the selective autophagy receptor p62 and the cytosolic DNA sensor STING. In this thesis, we further explore the function of Dram1 in zebrafish host defense against mycobacterial infection. Furthermore, we study the role of the selective autophagy receptors p62 and Optineurin in this defense mechanism.

DRAM1 has been identified and characterized as a lysosomal membrane protein by Crighton et al. in 2006. Expression of *DRAM1* is induced by DNA damage and its expression is regulated by tumour suppressor factor p53 [7]. It has been reported that DRAM1 functions as a regulator of autophagy and apoptosis in the context of diverse cellular processes, such as immunity, and cellular differentiation [8]. In addition to its link with TB [5], DRAM 1 has also been implicated in several other diseases, including HIV and several forms of cancer [9-11]. Until now, 5 DRAM family members have been identified and partially characterized. They are DRAM1, DRAM2/TMEM77, DRAM3/TMEM150B, DRAM4/TMEM150C and DRAM5/TMEM150A. DRAM2 is most closely related to DRAM1 among the DRAM family members. Like DRAM1, DRAM2 is also involved in cell death and autophagy in response to cellular stress factors. Furthermore, DRAM2 has also been implicated in TB and cancer [12, 13]. The expression of *DRAM3* has been detected in a broad range of normal tissues and tumour cells. It has been demonstrated that DRAM3 regulates autophagic flux and cell survival in response to starvation [14]. DRAM4 and DRAM5 have been identified as DRAM family members in an *in silico* study. DRAM4 could be detected at the plasma membrane but is primarily localized at lysosomes [15]. DRAM5 can form a complex with PI 4-kinase type IIIα (PI4KIIIα) at the plasma membrane to control the production of phosphatidylinositol 4,5-biphosphate $PI(4,5)P_2$ [15]. A potential role for DRAM4 and DRAM5 in regulation of autophagy or cell death has not been investigated yet. Thus, the function of these two DRAM family members requires further elucidation. In summary, DRAM family members play an important role in regulating cellular process in response to diverse stress factors and they are highly conserved from zebrafish, to mouse to human. We speculate that other DRAM family members, besides DRAM1 and DRAM2, could also be involved in host defense mechanisms (Chapter 1). Among all Dram family members in zebrafish, *dram1* is one that is most abundantly expressed in the immune cells of larvae and its expression is strongly induced by infection. Furthermore, our previous work had shown that increasing Dram1 activity can protect zebrafish larvae against Mm infection, based on which we proposed DRAM1 as a potential target for host-directed TB therapy [5]. Therefore, the studies in this thesis were focused on further elucidating the role of dram1 in the zebrafish model for TB.

Dram1 is required to restrict mycobacterial infection in lysosomal compartments and to prevent lytic cell death of infected macrophages

Using CRISPR/Cas9 technology we generated loss-of-function mutant alleles of zebrafish *dram1* to study its function during host defense against mycobacterial infection. Based on confocal image analysis we concluded that macrophages in *dram1* mutants fail to restrict Mm inside lysosomal compartments, which eventually results in cell death of the infected macrophages and excessive growth of extracellular mycobacteria. Furthermore, we demonstrated that the macrophage cell death in *dram1* mutants occurs in a Caspase 1 dependent manner (**Chapter 2**).

The lysosomal protein Dram1 promotes the maturation of autophagosomes by facilitating their fusion with lysosomes [5, 16]. In absence of Dram1, this process is affected, which can explain the reduced acidification of Mm-containing vesicles (**Chapter 2**). On a molecular level, DRAM2 has been reported to interact with the Beclin1-VPS34-UVRAG complex to facilitate the displacement of Rubicon in order to promote the maturation of autophagosomes [12]. Given the large degree of similarity in protein structure between DRAM1 and DRAM2 [17, 18], we speculate that Dram1 might utilize a similar mechanism as DRAM2 in mediating autophagosome maturation.

Mycobacteria-infected macrophages can undergo at least three general types of cell death: apoptosis, necrosis/necroptosis and pyroptosis [19]. Apoptosis is a non-lytic form of cell death, which avoids activation of an inflammatory response. This means that intracellular mycobacteria are encapsulated within the apoptotic envelope until the remains of the apoptotic cell are phagocytIosed by newly recruited macrophages [20-22]. Since uncontrolled extracellular growth of the bacteria is avoided during apoptosis of infected macrophages, this type of cell death is generally considered as host beneficial. Necrosis and pyroptosis, on the other hand, are lytic forms of cell death, meaning that the bacterial content of dying infected macrophages ends up in the extracellular environment. While necrosis is an 'accidental' form of cell death, this type of cell death can also occur as a regulated process, referred to as necroptosis. Pyroptosis is another regulated form of lytic cell death that is characterized by the formation of gasdermin pores in the cell membrane [23]. Pyroptosis is usually dependent

on caspase 1 activation and triggers an inflammatory response [24]. In this study, we found that Dram1 deficiency leads to impaired maturation of mycobacteria-containing vesicles, ultimately resulting in caspase 1 dependent cell death and extracellular overgrowth of bacteria. An important remaining question is how the reduced acidification of mycobacteria-containing vesicles in *dram1* mutants is related to the initiation of pyroptotic cell death in mycobacteria-containing macrophages. This question will need to be addressed in future studies. Other follow up studies regarding the molecular function of Dram1 in host defense should focus on identifying the interaction partners of Dram1/DRAM1 during homeostasis and infection. Collectively, with the analysis of *dram1* mutants presented in this thesis we have provided new evidence for the function for Dram1 in maturation of mycobacteria-containing vesicles, and have uncovered a link between this process and the control of cell survival versus the initiation of programmed cell death (Fig1).

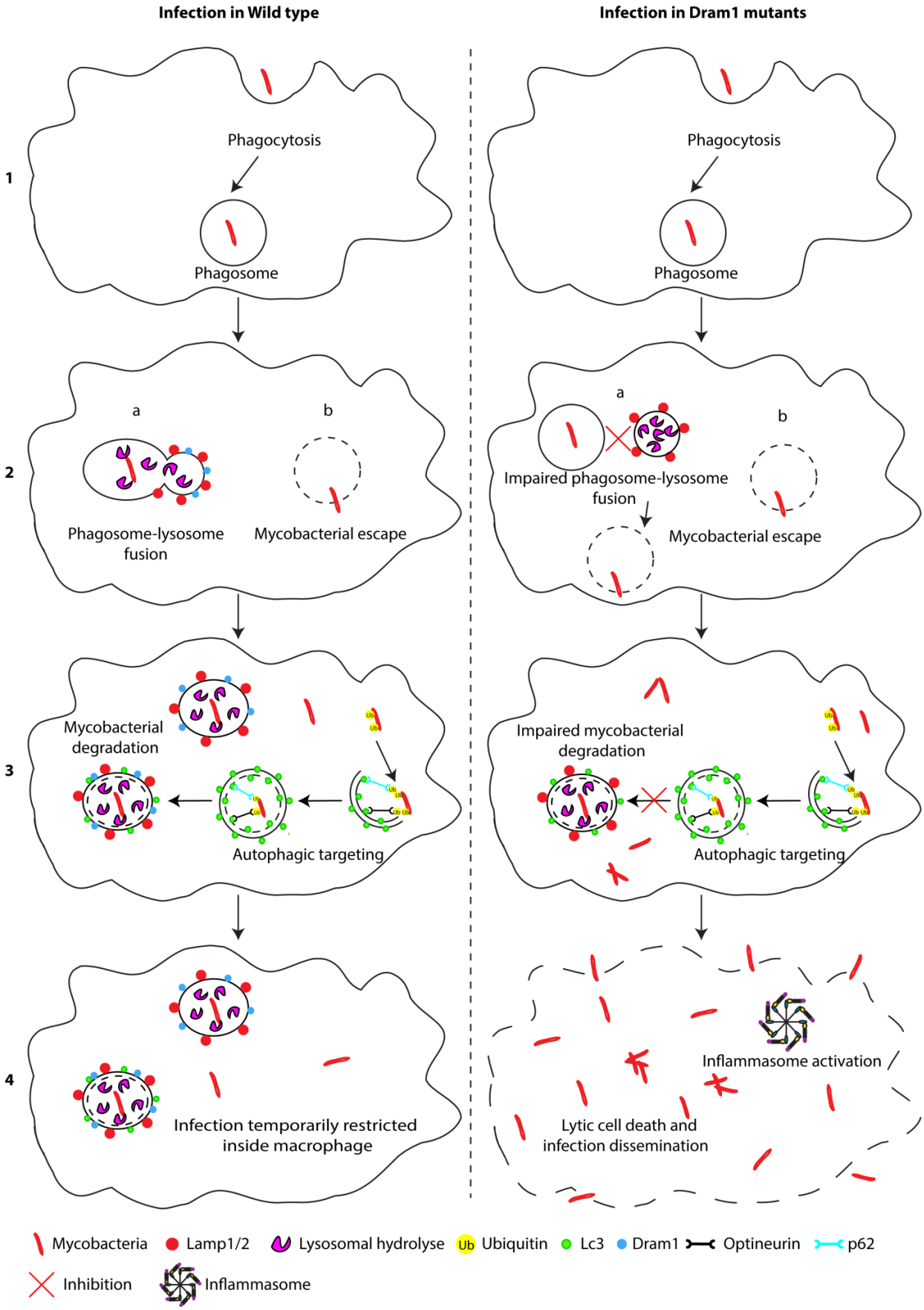
Figure 1: Schematic representation of a macrophage restricting mycobacterial infection via autophagic defense in Wild type and Dram1 deficient larvae (Figure on next page)

1. The invading mycobacteria are phagocytosed by macrophages and detected by pattern recognition receptors, such as Toll-like receptors (TLRs). During phagocytosis, mycobacteria are captured in phagosomes.

2. Whether the mycobacteria-containing phagosome matures into a bactericidal phagolysosome is a crucial factor in the outcome of infection. a) The phagosome fuses with a lysosome to degrade mycobacteria. b) However, mycobacteria have evolved diverse mechanisms to prevent fusion between phagosomes and lysosomes, such as the ESX-1 secretion system that facilitates mycobacterial escape from phagosomes. In the absence of the lysosomal protein Dram1, fusion between phagosomes and lysosomes is impaired, which contributes to mycobacterial escape from phagosomes

3. A proportion of mycobacteria have escaped from the phagosome into the cytosol. Their presence in the cytosol can be sensed and results in ubiquitination of mycobacteria. The DNA damage regulated autophagy modulator 1 (Dram1) activates selective autophagy against ubiquitinated mycobacteria. During this process, the ubiquitin receptors p62 and Optineurin are involved in the delivery of ubiquitinated mycobacteria to autophagosomes. The sequestered mycobacteria will be degraded in autophagolysosomes. However, the targeting of mycobacteria by autophagy is affected in the absence of Dram1 and the formation of autophagolysosomes is impaired.

4. Mycobacterial infection is temporarily restricted inside macrophages to avoid the dissemination of the infection. However, the mycobacteria are not effectively restricted by macrophages in Dram1-deficient larvae, which leads to pyroptotic cell death and extracellular overgrowth of mycobacteria.


Infection in Wild type
Infection in Dram1 mutants
1
Phagocytosis
Phagosome
2
a
b
Phagosome-lysosome fusion
Mycobacterial escape
Impaired phagosome-lysosome fusion
3
Mycobacterial degradation
Autophagic targeting
Impaired mycobacterial degradation
4
Infection temporarily restricted inside macrophage
Inflammasome activation
Lytic cell death and infection dissemination
Mycobacteria
Lamp1/2
Lysosomal hydrolyse
Ubiquitin
Lc3
Dram1
Optineurin
p62
Inhibition
Inflammasome

Dram1 deficiency affects gene expression of metabolic signaling pathways

In **chapter 2** of this thesis, we described that Dram1 deficiency did not significantly affect autophagy activity and cell death under basal, uninfected conditions. To better understand the function of Dram1 during homeostasis and mycobacterial infection, we performed RNA deep sequencing to analyse the transcriptomes of infected and uninfected *dram1* mutants and wild type sibling (**Chapter 3**). Under uninfected conditions, we found that the proteasome, ribosome, mitochondria, and polymerase pathways were affected to a relatively minor extent due to the absence of Dram1. This suggests that *dram1* mutants have deficiencies or alterations in these metabolic pathways. However, these metabolic alterations did not have a detectable impact on embryo and larval development. Furthermore, we found that Dram1 deficiency does not affect survival or fertility in adult zebrafish (**Chapter** 2). Taken together, we propose that the main function of Dram1/DRAM1 lies in facilitating an appropriate response to certain cellular stress factors, while its functions are somewhat redundant under homeostatic conditions (**Chapter 2** and **Chapter 3**).

Indeed, during mycobacterial infection various gene expression pathways were significantly changed in Dram1-deficient larvae compared with wild type individuals. Specifically, we found that gene expression of regulators of necroptotic and pyroptotic cell death (*hsp90* and *caspa*) was significantly altered in *dram1* mutants compared to wild type larvae infected with the same dose of mycobacteria. In contrast, while expression of *caspase 8* and *caspase 9* was slightly altered, we detected no differences in regulation of apoptosis effector genes in response to infection between infected *dram1*$^{+/+}$ and *dram1*$^{\Delta 19n/\Delta 19n}$ larvae. The analysis of the transcriptomics data confirmed our previous results that macrophages of Dram1-deficient larvae are more likely to undergo pyroptotic cell death when infected with mycobacteria (**Chapter 2**). While we have not addressed this experimentally yet, the results suggest that Dram1-deficient macrophages are more likely to undergo necroptosis in response to mycobacterial infection as well. Previously, it has been shown that DRAM1 mediates cell death of HIV-infected T-cells via lysosomal membrane permeabilization [9], and it would be interesting to investigate whether this is also the case in mycobacteria-infected macrophages. This could

potentially present a direct link between the reduced acidification of mycobacteria-containing vesicles and initiation of lytic cell death in infected Dram1-deficient macrophages.

We found that the gene expression of TLRs was also significantly altered in infected *dram1* mutant larvae compared to infected wild types. The expression of the plasma membrane receptor *tlr2* was reduced in the absence of Dram1, while the expression of the endosomal receptors *tlr*3 and *tlr*9 was significantly increased in Dram1 deficient larvae during mycobacterial infection [25, 26]. This differential expression of pattern recognition receptors might reflect the increased extracellular localization of mycobacteria in the absence of Dram1, which potentially results in adjustments in the expression of plasma membrane and endosomal TLRs. For instance, we speculate that Tlr2 molecules on the plasma membrane are continuously recognizing extracellular mycobacteria, which can result in down regulation of *tlr2* itself as a negative feedback loop. Collectively, we have demonstrated that Dram1 affects transcriptional regulation of metabolic processes; promotes maturation of mycobacteriacontaining vesicles; is required to prevent lytic cell death of macrophages in response to mycobacterial infection; and affects Toll-like receptor recognition of mycobacteria (**Chapters 2 and 3**).

The selective autophagy receptors Optineurin and p62 are required for autophagic targeting of mycobacteria

Recently, the relevance of autophagic defense mechanisms in host immune responses against Mtb infection has been challenged [27, 28]. These reports propose that Mtb utilises virulence mechanisms to suppress autophagic defense mechanisms and that a potential host beneficial function of autophagy factors depends on their role in other processes. Furthermore, mutation of the selective autophagy receptor p62 in mice did not affect the outcome of Mtb infection in this work [27]. In contrast, other studies in mice and zebrafish demonstrated that p62 is required for autophagic targeting of mycobacteria *in vitro* and *in vivo* [4, 5]. Here, we made zebrafish CRISPR/Cas9 loss-of-function mutants of p62 and another selective autophagy receptor, Optineurin, which, like p62, shows induced gene expression during Mm infection in our model. We found that both p62 and Optineurin restrict mycobacterial growth by sequestering

ubiquitinated bacteria to Lc3-positive vesicles (**Chapter 4**). Our findings provide *in vivo* confirmation of the importance of selective autophagy as an innate immune defense mechanism against intracellular mycobacterial pathogens. To the best of our knowledge, this is also the first time that a host protective function for Optineurin has been shown in defense against mycobacterial infection (**Chapter 4**).

Since a combined silencing of p62 and Optineurin did not have an additive effect on the mycobacterial burden compared to single mutation of p62 or Optineurin, we suspect that these receptors are part of the same defense pathway and are mutually dependent in promoting autophagic targeting of ubiquitinated cytosolic mycobacteria. Overexpression of either p62 or Optineurin increased host resistance to mycobacterial infection. To further investigate a potential mutual dependent relationship for these ubiquitin-binding receptors, it will be interesting to overexpress p62 in the Optineurin knock out situation and *vice versa*. If the two receptors truly depend on each other for their host protective effect, overexpression of one of the two will not have a beneficial effect in the absence of the other.

Optineurin and p62 have also been linked to the regulation of cell death [29, 30]. Another important question stemming from our studies is whether Optineurin or p62 are involved in cell death processes during mycobacterial infection. And if so, is this part of the same process of cell death modulation as Dram1? Collectively, this study provided *in vivo* evidence that p62 is involved in autophagic defense against mycobacteria during the critical early steps in the infection process when macrophages are parasitized and granuloma formation is initiated. During this phase, host defense relies on the function of the innate immune system. A similar situation occurs in patients with a compromised adaptive immune system and therefore these patients might benefit from stimulating the autophagic host defense mechanisms. In addition to consolidating the function of p62 in anti-mycobacterial host defense, our study provides the first evidence for a similar role for Optineurin in ubiquitin-dependent autophagic targeting of mycobacteria.

Conclusion

The proper control of cell death during mycobacterial infection is a critical factor in the battle between the host immune system and these pathogens [32]. Despite recent controversies, the current view remains that autophagy is an important host defense mechanism to restrict replication of mycobacteria inside immune cells [4, 6, 27]. Dying infected macrophages release a large number of mycobacteria which are disseminated into other tissues by newly recruited macrophages [3]. Since mycobacterial proliferation predominantly occurs inside immune cells, modulation of the cell-mediated immune response is a promosing potential target for host-directed therapy to control TB or other mycobacterial infections [33]. Based on our studies we propose that the defense pathway mediated by Dram1 is a promising target for host-directed therapy against TB by stimulating autophagic defense and preventing lytic cell death of infected immune macrophages (Chapter 2 and Chapter 3). The current bottleneck is to figure out how to pharmacologically stimulate Dram1/DRAM1 in animal models or human patients. A possible approach would be to directly inject DRAM1 recombinant protein into TB patients to artificially elevate DRAM1 protein levels. DRAM1 protein might directly participate in defense against Mtb. However, it remains unsure whether DRAM1 can be delivered to the appropriate membrane location in infected cells to exert its function. Thus, the more practical approach is to study mechanisms of DRAM1 activation in *in vivo* and *in vitro* models for TB (and other members of the DRAM family), to screen for endogenous modulators or upstream partners that can be used as drugable targets to activate the host defense pathway mediated by DRAM1.

The zebrafish *dram1* knockout mutants generated and characterized here (**Chapter 2)** revealed that deficiency in Dram1 results in uncontrolled mycobacterial infection not only due to impaired maturation of mycobacteria containing vesicles and defects in autophagic targeting, but also due to increased pyroptotic cell death of infected macrophages. In **Chapter 3**, we provide a fundamental characterization of the effect of Dram1 deficiency on the gene expression profiles in health and during mycobacterial infection, which revealed effects on metabolic pathways, lytic cell death, and Toll-like receptor signaling. Finally, in **Chapter 4** we demonstrate that the selective autophagy receptors Optineurin and p62 function in innate host

defense against mycobacterial infection by targeting ubiquitinated bacteria to autophagic compartments. Overall, this thesis presents new *in vivo* evidence for the important function of selective autophagy to inhibit mycobacterial proliferation inside macrophages. Furthermore, the results support that stimulating the innate host defense processes that are dependent on Dram1, p62 and Optineurin could be a useful strategy to explore for adjunctive treatment of TB patients.

References

1. Flynn JL, Chan J. IMMUNOLOGY OF TUBERCULOSIS. Annu Rev Immunol 2001; 19:93-129.
2. Meijer AH. Protection and pathology in TB: learning from the zebrafish model. Semin Immunopathol 2016; 38:261-73.
3. Davis JM, Ramakrishnan L. The role of the granuloma in expansion and dissemination of early tuberculous infection. Cell 2009; 136:37-49.
4. Watson RO, Manzanillo PS, Cox JS. Extracellular M. tuberculosis DNA targets bacteria for autophagy by activating the host DNA-sensing pathway. Cell 2012; 150:803-15.
5. van der Vaart M, Korbee CJ, Lamers GE, Tengeler AC, Hosseini R, Haks MC, et al. The DNA damage-regulated autophagy modulator DRAM1 links mycobacterial recognition via TLR-MYD88 to autophagic defense [corrected]. Cell Host Microbe 2014; 15:753-67.
6. Deretic V. Autophagy in leukocytes and other cells: mechanisms, subsystem organization, selectivity, and links to innate immunity. J Leukoc Biol 2016; 100:969-78.
7. Crighton D, Wilkinson S, O'Prey J, Syed N, Smith P, Harrison PR, et al. DRAM, a p53-induced modulator of autophagy, is critical for apoptosis. Cell 2006; 126:121-34.
8. Mrschtik M, Ryan KM. Another DRAM involved in autophagy and cell death. Autophagy 2016; 12:603-5.
9. Laforge M, Limou S, Harper F, Casartelli N, Rodrigues V, Silvestre R, et al. DRAM triggers lysosomal membrane permeabilization and cell death in CD4(+) T cells infected with HIV. PLoS Pathog 2013; 9:e1003328.
10. Galavotti S, Bartesaghi S, Faccenda D, Shaked-Rabi M, Sanzone S, McEvoy A, et al. The autophagy-associated factors DRAM1 and p62 regulate cell migration and invasion in glioblastoma stem cells. Oncogene 2013; 32:699-712.
11. Humbert M, Mueller C, Fey MF, Tschan MP. Inhibition of damage-regulated autophagy modulator-1 (DRAM-1) impairs neutrophil differentiation of NB4 APL cells. Leuk Res 2012; 36:1552-6.
12. Kim JK, Lee HM, Park KS, Shin DM, Kim TS, Kim YS, et al. MIR144* inhibits antimicrobial responses against Mycobacterium tuberculosis in human monocytes and macrophages by targeting the autophagy protein DRAM2. Autophagy 2017; 13:423-41.

13. Bai S, Tian B, Li A, Yao Q, Zhang G, Li F. MicroRNA-125b promotes tumor growth and suppresses apoptosis by targeting DRAM2 in retinoblastoma. Eye (Lond) 2016; 30:1630-8.

14. Mrschtik M, O'Prey J, Lao LY, Long JS, Beaumatin F, Strachan D, et al. DRAM-3 modulates autophagy and promotes cell survival in the absence of glucose. Cell Death Differ 2015; 22:1714-26.

15. Chung J, Nakatsu F, Baskin JM, De Camilli P. Plasticity of PI4KIIIalpha interactions at the plasma membrane. EMBO Rep 2015; 16:312-20.

16. Zhang XD, Qi L, Wu JC, Qin ZH. DRAM1 regulates autophagy flux through lysosomes. PLoS One 2013; 8:e63245.

17. O'Prey J, Skommer J, Wilkinson S, Ryan KM. Analysis of DRAM-related proteins reveals evolutionarily conserved and divergent roles in the control of autophagy. Cell Cycle 2009; 8:2260-5.

18. Park SM, Kim K, Lee EJ, Kim BK, Lee TJ, Seo T, et al. Reduced expression of DRAM2/TMEM77 in tumor cells interferes with cell death. Biochem Biophys Res Commun 2009; 390:1340-4.

19. Srinivasan L, Ahlbrand S, Briken V. Interaction of Mycobacterium tuberculosis with host cell death pathways. Cold Spring Harb Perspect Med 2014; 4.

20. Martin CJ, Booty MG, Rosebrock TR, Nunes-Alves C, Desjardins DM, Keren I, et al. Efferocytosis is an innate antibacterial mechanism. Cell Host Microbe 2012; 12:289-300.

21. Behar SM, Martin CJ, Booty MG, Nishimura T, Zhao X, Gan HX, et al. Apoptosis is an innate defense function of macrophages against Mycobacterium tuberculosis. Mucosal Immunol 2011; 4:279-87.

22. Stutz MD, Clark MP, Doerflinger M, Pellegrini M. Mycobacterium tuberculosis: Rewiring host cell signaling to promote infection. J Leukoc Biol 2018; 103:259-68.

23. Galluzzi L, Vitale I, Aaronson SA, Abrams JM, Adam D, Agostinis P, et al. Molecular mechanisms of cell death: recommendations of the Nomenclature Committee on Cell Death 2018. Cell Death Differ 2018; 25:486-541.

24. Wallach D, Kang TB, Dillon CP, Green DR. Programmed necrosis in inflammation: Toward identification of the effector molecules. Science 2016; 352:aaf2154.

25. Nishiya T, DeFranco AL. Ligand-regulated chimeric receptor approach reveals distinctive subcellular localization and signaling properties of the Toll-like receptors. J Biol Chem 2004; 279:19008-17.

26. Means TK, Jones BW, Schromm AB, Shurtleff BA, Smith JA, Keane J, et al. Differential Effects of a Toll-Like Receptor Antagonist on Mycobacterium tuberculosis-Induced Macrophage Responses. The Journal of Immunology 2001; 166:4074-82.

27. Kimmey JM, Huynh JP, Weiss LA, Park S, Kambal A, Debnath J, et al. Unique role for ATG5 in neutrophil-mediated immunopathology during M. tuberculosis infection. Nature 2015; 528:565-9.

28. Behar SM, Baehrecke EH. Autophagy is not the answer. NATURE 2015; 528:482-3.

29. Fan L, Yin S, Zhang E, Hu H. Role of p62 in the regulation of cell death induction. Apoptosis 2018; 23:187-93.

30. Slowicka K, van Loo G. Optineurin Functions for Optimal Immunity. Front Immunol 2018; 9:769.

31. Shaid S, Brandts CH, Serve H, Dikic I. Ubiquitination and selective autophagy. Cell Death Differ 2013; 20:21-30.

32. Mahamed D, Boulle M, Ganga Y, Mc Arthur C, Skroch S, Oom L, et al. Intracellular growth of Mycobacterium tuberculosis after macrophage cell death leads to serial killing of host cells. Elife 2017; 6.

33. Kolloli A, Subbian S. Host-Directed Therapeutic Strategies for Tuberculosis. Front Med (Lausanne) 2017; 4:171.