***Mycobacterium tuberculosis* and HIV coinfection**

Effects on innate immunity and strategies to
boost the immune response

Anna-Maria Andersson

Linköping University Medical Dissertation
No. 1659

***Mycobacterium tuberculosis* and HIV coinfection**

Effects on innate immunity and strategies to boost the
immune response

Anna-Maria Andersson

Department of Clinical and Experimental Medicine (IKE)

Division of Microbiology and Molecular Medicine

Faculty of Medicine and Health Sciences

Linköpings universitet, SE-581 83 Linköping, Sweden

Linköping 2019

© Anna-Maria Andersson 2019

Cover: Confocal microscopy images of a macrophage that have ingested *Mycobacterium tuberculosis* (green) and remnants of apoptotic neutrophils (yellow).

Paper I and II are reprinted with permission from the respective publishers

ISSN 0345-0082

ISBN 978-91-7685-142-5

Printed by LiU-Tryck, Linköping 2019

Supervisor

Robert Blomgran, Linköping University, Sweden

Co-supervisors

Marie Larsson, Linköping University, Sweden

Olle Stendahl, Linköping University, Sweden

Faculty opponent

Susanna Brighenti, Karolinska Institutet, Sweden

ABSTRACT

Tuberculosis (TB) still remains a big threat today, being the leading cause of death by a single infectious agent. The TB epidemic is fueled by HIV along with the increasing drug-resistance which prolongs the already long treatment duration and decreases the success rate for curing TB. In most cases an infection results in latency but HIV patients have a 20-30 times higher risk of developing active TB. There are around 36.9 million people living with HIV globally, with the highest burden in Africa. Although there are effective treatments against the disease, there is no cure for AIDS and the availability of the lifelong treatment is limited in low-income countries where the burden is highest. HIV infection causes an immunodeficiency characterized by the progressive loss of CD4 T cells which increases the risk of opportunistic infections, and infection by *Mycobacterium tuberculosis* (Mtb), the causative agent of TB. Mtb spreads through aerosols from one person with active tuberculosis to a healthy person. Upon inhalation the bacteria are phagocytosed by alveolar macrophages that secrete cytokines and chemokines to recruit more cells, such as dendritic cells, macrophages and lymphocytes, leading to the formation of a granuloma. During a single TB infection the bacteria are usually contained within the granuloma, but HIV can disrupt the stable granuloma, causing a rupture and dissemination of Mtb. This inflammatory site is also beneficial to HIV since it promotes replication of the virus within infected cells. HIV and Mtb are two successful intracellular pathogens able to avoid immune defense mechanisms both of the innate and adaptive immunity in order to persist and replicate. Their virulence factors can manipulate or inhibit cell signaling, phagosome maturation, autophagy, ROS production, apoptosis and antigen presentation, to promote survival. Boosting of immune defenses with host-directed therapies (HDT) has been proposed as a treatment strategy against TB, either alone or adjunctive to the current regimen.

In this thesis, ways to boost the innate immune responses in Mtb and HIV coinfecting macrophages were investigated, along with studies of the effect of HIV on Mtb antigen presentation in coinfecting dendritic cells. The initial hypothesis was that autophagy induction through inhibition of mammalian target of rapamycin (mTOR) could suppress Mtb growth in HIV coinfecting macrophages. However, during a low grade infection, autophagy induction increased Mtb replication due to a decreased autophagic flux and acidification of Mtb phagosomes. A general autophagic flux was induced, although not localized to the Mtb phagosomes, thus not inducing a xenophagy (autophagy of intracellular pathogens). Other ways of inducing autophagy or boosting the response in coinfecting macrophages might be more beneficial and therefore the effect of efferocytosis was investigated. Uptake of apoptotic neutrophils by coinfecting macrophages did not induce autophagy but enhanced the control of Mtb by other means. Upon efferocytosis, the macrophages acquired active myeloperoxidase (MPO) from the neutrophils that suppressed Mtb growth. The coinfecting macrophages also produced more ROS after efferocytosis. The inhibition of Mtb growth could thus be mediated by MPO and the increased ROS production either directly or indirectly.

The possibility to boost the innate immunity could prove to be important during an HIV coinfection, when the adaptive immunity is deficient. In addition to the well-known decline in CD4 T cells during the course of HIV progression, we found that HIV infection of dendritic cells inhibited antigen presentation by suppressing the expression of HLA-DR and co-stimulatory molecules on coinfecting dendritic cells. Furthermore, HIV reduced secretion of pro-inflammatory cytokines and suppressed antigen processing through inhibition of autophagy. This impaired antigen presentation in coinfecting dendritic cells resulted in a decreased activation and response of Mtb-specific CD4 T cells.

In conclusion, this thesis shows how HIV can manipulate antigen presentation in Mtb coinfecting dendritic cells and subsequently inhibit the adaptive immune response. It also contributes to insights on how efferocytosis of apoptotic neutrophils can boost the innate immune responses during coinfection. Lastly, autophagy induction through mTOR inhibition does not enhance protection against TB. Induction of autophagy should therefore be handled with care, particularly during HIV coinfection.

POPULÄRVETENSKAPLIG SAMMANFATTNING

Tuberkulos orsakas av bakterien *Mycobacterium tuberculosis* (Mtb) som överförs från en infekterad person till en frisk person via aerosoler från hostningar eller nysningar. När bakterien når lungorna tas den upp av specialiserade immunceller, så kallade lungmakrofager. Dessa i sin tur skickar ut signalmolekyler för att rekrytera fler immunceller som tillsammans avskärmar bakterierna genom att bilda ett så kallat granulom. Oftast resulterar detta i en latent infektion och patienten upplever inga symptom. I 5-10 % av fallen blir granulomet instabilt, vilket leder till spridning av bakterierna och utveckling av aktiv tuberkulos med medföljande symptom. HIV patienter har 20-30 gånger större risk att utveckla aktiv tuberkulos, då detta virus orsakar instabilitet av granulomet. HIV som är orsaken till AIDS påverkar det adaptiva immunförsvaret genom att minska mängden T celler som är viktiga i försvaret mot mykobakterie-infektioner. En samtidig infektion med tuberkelbakterien och HIV är en dödlig kombination då båda patogenerna förvärrar varandras sjukdomsförlopp. Behandlingstiden för båda sjukdomarna är väldigt lång och i fallet med HIV så finns det inget botemedel utan det kräver livslång behandling. Det är inte heller alla som har tillgång till behandling, framför allt inte i de afrikanska länder där HIV främst förekommer. Detsamma gäller tuberkulos, men det som främst försvårar behandlingen är utvecklingen av resistens som kräver andra antibiotika under en längre period än det normala 6 månader. På grund av detta kvarstår tuberkulos och HIV som globala hälsopotentialer även idag. Tuberkulos är den ledande dödsorsaken från en enskild patogen och ca 2 miljarder människor uppskattas vara infekterade. År 2017 dog 1.6 miljoner människor av tuberkulos varav 300 000 personer var HIV positiva.

Både HIV och tuberkelbakterien är framgångsrika patogener som kan överleva genom att manipulera olika processer och signalvägar i immunceller. Exempel på försvarsmekanismer i infekterade immunceller är fagosomal mognad och autofagi som båda resulterar i en sänkning av pH inuti vakuolen där patogenen befinner sig (fagosomen). Dessutom kan immuncellen initiera en kontrollerad celledöd (apoptos) och dessa döda celler kan sedan tas om hand av andra immunceller, i en process som kallas efferocytos. Aktivering av det adaptiva immunförsvaret genom antigenpresentation är ytterligare en försvarsmekanism för att eliminera patogener. Försök att stimulera dessa immunsvår skulle kunna förbättra avdödandet av tuberkelbakterien, och kan vara ett bra behandlingsalternativ, särskilt i kombination med nuvarande antibiotikabehandling.

I denna avhandling har syftet varit att undersöka olika sätt att stimulera det medfödda immunförsvaret och utreda hur HIV kan påverka immunceller för att minska immunsvaret mot tuberkelbakterien. Autofagistimulering studerades som ett sätt att förbättra eliminering av tuberkelbakterien i HIV/Mtb dubbelinfekterade makrofager. Generell autofagistimulering ökade inte eliminationen av tuberkelbakterien utan resulterade i ökad tillväxt av bakterien på grund av hämmad surgörning i fagosomen. Vi undersökte även om autofagistimulering kan åstadkommas på andra mer förmånliga sätt, såsom vid efferocytos. Apoptotiska immunceller (neutrofila granulocyter) som togs upp av HIV/Mtb dubbelinfekterade makrofager hämmade tillväxten av tuberkelbakterien, men utan att stimulera autofagi. Dessa celler producerade

istället mer syreradikaler med hjälp av ett enzym från granulocyterna och stimulerade därmed avdödning av tuberkelbakterien i HIV/Mtb dubbelinfekterade makrofager. Denna möjlighet till förstärkning av det medfödda immunförsvaret mot tuberkelbakterien kan vara särskilt viktig vid samtidig HIV infektion när det adaptiva immunförsvaret är bristfälligt.

Utöver den välkända progressiva förlusten av T celler, upptäcktes även att HIV kan manipulera andra immunceller (dendritiska celler) och därmed minska det adaptiva immunsvaret mot tuberkelbakterien. Dendritiska cellers uppgift är att presentera antigen (del av patogen som kan stimulera ett immunsvaret) för celler tillhörande det adaptiva immunförsvaret (T celler). HIV hämmade denna antigenpresentation genom att dämpa autofagiprocessen, i den dendritiska cellen. Detta resulterade i ett minskat T cell svar mot tuberkelbakterien.

Sammanfattningsvis så innehåller denna avhandling information om hur HIV kan hämma antigenpresentation i infekterade dendritiska celler, och därmed dämpa det adaptiva immunsvaret mot tuberkelbakterien. Avhandlingen bidrar också till insikter i hur det medfödda immunförsvaret kan förstärkas för att öka skyddet mot tuberkelbakterien och varför stimulering av autofagi bör undvikas vid HIV/Mtb dubbelinfektion.

LIST OF PAPERS

Paper I

Andersson AM, Andersson B, Lorell C, Raffetseder J, Larsson M, Blomgran R. Autophagy induction targeting mTORC1 enhances *Mycobacterium tuberculosis* replication in HIV co-infected human macrophages. Sci Rep. 2016 Jun 15;6:28171. doi: 10.1038/srep28171.

Paper II

Singh SK, **Andersson AM**, Ellegård R, Lindestam Arlehamn CS, Sette A, Larsson M, Stendahl O, Blomgran R. HIV Interferes with *Mycobacterium tuberculosis* Antigen Presentation in Human Dendritic Cells. Am J Pathol. 2016 Dec;186(12):3083-3093. doi: 10.1016/j.ajpath.2016.08.003. Epub 2016 Oct 13.

Paper III

Andersson AM, Larsson M, Stendahl O, Blomgran R. The enhanced control of *Mycobacterium tuberculosis* in HIV coinfecting macrophages by apoptotic neutrophils is myeloperoxidase dependent. Submitted

ABBREVIATIONS

ABAH	4-Aminobenzoic acid hydrazide
AIDS	Acquired immune deficiency syndrome
APC	Antigen presenting cell
ART	Antiretroviral therapy
Atg	Autophagy related
BCG	Bacille Calmette-Guérin
CFP-10	10 kDa culture filtrate protein
cGAS	Cyclic GMP-AMP synthase
CLR	C-type lectin receptor
CR	Complement receptor
CTL	Cytotoxic T lymphocytes
DC	Dendritic cell
DC-SIGN	Dendritic cell-specific intercellular adhesion molecule-3 grabbing non-integrin
EEA1	Early endosome antigen 1
Eis	Enhanced intracellular survival
ESAT-6	6 kDa early secreted antigenic target
ESX-1	ESAT-6 secretion system-1
FasL	Fas ligand
fMLP	N-formyl-methionyl-leucyl-phenylalanine
HIV	Human immunodeficiency virus
IFN	Interferon
IRIS	Immune reconstitution inflammatory syndrome
LAM	Lipoarabinomannan
LAMP	Lysosomal-associated membrane proteins
LC3	Microtubule-associated protein light chain 3
LM	Lipomannan
LpdC	Lipoamide dehydrogenase C
ManLAM	Mannose-capped lipoarabinomannan
MAPK	Mitogen-activated protein kinases
MDR-TB	Multi-drug resistant TB
MHC	Major histocompatibility complex
Mincle	Macrophage-inducible C-type lectin
MOI	Multiplicity of infection
MPO	Myeloperoxidase
MR	Mannose receptor
Mtb	<i>Mycobacterium tuberculosis</i>
MTBC	<i>Mycobacterium tuberculosis</i> complex
mTORC1	Mammalian target of rapamycin complex 1
MyD88	Myeloid differentiation primary response protein 88
NADPH	Nicotinamide adenine dinucleotide phosphate
Ndk	Nucleoside diphosphate kinase

Nef	Negative regulatory factor
NETs	Neutrophil extracellular traps
NFκB	Nuclear factor kappa-light-chain-enhancer of activated B cells
NLR	Nucleotide oligomerization domain-like receptor
NNRTI	Non-nucleoside reverse transcriptase inhibitor
NOD	Nucleotide oligomerization domain
NOX	Nicotinamide adenine dinucleotide phosphate oxidase
NRTI	Nucleoside reverse transcriptase inhibitors
PDIM	Phthiocerol dimycocerosates
PI3K	Phosphatidylinositol 3-kinase
PI3P	Phosphatidylinositol-3-phosphate
PMA	Phorbol 12-myristate 13-acetate
POA	Pyrazinoic acid
PPD	Purified protein derivative
PR	Protease
PRR	Pattern recognition receptor
RD1	Region of difference 1
ROS	Reactive oxygen species
RT	Reverse transcriptase
SOD	Superoxide dismutase
SR	Scavenging receptor
SQSTM1	Sequestosome 1
T7SS	Type VII secretion systems
TACO	Tryptophan-aspartate containing coat protein
Tat	Trans-activator of transcription
TB	Tuberculosis
TCR	T cell receptor
TDM	Trehalose dimycolate
Th cell	T helper cell
TLR	Toll-like receptor
TNF	Tumor necrosis factor
TRAIL-R	TNF-related apoptosis-inducing ligand receptor
Treg	Regulatory T cells
ULK1	Unc-51-like kinase 1
Vif	Virion infectivity factor
Vpr	Viral protein R
Vpu	Viral protein unique
XDR-TB	Extensively drug-resistant tuberculosis

TABLE OF CONTENTS

ABSTRACT i

POPULÄRVETENSKAPLIG SAMMANFATTNING iii

LIST OF PAPERS v

ABBREVIATIONS vi

BACKGROUND 1

Tuberculosis 1

Epidemiology 1

Mycobacterium tuberculosis 1

 Virulence factors 2

Pathogenesis 2

Symptoms and diagnosis 4

Treatment strategies 5

Pathogenesis 6

The HIV particle 7

HIV life cycle 8

Treatment and prevention 8

HIV and *Mycobacterium tuberculosis* coinfection 9

Innate immune cells 11

Macrophages 11

Dendritic cells 11

Neutrophils 12

Sensing of Mtb/HIV and signaling responses 12

Phagocytosis and phagosome maturation 14

Autophagy 15

Reactive oxygen species 17

Apoptosis 19

Activation of adaptive immunity 20

Antigen presentation 20

 Effects of Mtb and HIV on antigen presentation 21

T cell responses 22

AIMS 25

RESULTS27

Paper I27

Autophagy induction targeting mTORC1 enhances Mycobacterium tuberculosis replication in HIV co-infected human macrophages.....27

Paper II29

HIV interferes with Mycobacterium tuberculosis antigen presentation in human dendritic cells.....29

Paper III31

The enhanced control of Mycobacterium tuberculosis in HIV coinfecting macrophages by apoptotic neutrophils is myeloperoxidase dependent.31

DISCUSSION33

GENERAL CONCLUSIONS37

REFERENCES39

ACKNOWLEDGEMENTS57

BACKGROUND

Tuberculosis

Tuberculosis (TB) is an ancient disease that has caused several epidemics throughout history, with signs of the disease found in Egyptian mummies. The genus *Mycobacterium* is believed to have existed for about 150 million years, with *Mycobacterium tuberculosis* (Mtb) originating around 15 000 years ago. In 1882, the German microbiologist Robert Koch was the first to discover the causative agent of TB, which was later named *Mycobacterium tuberculosis*. Although being an old disease it took a long time before the realization that it was a transmittable disease which was caused by a bacteria. With these important findings the spread of TB could be prevented and was halted further by the discovery of a vaccine that was started being used in children in 1921. Albert Calmette and Camille Guérin successfully attenuated *Mycobacterium bovis* to create this vaccine called bacille Calmette-Guérin (BCG). Later came the discovery of treatments for tuberculosis with the antibiotics streptomycin in 1944 and isoniazid in 1952. (1). However, TB remains a big threat fueled by the HIV epidemic and the growing drug resistance problem.

Epidemiology

According to the latest TB report from the World Health Organization (WHO), TB is the top ten cause of death worldwide and the leading cause of death by a single infectious agent. 1.7 billion people are estimated to be infected with TB, of which only around 5-10% will develop active disease. The biggest risk factor for developing active TB is HIV (20-30 times higher risk), but other examples are undernutrition, diabetes, smoking and alcohol consumption. In 2017 an estimate of 10 million people fell ill with TB and 1.3 million people died of the disease with additionally 300 000 deaths among HIV coinfecting people. The following countries had the highest incidence rates: India, China, Indonesia, the Philippines, Pakistan, Nigeria, Bangladesh and South Africa, which together accounted for two thirds of the new TB cases in 2017. Furthermore the multi-drug resistant TB (MDR-TB) burden is highest in India, China and the Russian Federation and around 8.5% of MDR-TB cases had extensively drug-resistant TB (XDR-TB) in 2017 (2).

Mycobacterium tuberculosis

There are several species of *Mycobacterium* of which most are environmental bacteria. Human tuberculosis is mainly caused by members of the *Mycobacterium tuberculosis* complex (MTBC) but in immune-compromised individuals also non-tuberculous mycobacteria can cause disease. MTBC can be divided into human-adapted MTBC and animal-adapted MTBC. The human-adapted MTBC consists of *M. tuberculosis sensu stricto* (with the phylogenetic lineages L1, L2, L3, L4, L7) and *M. africanum* (with lineages L5 and L6). Examples of

some members of animal-adapted MTBC are *M. bovis*, *M. caprae*, *M. microti* and *M. pinnipedii* (3).

Mtb is a slow-growing bacterium with a generation time of around 24h, forming colonies on agar after around 3-4 weeks. Mtb is a rod-shaped non-motile intracellular bacteria about 1-4µm in length. It has a robust and thick cell wall that shields it from the environment and limits drug permeability. Due to its unique cell wall, Mtb is classified as an acid fast bacterium, meaning that it resists decolorization by acidic alcohol and can be visualized through Ziehl–Neelsen acid-fast staining (4). The complex cell envelope consists of a bilayer phospholipid plasma membrane surrounded by a layer of peptidoglycans that attaches the arabinogalactans that are linked with mycolic acid. The envelope is surrounded by a capsule mainly containing polysaccharides. The mycolic acids are responsible for the low permeability of the cell envelope and are essential for Mtb survival (5).

Virulence factors

Lipomannan (LM), lipoarabinomannan (LAM) and mannose-capped lipoarabinomannan (manLAM) are lipoglycans within the cell wall of Mtb which are important for virulence. ManLAM can bind to mannose receptors on macrophages and dendritic cells and DC-SIGN on dendritic cells and can promote the uptake of Mtb but also inhibit a pro-inflammatory response and phagosomal maturation (5). Furthermore, the cell wall lipid phthiocerol dimycocerosates (PDIM) has been shown to contribute to phagosomal escape of Mtb (6). Apart from components of the cell wall, Mtb also secrete some virulence factors. The genome of mycobacteria encodes for five type VII secretion systems (T7SS) of which the ESAT-6 secretion system-1 (ESX-1) is the most important, secreting the two major virulence factors; 6 kDa early secreted antigenic target (ESAT-6) and 10 kDa culture filtrate protein (CFP-10) that together form a heterodimer. ESX-1 is encoded by the genetic locus region of difference (RD1) which is deleted in the vaccine strain *Mycobacterium bovis* bacille Calmette-Guérin (BCG) and other attenuated strains of Mtb. The secretion of ESAT-6 is essential for virulence of Mtb and has been associated with phagosomal escape, cytolysis, necrosis and apoptosis (7). ESX-1 is also important for blocking phagosomal acidification and impairing autophagic flux. Other effectors that also have been associated with blocking of phagosomal maturation are PtpA, SapM and EsxH (8).

Pathogenesis

Mtb spreads through aerosols from cough of an individual with active tuberculosis to a new host that gets infected upon inhalation. Mtb first encounter alveolar macrophages that recognize the bacteria through different surface receptor such as scavenging receptors (SR), complement receptors (CR) and C-type lectin receptors (CLRs). These receptors mediate the uptake of the bacteria and initiate signaling pathways leading to production of cytokines and chemokines which promotes recruitment of more cells (9,10). The alveolar macrophages are not well equipped to control Mtb alone but by recruiting more macrophages, and other cells

such as dendritic cells (DCs) and lymphocytes to the site of infection the bacteria can be contained through the formation of a granuloma (9). The granuloma is an organized structure of cells which is the hallmark of tuberculosis. Typically, macrophages are localized in the middle together with some dendritic cells and neutrophils, which are all surrounded by lymphocytes (mainly T cells). The environment inside the granuloma is deprived of both oxygen and nutrients. The granulomas are very heterogeneous even within the same individual, but can be divided into three types; solid granulomas which contain Mtb (latency), necrotic granulomas that are found during early stages of active disease, and caseous granulomas found during severe tuberculosis when the bacteria disseminates (11,12). Solid granulomas are well organized and surrounded by a fibrotic wall, within which the Mtb burden is low. In necrotic granulomas the infected cells in the center of the granuloma have become necrotic forming a necrotic zone called caseum. Upon progression into caseous granulomas, the center becomes liquefied and the oxygen and nutrient supplies are reestablished promoting growth of Mtb (11).

Most of the macrophages involved in granuloma formation are the differentiated epithelioid macrophages. Furthermore, another characteristic cell type often found in granulomas are multinucleated giant cells (MGCs), which are formed when several macrophages fuse together. The MGCs are not able to phagocytose bacteria, but still has the capacity to present antigens (13). Mtb also induce the differentiation of macrophages into foamy macrophages, which accumulate lipids within intracellular lipid bodies or droplets. These cells also phagocytose poorly and lack bactericidal activities, instead it is believed that they provide Mtb with nutrients and allow the bacteria to persist in a dormant state (14). Although macrophages are abundantly found in granulomas, neutrophils are also recruited as the first line of defense. They get activated by LAM of Mtb and respond by producing oxygen radicals and secreting chemokines to recruit more leukocytes and to organize the granuloma. In addition to macrophages and neutrophils, dendritic cells are also found in granulomas and although these cells are less effective at phagocytosing and killing Mtb than macrophages, they control the infection through activation of lymphocytes. After ingestion of Mtb, the dendritic cells travel from the granuloma to the lymph nodes where they present antigens to T cells to mount a T-cell response. T cells are very important for the organization of the granuloma as well as for the control of the bacteria. The T cells can activate the macrophages through the secretion of cytokines (for example IFN- γ), making them better at controlling the infection (15).

There is still a question of whether or not the granuloma favors the host or the bacteria. On one hand Mtb is contained by the granuloma and the bacterial replication is suppressed, but on the other hand the bacteria have adapted to this environment and are able to persist for decades and can eventually cause a rupture leading to bacterial dissemination. In most cases the infection results in asymptomatic latency and the granuloma remains intact, however in 10% of the cases the granuloma gets compromised, leading to active tuberculosis (9,16). HIV patients have a higher risk of developing active disease due to the immunosuppression (2). It is believed that the lack of oxygen and nutrients in solid granulomas forces Mtb into dormancy

and that this non-replicating state is especially difficult to treat since most antibiotics target the replication machinery and thus a long treatment period is required (17).

Figure 1. Solid granuloma. The hallmark of tuberculosis is the granuloma, an organized structure of cells within which *Mtb* is contained. The innate immune cell types within the granuloma are macrophages, epithelioid macrophages, foamy macrophages, multinucleated giant cells, neutrophils and dendritic cells. These cells are surrounded by lymphocytes and an outer fibrotic wall.

Symptoms and diagnosis

TB can be asymptomatic, with more symptoms as the disease progresses. The initial symptoms can be fever, night sweats, fatigue and weight loss. Later the patient can develop a cough and mild hemoptysis, and in severe cases shortness of breath and chest pain (18). In order to treat the disease, a proper diagnosis has to be made to detect it. The diagnosis options for low-income countries where the TB burden is highest are limited. These countries mainly rely on the cheap and quick sputum smear microscopy, making it difficult to detect early stages of TB. However, additional diagnostic methods have been developed. WHO has recommended the rapid molecular test called Xpert MTB/RIF assay which gives the results within two hours in addition to detecting rifampicin resistance and being more accurate than sputum smear microscopy for diagnosis. The use of Xpert MTB/RIF has expanded greatly since 2010 when it was first recommended and it has great advantages to other tests such as the reference standard culture-based methods, which take up to 12 weeks for results (2). Another test is the tuberculin skin test (TST) that is based on the injection of purified protein derivative (PPD) into the skin which causes a hypersensitivity reaction within 48-72 hours in people with a mycobacterium-specific immunity. This test is used for diagnosis of latent TB, but with limited specificity since it cannot distinguish *Mtb* from other mycobacteria such as BCG (19). The TST could give a false negative result due to poor sensitivity in immune-compromised

individuals for example in HIV infected patients. Another more sensitive and specific test for latency, based on an individual's immune reaction is the interferon- γ release assay (IGRA), which measures the interferon- γ levels upon Mtb antigen (ESAT-6 and CFP-10) stimulation of mononuclear cells of the peripheral blood (20).

Treatment strategies

The treatment of TB includes a combination of the four first-line drugs isoniazid, rifampicin, ethambutol and pyrazinamide during two months followed by a four months treatment with only isoniazid and rifampicin (2,21). This treatment is successful for at least 85% of the cases with drug-susceptible TB. Drug resistant TB, including multidrug-resistant (MDR) or extensively drug-resistant (XDR) TB, requires a longer treatment period with more toxic drugs and for MDR-TB the success rate is only about 55% (2).

The effective TB drugs isoniazid and ethambutol target the cell wall of Mtb, more specifically the synthesis of the mycolic acids and arabinogalactan, respectively (22). Rifampicin blocks mRNA synthesis and subsequent protein synthesis by inhibiting the β -subunit of the DNA-dependent RNA polymerase of Mtb. Resistance is caused by mutations in the β -subunit, making it impossible for rifampicin to bind to it. Pyrazinamide is an important drug in the regimen since it kills semi-dormant Mtb that resides in an acidic environment, where other TB drugs cannot act. Pyrazinamide passively diffuses into the bacteria, where it is converted into its active form pyrazinoic acid (POA) by an Mtb enzyme that if mutated causes resistance. The POA is expelled out of the bacteria and can bind protons in the acidic environment that it can bring into the bacteria, causing acidification inside of Mtb, leading to inhibition of vital enzymes and disruption of membrane function (23).

There is currently only one existing vaccine, called BCG, but it only protects against severe childhood TB and not against pulmonary TB in adults (2). A new and improved vaccine is therefore warranted. Furthermore, the increasing drug resistance seems to be progressing faster than the development of new antibiotics and this has made researchers focus on how to improve the host immune response. Host-directed therapies (HDTs) might be a promising strategy to fight off the infection, either alone or as adjunctive treatment to the current regimen. HDTs can for example include drugs that either boost the immune defenses against TB, reduce the inflammatory response and tissue damage, or interfere with host mechanisms that are exploited by Mtb. Boosting of macrophage responses may include inducing production of free radicals, antimicrobial peptides, cytokines/chemokines, or inducing processes such as apoptosis, phagosomal maturation or autophagy (24). Examples of HDTs that have shown positive results *in vitro* or in mouse or zebrafish models are drugs that target the granuloma to increase antibiotic access, drugs that induce autophagy, decrease inflammation, or enhance T cell responses, or antibodies that target Mtb specifically. However, clinical studies using HDT agents such as vitamin D, the corticosteroid prednisolone or IFN- γ have shown controversial results (25) and the pursuit for effective HDTs continues. In the pipeline right now is a phase 2 trial (NCT02968927) investigating efficacy of adjunctive therapy with the TB HDT candidates Everolimus (mTOR inhibitor), Auranofin (antirheumatic

drug), Vitamin D3 and the novel anti-inflammatory compound CC-11050. Furthermore, the adjunctive potential of Ibuprofen (NCT02781909), N-acetyl cysteine (NCT03702738) and Pravastatin (NCT03456102) are also started to be investigated in clinical trials for treatment of TB.

HIV

Pathogenesis

Human immunodeficiency virus (HIV) is the cause of acquired immune deficiency syndrome (AIDS) which was first recognized in the 1980s. The first cases of the disease appeared in 1981 but the causative virus was not discovered until 1983 (26,27). AIDS is a severe disease that if left untreated leads to death, often by an opportunistic infection or *Mycobacterium tuberculosis* infection (28). HIV can be found in several body fluids of an infected individual, for example in blood, semen, vaginal and rectal secretions and in breast milk. The most common route of HIV transmission is through sexual intercourse but the virus can also be transmitted from mother to infant, or through blood upon needle sharing (29). During acute HIV infection, the risk of transmission is high due to the very high replication of the virus, peaking at around 2-4 weeks after infection. Patients can be experiencing unspecific symptoms such as headache, fever and rashes. This is followed by the chronic stage which can persist for years and be asymptomatic. It is characterized by lower virus replication and antibody production along with a gradual decline of CD4 T cells. The last stage, AIDS is characterized by a further reduction in CD4 T cells together with high viral load, causing an immunodeficiency that increases the risk of opportunistic infections. Diagnosis and monitoring of the disease is based on measurement of HIV antibodies, p24 antigen, viral load in plasma and CD4 count (28).

CD4 T cells are the primary target cells for HIV, but the virus can also infect other CD4-positive cells such as macrophages, dendritic cells and astrocytes (30). In addition to binding of CD4, the virus also binds to a chemokine co-receptor, either CCR5 or CXCR4, depending on the strain of HIV. R5 (M-tropic) virus is present during the whole course of the infection and typically binds to CCR5 on monocytes/macrophages and T cells, but cannot infect T cell lines since they only have CXCR4. X4 (T-tropic) virus, which is typically more prominent during the later stages of infection and is associated with disease progression, normally binds to CXCR4 on T cells and T cell lines. Whether or not this X4 virus can infect macrophages (which also express CXCR4) is controversial. Dual tropic virus (R5X4), as the name implies, can bind to both co-receptors. The virus can also switch from R5 to R5X4 or X4 as the disease progresses. T cells can be infected by any strain of HIV since they express both CCR5 and CXCR4. While CCR5 is mainly expressed on memory T cells, CXCR4 expression is more widespread but is mainly found on naïve T cells (31).

Immature dendritic cells at the mucosa, called Langerhans' cells, are believed to be the first cells to encounter HIV. They are important for the transfer of the virus to T cells, by migrating to the lymph nodes (31). Upon HIV transmission at the genital mucosa, the virus can either

be internalized by Langerhans' cells or it can directly infect intraepithelial CD4 T cells. The free virus or the infected cells can then make their way into the stroma where they either migrate to the lymph node or blood circulation, or make contact with dendritic cells, T cells and macrophages. The virus can either directly infect dendritic cells, become surface-bound to a C-type lectin receptor such as DC-SIGN or upon DC-SIGN binding become internalized into the endocytic compartment of these cells. In any case, the resulting accumulation of virus from dendritic cells efficiently passes to CD4 T cells across an infectious synapse. The interaction between the dendritic cell and the CD4 T cell also results in activation of the CD4 T cell, promoting gene transcription and HIV replication (32).

The HIV particle

The HIV virus particle is around 100 nm in diameter and consists of an envelope (env) with an associated matrix which surrounds the capsid that protects the inner core. The inner core contains two copies of single-stranded RNA genome, polymerase and viral enzymes (30,33). HIV belongs to the genus *Lentivirus*, within the family of *Retroviridae* meaning that the virus is a retrovirus which viral RNA is transcribed into DNA by the action of a reverse transcriptase (30). This transcription from RNA to DNA was first discovered in 1970 simultaneously and independently by Howard Temin and David Baltimore (34), having an important impact on future understanding of the HIV life cycle along with treatment development.

Figure 2. The HIV particle. The glycoproteins gp120 and gp41 are attached to the lipid bilayer creating the envelope of the HIV particle. Underneath the envelope is the matrix protein p17 which surrounds the capsid that contains enzymes and the genome consisting of two copies of single-stranded RNA molecules.

HIV life cycle

HIV enters the target cell by binding its glycoprotein gp120 of the envelope to the cell surface receptor CD4. Upon binding, the gp120 becomes rearranged, allowing subsequent binding to the chemokine co-receptors CCR5 or CXCR4. This is followed by the insert of the transmembrane glycoprotein gp41 of the envelope into the plasma membrane, creating a pore where the viral envelope and the plasma membrane fuses, allowing the viral capsid to enter the cytoplasm (33,35,36). Once the virion is uncoated, its RNA genome is reversed transcribed into double-stranded complementary DNA (cDNA) by viral reverse transcriptase (RT) and is transported into the nucleus by the help of viral protein R (Vpr). Once in the nucleus the cDNA gets incorporated into the host genome by the viral enzyme integrase (IN). In this stage the virus can remain latent, but upon activation of the host cell and induction of cell division, also the viral genome will be transcribed. The HIV genome carries nine genes that encodes for 19 proteins that can be divided into three classes; major structural proteins (encoded by Gag, Pol and Env), regulatory proteins (encoded by Tat (trans-activator of transcription) and Rev) and accessory proteins (encoded by Vif (virion infectivity factor), Vpr, Vpu (viral protein unique) and Nef (negative regulatory factor)). The mRNA translated proteins, along with newly transcribed copies of genomic RNA, all migrate to the cell surface for assembly of new virus particles. These viral particles bud off the plasma membrane after viral glycoproteins gp120 and gp41 have been incorporated, providing the lipid envelope. The final step is maturation of the virus particle, exerted by viral proteases (PR) that cleaves HIV Gag and Gag-Pol polyproteins into matrix protein (MA), capsid (CA), nucleocapsid (NC), PR, RT and IN, making the virus ready to infect new cells (33,36). It takes around 24 hours from that the HIV binds to the CD4 receptor until new virus particles are produced and released from the cell. However in long living cells like macrophages or memory T cells the virus can remain latent for years and only upon activation of these cells, infectious HIV can be produced (30). In tissues such as brain, lungs and liver, macrophages are the main reservoirs for HIV (31). Infected T helper (Th) cells on the other hand, have a shorter life span and are either lysed by the virus or by cytotoxic T lymphocytes (CTL), leading to the decrease of these cells and subsequent immunodeficiency (30).

Treatment and prevention

The first drug against HIV called zidovudine (ZDV) or azidothymidine (AZT) started being used already in 1987 and although effective, this monotherapy soon resulted in HIV resistance. The improved prognosis of the disease was only accomplished later with a treatment strategy that included several efficient drugs used in combination (27). Although effective antiretroviral therapy (ART) is available against HIV, the treatment is not a cure and cannot kill the virus but only suppresses it and therefore requires life-long treatment. ART can however increase the number of CD4 T cells in the blood and reduce the viral load, thereby greatly reducing the risk

of transmission and the patients can have a rather normal life on ART. ART targets several steps of the HIV life cycle in order to prevent viral replication (37). The standard regimes consist of 3 or more drugs in combination, including two nucleoside reverse transcriptase inhibitors (NRTI) together with a non-nucleoside reverse transcriptase inhibitor (NNRTI), protease inhibitor or integrase inhibitor, all disrupting different parts of the HIV life cycle. WHO are now, as first line regimen, recommending using two NRTI (Tenofovir and Lamivudine) together with the integrase inhibitor Dolutegravir as preferred to the previously used NNRTI Efavirenz (38). Within three months of treatment most patients have a great decrease in plasma viral load down to below the detection limit (37).

HIV infection is most common in Africa where 25.7 million people out of globally 36.9 million people are living with HIV (in 2017). Furthermore, the access to ART is limited especially in developing countries and in 2017 only 59% of the people globally living with HIV (21.7 million) received the treatment. This is a major issue since without ART, the infection easily transmits to healthy people, causing 1.8 million people to be newly infected and 0.9 people to die of the disease in 2017 (39). Therefore, prevention strategies has been employed in order to decrease the risk of transmission. Some examples are the use of male and female condoms, testing for HIV, voluntary medical male circumcision, antiretroviral medicines as pre-exposure prophylaxis (PrEP) for HIV-negative partners and post-exposure prophylaxis (PEP) within 72h of exposure to prevent infection. Prevention of mother to child transmission during pregnancy, labour, delivery or breastfeeding is achieved through antiretroviral drugs provided to both the mother and the baby (40).

Although effective treatment and prevention strategies, HIV remains a big threat due to the life-long treatment and the limited availability of ART and thus the best hope for eradicating the disease would be the development of a vaccine.

HIV and *Mycobacterium tuberculosis* coinfection

The HIV-caused immunosuppression creates a high risk for coinfections, most commonly with Mtb, either through reactivating latent TB or acquiring a new infection. The reduction in CD4 T cells caused by the virus likely contributes to the susceptibility to TB since these T cells are important in the control of TB. It is well-known that HIV impairs the control of Mtb infection and that active TB increases the risk of death in HIV infected individuals (41–43). An HIV co-infection affects the TB granuloma formation and appearance, seen by a reduction in lymphocytes and epithelioid macrophages along with larger areas of necrosis and higher Mtb burden (44). However, it's not only HIV that can promote replication of Mtb, but Mtb can also induce HIV replication and thus the coinfection accelerates the progression of both diseases. This Mtb-induced HIV replication was shown to be dependent on a simultaneous pro-inflammatory response (45), showing that activated cells support HIV replication. Further studies have confirmed an increase in cytokines in the pleural fluid compared to plasma of co-infected individuals and also discovered a higher viral replication in macrophages and lymphocytes in these Mtb located sites (46–48). HIV has also been shown to preferentially

replicate in Mtb specific CD4 T cells, leading to the depletion of these cells (49). In summary, the pro-inflammatory environment in Mtb located sites increases the replication of HIV, leading to a reduction in CD4 T cells with subsequent disruption of the granuloma and reduced control of Mtb, resulting in dissemination of both diseases.

Upon ART treatment the number of central and naïve CD4 T cells increase, along with Mtb specific T cells (50). However this treatment may not always be beneficial during coinfection and can lead to exacerbation of TB, resulting in the immune reconstitution inflammatory syndrome (IRIS). IRIS can be divided into two groups; paradoxical TB IRIS (patients receive TB treatment before ART) or unmasking TB IRIS (patients with latent or undiagnosed TB that becomes active upon receiving ART). The TB symptoms are believed to be enhanced by the dysregulated recovering immune responses with exaggerated inflammation and sustained Th1 responses against Mtb antigens, following ART (51,52). The granulomas can get disrupted by this, since they are dependent on a balance of pro- and anti-inflammatory cytokines for optimal control of Mtb.

In addition to infecting and decreasing CD4 T cell numbers, HIV can also infect macrophages and affect their function, reducing their phagocytic capacity by the action of Nef and inhibiting phagosomal maturation through Vpr (53–55). Thus, macrophages are a reservoir for both HIV and Mtb and the manipulation of these cells by either one of the pathogens can promote each other's replication during coinfection (56). The HIV protein Nef can for example inhibit apoptosis of Mtb infected macrophages (57), preventing engulfment of apoptotic bodies by surrounding macrophages that could kill the bacteria and present antigens to T cells to promote a T cell response (described later). Furthermore coinfection with Mtb leads to the activation of macrophages, resulting in production of pro-inflammatory cytokines and chemokines that are sustained by HIV inhibition of IL-10, causing a failure of immunoregulation (58,59). The cytokines and chemokines recruit and activate T cells, providing another niche for HIV to replicate within (59). Pro-inflammatory cytokines such as IL-6 and TNF- α promote HIV replication (60), through activation of the transcription factor NF- κ B that promotes transcription of the HIV integrated genome, leading to replication of HIV (59). Furthermore Mtb can increase the expression of CXCR4 in alveolar macrophages, favoring entry and replication of X4 viruses (61), as well as increase CXCR4 and CCR5 on CD4 T cells (62). In addition, HIV can promote Mtb replication by disrupting macrophages' microbicidal activity against Mtb, as well as decreasing antigen presentation (described later).

The following chapters will focus on the role of different immune defense mechanisms and how these are manipulated by HIV and Mtb, showing how the pathogens can evade the immune response and increase pathogenesis, making the coinfection so fatal.

Innate immune cells

Macrophages

Macrophages play a big role during Mtb pathogenesis since they are the primary cells to be infected in the lungs. Mtb is engulfed by alveolar macrophages which have good phagocytic ability but are weakly bactericidal with limited oxidative burst and poor antigen presenting capacity, creating a niche for the bacteria (63). Alveolar macrophages relocate from the airways to the lung interstitium upon Mtb infection and thereafter initiate dissemination to monocyte-derived macrophages and neutrophils (64). The main functions of alveolar macrophages are to maintain steady-state in the alveolar microenvironment by removing debris and dead cells, but they can also initiate an inflammatory response upon infection (65).

Macrophages, which are differentiated from circulating monocytes, have many different mechanisms for defending against Mtb, including phagosome acidification, autophagy, ROS and cytokine production. Macrophages are heterogeneous and can be polarized into the two main groups M1 or M2 macrophages depending on the environment. The classically activated M1 macrophages are induced by LPS, IFN- γ and TNF- α . They produce pro-inflammatory cytokines and are important during host response against intracellular bacteria such as Mtb. The alternatively activated M2 macrophages are induced by IL-4, IL-13, IL-10 and TGF- β . They are on the other hand immunosuppressive and poor antigen presenters (66). Alveolar macrophages cannot be classified into M1 or M2 since they exhibit characteristics of both phenotypes and exert both pro- and anti-inflammatory actions (65). Mtb can manipulate the polarization of macrophages in order to survive and replicate inside the cells. By inducing the production of IL-10 from Mtb infected macrophages, M2 polarization is promoted causing increased susceptibility to Mtb (67). Furthermore, Mtb can also induce the differentiation of macrophages into foamy macrophages, through ESAT-6 (68). These cells could serve as a reservoir for dormant Mtb (14), exhibiting reduced antigen processing capacity and increased secretion of TGF- β (66).

Dendritic cells

Dendritic cells (DCs) are important for Mtb antigen presentation and activation of the adaptive immunity, by migrating to the lymph nodes. Upon infection or uptake of antigens DCs go through a maturation process. Immature DCs are specialized in antigen capture and express high CCR1, CCR5 and CCR6, while expressing low CCR7 (lymph node homing molecule), CD40, CD54, CD80, CD83, CD86 and CD58. When the DCs have phagocytosed a pathogen/antigen they migrate to the draining lymph node while they undergo a maturation process. Their functions are now specialized for antigen processing and presentation and the capacity to capture antigens is downregulated. These mature DCs have a high surface expression of MHC II, along with the opposite low/high expression of the already mentioned markers (69). Mtb is able to modulate DC functions by for example impairing antigen

processing and preventing DC maturation (70) or on the contrary by promoting maturation and MHC expression before antigen processing and loading occurs (71).

Neutrophils

Neutrophils are important cells of the innate immunity since they are the first line of defense against pathogens. They are also the first cells to infiltrate the lungs upon Mtb infection (66). Their main functions are phagocytosis and killing of invading bacteria through several mechanisms such as degranulation, production of reactive oxygen species (ROS) and formation of neutrophil extracellular traps (NETs) (72). The neutrophils secrete ROS, elastase, collagenase and myeloperoxidase, all of which can damage both the bacteria and the host cells (73). Their role during TB is complex and they can contribute to both defense and tissue damage (66). However, depletion of neutrophils in whole blood leads to impaired growth restriction of Mtb due to lack of human neutrophil peptides (74), indicating the importance of these cells and their antimicrobial peptides in the defense against TB. Mtb infection of neutrophils leads to cell death, either by apoptosis (75,76) or necrosis (77). Apoptosis of neutrophils promotes their uptake by surrounding phagocytes in a process called efferocytosis, leading to inhibition of Mtb replication (78,79), while macrophage uptake of Mtb-induced necrotic neutrophils results in Mtb growth (77). ESAT-6 induced necrosis can also result in formation of neutrophil extracellular traps (NETs) with extruded DNA and granular proteins in a myeloperoxidase-dependent process (80,81). NETs function as a tool for host defense by trapping the pathogen and participating in the killing (73). Mtb-induced NETs can be phagocytosed by macrophages and activate a pro-inflammatory response (82).

Innate immune defense mechanisms and counterstrategies by the pathogens

Sensing of Mtb/HIV and signaling responses

Recognition of bacterial or viral pathogen-associated molecular patterns (PAMPs) induce activation of membrane-bound or cytosolic pattern recognition receptors (PRRs) (83). Surface receptors on cells that encounter pathogens can mediate signaling and uptake. The main families of surface receptors to interact with Mtb are Toll-like receptors (TLRs), c-type lectin receptors (CLRs) and scavenger receptors. The complement receptor 3 can also interact with Mtb and upon pathogen opsonization mediate its uptake (84). CLRs are a class of PRRs that upon recognition of Mtb mediate uptake and cytokine signaling. Examples of some CLRs are the mannose receptor (MR), Dectin-1, and DC-SIGN. The MR is one of the main Mtb receptors of macrophages, and facilitates uptake of Mtb, while DC-SIGN is abundantly expressed on DCs and is important for uptake of Mtb (83). The mycobacteria cell wall component ManLAM can upon binding to DC-SIGN cause impaired DC maturation and production of IL-10, thereby promoting immunosuppression (85). MR binding of LAM or ManLAM also induces an anti-inflammatory response along with inhibition of phagosome maturation (84). Dectin-1 induces

production of TNF- α , IL-6, IL-1 β , and IL-23 in DCs upon Mtb recognition which leads to Th17 generation (86). Yet another CLR is Macrophage-inducible C-type lectin (Mincle), a receptor essential for the recognition of Mtb's glycolipid trehalose dimycolate (TDM) which activates macrophages and promotes Th1 and Th17 polarization of T cells through DCs (87).

Other common PRRs are TLRs and Nucleotide oligomerization domain (NOD)-like receptors (NLRs), which upon activation induce production of pro-inflammatory cytokines such as TNF, IL-1 β and IL-12 that are important for elimination or control of pathogens. Both TLRs and NLRs activate multiple signaling pathways including NF- κ B and mitogen-activated protein kinases (MAPKs). TLR signaling can also lead to the assembly of the inflammasome (a multiprotein complex including NLR proteins) which activates caspase-1, which in turn cleaves pro-IL-1 β and pro-IL-18 into their active and secreted forms (88).

Mtb possess many agonists for TLRs, including TLR4 and TLR9, but mainly TLR2 (84). Mycobacterial cell wall lipids induce production of pro-inflammatory cytokines such as TNF in a TLR2 dependent manner (89). Many members of the TLR family are dependent on the adaptor protein myeloid differentiation primary response protein 88 (MyD88) for downstream signaling and a lack of this protein makes the host susceptible to Mtb (90). The role of TLRs in the protection against TB is complex and not all TLR signaling is beneficial for the host. Prolonged TLR2 signaling in macrophages by Mtb can downregulate some immune functions such as antigen presentation (91), however knockout of TLR2 results in susceptibility to Mtb (92). Furthermore Mtb can also manipulate the TLR2 signaling through its lipoprotein LprG, that induces TLR2 and causes inhibition of antigen processing in macrophages (93).

Secreted TNF and IL-12 upon TLR activation stimulate IFN- γ production mainly from natural killer (NK) cells and T cells (84). Both IFN- γ and TNF- α are important pro-inflammatory cytokines involved in controlling Mtb infection. They can either act in concert, or separately, activating macrophages to increase their antimicrobial activities. Too much TNF can however result in tissue damage and disease progression, and thus an optimal amount is needed for protection (94). During HIV coinfection, addition of TNF- α or IFN- γ increased the growth of Mtb in human macrophages, while they in HIV uninfected cells could control Mtb growth (95). IFN- γ can promote killing of Mtb through nitric oxide production, autophagy induction and phagosomal maturation, as seen in mouse macrophages (96–98), while IFN- γ stimulation of human macrophages can result in extracellular trap formation and necrosis, promoting Mtb growth in an ESX-1 dependent manner (99). Thus, the role of IFN- γ and TNF- α seems to differ depending on dose and cell type, but are generally believed to be important during Mtb infection.

The PRRs interferon inducible protein 16 (IFI16) and cyclic GMP-AMP synthase (cGAS) can recognize cytosolic DNA from Mtb or reversed transcribed HIV DNA if it is not successfully masked by the viral capsid in a complex with CypA (100,101). Upon DNA recognition, both PRRs activate STING that recruits the signaling cofactors TBK1 and IKK- α/β to activate the transcription factors IRF3 and NF- κ B. NF- κ B induces production of pro-inflammatory cytokines and chemokines. IRF3 can be suppressed by the viral proteins Vpu, Vif and Vpr but its

activation induces the expression of HIV restriction factors and type I IFN. Furthermore the produced IFN- α/β can bind to its receptor on HIV infected cells, leading to activation of the STAT1-STAT2 complex and IRF9, that induces the expression of anti-HIV IFN-stimulated genes (ISGs) encoding IFI16 and cGAS. Furthermore, HIV can also be recognized by endosomal TLR7 and TLR8 which can activate the NLRP3 inflammasome leading to the release of IL-1 β . This cytokine is a potent inducer of other pro-inflammatory cytokines that can activate and recruit innate immune cells (100). Although IFNs can be harmful to HIV, other cytokines can have a positive effect on viral replication, by activating infected cells. Upon HIV infection, the glycoprotein gp120 induces TNF- α secretion by macrophages, mediated by PI-3K and MAPK activation (102). TNF- α has been shown to promote HIV transcription through induction of NF- κ B (103). Several HIV proteins (Vpr, Tat and Nef) can mimic TNF signaling in HIV infected cells and thereby also promote HIV replication (104,105).

Phagocytosis and phagosome maturation

Mtb can bind to a number of receptors, which will influence and determine the ability of the macrophages to control the infection. By interaction with certain receptors, Mtb can promote its own survival even before entering the phagosome (84). Upon binding to surface receptors, phagocytosis is initiated, leading to the generation of a phagosome where the pathogen resides. Once in the phagosome, the bacteria will experience a gradual decrease of pH due to a number of fusion events with early to late endosomes. This chain of events is called phagosome maturation, and results in the fusion of the late phagosome with a lysosome, creating a phagolysosome wherein most bacteria are being degraded (106). The process starts with the recruitment of Rab5 from early endosomes. This small GTPase interacts with Vps15 (of the Class III PI3K complex) which assists in the recruitment of Vps34 (phosphatidylinositol 3-kinase (PI3K)) that generates phosphatidylinositol-3-phosphate (PI3P) on the early phagosomal membrane. PI3P is a docking site for the early endosome antigen 1 (EEA1) which together with Rab5 are necessary for the maturation process to proceed. In the late phagosome, recognized by the presence of Rab7 (replacing Rab5), the pH has dropped considerably (pH 5.5) due to the acquisition of several V-ATPases. The phagosome is now also enriched with lysosomal-associated membrane proteins (LAMP). The phagolysosome is characterized by even lower pH (pH 4.5), a greater number of V-ATPases in the membrane and an accumulation of active hydrolases and antimicrobial peptides (106–108). This environment is bactericidal to most pathogens, including Mtb although this bacteria is more resistant to acidic compartments than most other microbes. Still in order to persist inside the phagosome, Mtb is able to block these fusion events and reside in an early phagosome (109).

Mtb has several effectors and virulence factors able to obstruct phagosome maturation. Among them are nucleoside diphosphate kinase (Ndk) and the tyrosine phosphatase PtpA that interferes with Rab conversion (106). ManLAM can by binding to the mannose receptor impair phagosome maturation, through activation of the protein tyrosine phosphatase (SHP-1) which inhibits the activity of PI3K (110,111). The secreted SapM is able to inhibit the recruitment of EEA1, by removing PI3P through dephosphorylation (106). Furthermore, the

host protein tryptophan-aspartate containing coat protein (TACO) is recruited to phagosomes and is normally released upon fusion with lysosomes, but the mycobacterial lipamide dehydrogenase C (LpdC) can bind and retain this protein on the phagosomes and thereby block the phagosome maturation (112,113). The TACO can however be downregulated by Vitamin D3 together with retinoic acid (114), which may lead to control of Mtb. Yet another way of which Mtb prevents phagosome maturation is through the ubiquitination and degradation of V-ATPase, which prevents acidification (115). HIV is also able to block phagosome maturation, by the action of the HIV protein Vpr (55), but at the same time the virus also utilizes the endocytic pathway in macrophages in order to replicate inside the cells. The budding of HIV virions is directed into the lumen of late endosomes, and these vesicles are then moved to the cell surface for virus release (116,117). Thus, although the endocytic pathway is important for protection against pathogens, HIV can utilize it and both Mtb and HIV can block the phagosome maturation, in order to persist inside cells. This block can however be overcome by pro-inflammatory cytokine (INF- γ and LPS) activation of macrophages (97).

Phagosomal escape is another essential part of Mtb virulence. By translocating to the cytosol Mtb makes itself exposed to cytosolic sensor pathways such as the NLRP3 inflammasome, the cGAS-STING pathway and autophagy. The phagosomal rupture can either be complete and the bacteria then translocate to the cytosol, or it can be a partial rupture and the bacteria then only gain access to the cytosol and can release bacterial effectors to inhibit host defenses (118). The phagosomal rupture is dependent on Mtb's ESX-1 secretion system and is enhanced by restriction of phagosomal acidification (119). Furthermore, the Mtb cell wall lipid phthicerol dimycocerosates (PDIM) contributes to the phagosomal escape, causing necrosis of the host cell which favors spreading to other cells (6). The phagosomal escape can enable replication in the cytosol unless the bacteria is targeted by other intracellular surveillance pathways, such as autophagy.

Autophagy

There are different types of autophagy, such as microautophagy and chaperone-mediated autophagy but herein the focus will be on the main one, called macroautophagy (further referred to as autophagy). The discovery of autophagy started with the discovery of lysosomes in 1955 by Christian de Duve who in 1963 coined the term autophagy (120,121). Since then there have been several advances in methods and the knowledge about autophagy has grown extensively (121).

The characteristics of autophagy; the formation of the double-membrane vacuole called the autophagosome, was first detected during starvation conditions (122). Indeed, autophagy is a process targeting pathogens (called xenophagy) or old organelles for degradation as a part of the innate immune defense or for energy supply respectively. Upon initiation, a phagophore (isolation membrane) is formed that elongates to enwrap portions of the cytoplasm and upon closure creates an autophagosome containing its cargo. This

autophagosome then fuses with a lysosome, creating an autolysosome (autophagolysosome) where the content is degraded and the amino acids can be recycled by the cell (123–125).

After observing autophagy in yeast, a number of proteins required for autophagy were discovered (126,127), which in mammalian nomenclature are called autophagy related (Atg) proteins (128). This breakthrough led to the understanding of how the autophagosome is formed. Upon autophagy stimulation, the mammalian target of rapamycin complex 1 (mTORC1) is inhibited leading to the activation of the Unc-51-like kinase 1 (ULK1) complex that in turn is recruited to the PI3K complex where it phosphorylates Beclin 1 (Atg6 homologue), and initiates the phagophore formation (129,130). For elongation of the phagophore, Atg conjugation systems are necessary. In the Atg12 conjugation system, Atg12 gets conjugated with Atg5 and forms a complex together with Atg16L, that localizes to the phagophore and dissociates upon completion of the autophagosome. The Atg12 conjugation system is necessary for the Atg8/LC3 conjugation system, where the microtubule-associated protein light chain 3 (LC3) is processed into the cytosolic form LC3-I (125,129,131). LC3 I gets conjugated to phosphatidylethanolamine, forming LC3 II that is attached to both the inner and outer membranes of the autophagosomes (129,132,133). By measuring the amount of LC3 II, which represents the amount of autophagosomes, it is possible to monitor autophagy. However, over the process of autophagy when autophagosomes fuse with lysosomes the content is degraded leading to reduction of LC3 II expression (133,134). To study this autophagic flux, inhibitors that block this degradation such as Bafilomycin A1 (135,136) can be used when monitoring LC3 II levels (133,134).

Figure 3. Autophagy in homeostasis and during Mtb infection. Upon mTOR inhibition autophagy is induced, leading to the formation of a phagophore that encloses the target within an autophagosome. When the autophagosome fuses with a lysosome, an autolysosome is formed, wherein the target is degraded, in a process called autophagic flux. Mtb can inhibit the fusion with lysosomes and thereby evade autophagic flux and degradation.

Another autophagy protein that has been used to study the process is sequestosome (SQSTM1/p62), which binds directly to LC3, and is necessary for the degradation of ubiquitinated proteins during autophagy (137). SQSTM1 can also target intracellular bacteria to the autophagy pathway (138), and deliver ubiquitinated proteins with microbicidal properties to autolysosomes to facilitate killing of the bacteria (139). Thus, autophagy is an important part of the innate immune response and has been shown to contribute to the killing of a number of intracellular bacteria, including *Mycobacterium tuberculosis* (139). However, both Mtb and HIV has several ways to manipulate autophagy, causing an induction of the process, but at the same time blocking the later steps, the autophagic flux, in order to avoid killing. In HIV, Nef is the protein involved in this autophagic block through interaction with Beclin 1 (140), while the ESX-1 secretion system is essential for Mtb (141). Additionally the Mtb secreted protein enhanced intracellular survival (Eis) can also inhibit autophagy through induction of IL-10 expression that activates the mTOR pathway (142). Several autophagy inducers can reverse the block in autophagic flux exerted by the pathogens, and thereby promote killing. Some examples are Vitamin D, IFN- γ and the commonly used autophagy inducer rapamycin (141,143,144). However, virulent Mtb upregulate macrophage IL-6 production which can inhibit IFN- γ induced autophagy, and prevent killing (145). Many studies suggest a beneficial role of autophagy induction during Mtb infection and there are attempts of developing adjunctive treatments or vaccines that modulate autophagy. In this regard, rapamycin which is an immunosuppressive drug has surprisingly been shown to enhance BCG efficiency in mice at low concentrations (146). Moreover it has been shown in mice that inhalable particles of rapamycin decreased Mtb burden in the lungs, but the bacteria were only eliminated upon combination with TB drugs (147). Clinical studies are however needed to investigate if rapamycin could be an option for TB treatment. Additionally, several *in vitro* and *ex vivo* studies using D vitamin has shown a beneficial effect on host response against Mtb (144,148–151). However the effect of vitamin D supplementation on Mtb clearance in clinical studies has been controversial and only shown some promise (152–154). Thus, the search for effective host directive therapies continues.

Reactive oxygen species

The innate immune defense in phagocytic cells is not only limited to a decrease in pH but also includes reactive oxygen species (ROS) production. ROS has several sources, and can be produced from the mitochondria, from nicotinamide adenine dinucleotide phosphate (NADPH) oxidases (NOXs) located on cellular membranes or from the endoplasmic reticulum (ER) where it facilitates protein folding. To avoid damage of DNA, lipids and proteins from the highly reactive ROS, the cells have several antioxidants (glutathione and thioredoxin) and redox proteins (catalase, SOD) to regulate ROS. However, if ROS production is greater than the scavenging abilities, it leads to oxidative stress which can damage the host cells, but also kill pathogens. NOXs are important for killing of pathogens as well as for cell signaling and they are present in the membrane of phagosomes of phagocytic cells such as macrophages and neutrophils. Upon phagocytosis, an oxidative burst is induced and ROS is pumped into the phagosome by NOX2 which damages the engulfed pathogen (155,156). The oxidative

burst starts with the transfer of electrons by the NOX2 from cytosolic NADPH to phagosomal oxygen forming superoxide anions ($O_2^{\cdot-}$), which quickly dismutates to hydrogen peroxide (H_2O_2) which in turn transforms into toxic hydroxyl radicals (OH), all of which are part of the ROS family. The dismutation to hydrogen peroxide can either be spontaneous or mediated by superoxide dismutase (SOD). More toxic ROS can be produced by superoxide reacting with nitric oxide forming peroxynitrite, or peroxidase catalyzation of hydrogen peroxide into hypochlorous acid (HOCl) (157). HOCl is the most effective antimicrobial oxidant of neutrophils, generated by myeloperoxidase (MPO) which is released upon degranulation. During ROS production, protons are consumed, raising the pH in the phagosome and reducing it in the cytoplasm, but this is then balanced by proton transfer (155). NOX2 is also important for antigen presentation in DCs. The complex gets assembled onto the phagosome where it prevents the degradation of antigen by increasing the pH through a continuous production of low levels of ROS (158). Furthermore, ROS is important for cell signaling and can act as a second messenger to mediate apoptosis, proliferation, cytokine release and gene regulation. ROS can also modulate redox-sensitive transcription factors such as NFkB (155). Furthermore NOX2-derived ROS can be important for granuloma formation and NET formation (159).

The bactericidal effect of ROS on Mtb is controversial and Mtb has several ways to counteract redox stress. Some examples are the mycolic acids in the cell wall which form a barrier to protect against ROS, in addition to the secretion of the antioxidant enzymes SOD (SodA and SodC) and KatG, the latter accounting for catalase, peroxidase and peroxinitrate activity. KatG is required for ROS protection, but it also activates the efficient prodrug isoniazid to its active form which inhibits the synthesis of mycolic acid (160). KatG converts hydrogen peroxide into water and oxygen, and a loss of this catalase results in hypersensitivity to ROS, but resistance to isoniazid (161). Although the bacterium has ways to protect itself against exogenous ROS, Mtb (including MDR/XDR strains) has been shown to be more sensitive to endogenous ROS produced within the bacteria (162). Another study suggested that the toxicity of exogenous ROS on Mtb is concentration dependent; with low concentrations of H_2O_2 not affecting survival but resulting in induction of responsive genes to initiate DNA repair, while high concentrations of H_2O_2 was lethal to Mtb (163). Phagosomal ROS is generated through NOX2 upon Mtb infection. NOX2 is also important for apoptosis induction through TNF- α signaling. By inhibiting the activity of NOX2, Mtb is protecting itself against both ROS and apoptosis, another important defense mechanism (164). The effects of ROS may not be directly bactericidal to Mtb, but the radicals can mediate cell signaling events that are important for host defense (159).

In contrast to Mtb, HIV benefits from oxidative stress. HIV patients have a depletion of glutathione in plasma, lymphocytes and monocytes (165) along with reduced levels of SOD (166). At the same time, monocytes from HIV patients have increased ROS production, which correlated with viral load (167). HIV induces oxidative stress, with a number of its proteins (Tat, gp41, Vpr) involved in deregulating different pathways to increase ROS production (168). Tat can for example induce oxidative stress by activating NOX2 through the PI3K/Akt signaling pathways, leading to activation of NFkB (169). Furthermore, ROS induces HIV transcription through NFkB activation (170), while antioxidants reduce the transcription of the virus (171).

In summary, HIV induces ROS production that in turn promotes HIV replication, thereby creating a vicious circle.

Figure 4. ROS production in a neutrophil. Upon phagocytosis, ROS is produced inside the phagosome, starting with the production of superoxide anions (O_2^-) by the NADPH oxidase (NOX). This radical then dismutates to hydrogen peroxide (H_2O_2) that is converted into hypochlorous acid (HOCl) by myeloperoxidase (MPO). MPO is transported into the phagosome by the fusion of azurophil granules in the cytosol.

Apoptosis

Apoptosis (programmed cell death) is characterized by controlled breakdown of a cell, morphologically seen as cell shrinking, membrane blebbing and chromatin condensation. This results in the formation of apoptotic bodies that can be engulfed by surrounding phagocytes, in a process called efferocytosis (172). Efferocytosis of apoptotic cells is considered to be anti-inflammatory, through the induction of transforming growth factor (TGF)- β secretion (173), but during Mtb infection a pro-inflammatory response upon efferocytosis has been observed (174). It is also a defense mechanism during infection, killing the cell wherein the pathogens reside and replicating and delivering it to a non-infected cell. Efferocytosis of Mtb-infected macrophages by uninfected ones has been shown to decrease growth of Mtb through lysosome fusion (175).

Apoptosis is activated through an intrinsic or extrinsic pathway and is dependent on caspases. DNA damage or oxidative stress activates the intrinsic pathway leading to activation of the pro-apoptotic proteins Bax and Bak which creates a channel in the mitochondria for release of cytochrome c into the cytosol. This leads to the activation of procaspase-9 which initiates a proteolytic cascade that activates the executioner caspases-3, -6 and -7, leading to

apoptosis. Smac/Diablo are other pro-apoptotic proteins released from the mitochondria. The extrinsic pathway is induced by external stimuli (such as TNF- α and Fas ligand (FasL)) binding to death receptors such as the TNF receptor, Fas and TRAIL-R (TNF-related apoptosis-inducing ligand receptor). This leads to the formation of a death-inducing signaling complex that recruits the initiator caspases-8 or -10 that in turn activates the executioner caspases (172,176).

Another type of cell death is necrosis which is characterized by uncontrolled cell swelling and loss of cell membrane integrity, leading to the release of the cell content into the surroundings (172). Apoptosis is generally believed to promote killing of mycobacteria and promote antigen presentation, mounting a T-cell response, while necrosis is considered to promote spreading of the bacteria along with inflammation and tissue damage (177). Thus, it is not surprising that virulent mycobacteria inhibit apoptosis and promote necrosis (178). The attenuated Mtb strain H37Ra was shown to induce more apoptosis than the virulent H37Rv (179). Virulent Mtb has several ways to inhibit apoptosis including; the induction of IL-10 which decreases TNF- α mediated apoptosis (180), decreased Fas receptor expression (181), and upregulation of the expression of the antiapoptotic Mcl-1 protein (182). Apoptosis of Mtb infected macrophages can also be inhibited by HIV Nef by reducing TNF- α production (57). HIV can also manipulate apoptosis through regulation of pro- and anti-apoptotic proteins and FasL, TRAIL and TNF- α expression. The viral proteins gp120, Tat, Vpu, Nef, Vpr and protease inhibit apoptosis early during infection to promote viral replication but later when the infection is established they induce apoptosis. Apoptosis is mainly induced in immunocompetent cells such as CD4 T cells and uninfected bystander cells, while it is inhibited in latently infected cells (viral reservoirs) (183). This contributes to the depletion of CD4 T cells along with progression of the disease.

Activation of adaptive immunity

Antigen presentation

Macrophages and dendritic cells are the main antigen presenting cells (APCs) during Mtb infection. These cells get infected or pick up antigens to be presented to naïve T cells through the major histocompatibility complex (MHC), and thereby mount a T cell response. Dendritic cells are especially important in priming of T cell responses, while macrophages mainly present antigens to effector T cells (184). Typically intracellular antigens are presented to CD8 T cells by MHC I and exogenous antigens are presented to CD4 T cells by MHC II. However, through cross-presentation exogenous antigens can be presented by MHC I and if cytosolic proteins are degraded through pathways such as autophagy, they can be presented by MHC II. MHC I molecules are ubiquitously expressed by all nucleated cells, while MHC II are expressed mainly by APCs. During MHC I antigen presentation, the antigens are degraded by proteasomes and the resulting peptides of 8-9 amino acids are loaded onto the MHC I in the endoplasmic reticulum (ER) before transport of the complex to the cell surface and presentation to CD8 T cells. The MHC II is assembled in the ER, and is then transported to a

late endosomal compartment, where it can bind a specific peptide derived from a protein degraded in the endosomal pathway. The complex is then transported to the plasma membrane where it presents the antigen to CD4 T cells (185). The T cell receptor (TCR) on CD8/CD4 T cells interacts with the MHC I/II and its antigen, creating the first signal, determining the antigen specificity of the response. A second signal is required for T cell activation and is obtained through binding of the co-stimulatory molecules CD80 and CD86 to the primary costimulatory receptor CD28 on T cells. This is followed by the third signal in shape of cytokine secretion from the APC, inducing proliferation of T cells and polarizing the CD4 T cell response. The type of cytokines secreted depends on the stimuli that primed the APC through PRR, and will determine which type of CD4 T cell response will be mounted (186). There are also several co-stimulatory molecules on DCs that can either promote an activation or inhibition response. Examples of co-stimulatory molecules on DCs are: CD80/B7-1, CD86/B7-2, CD83, CD40 and PDL-1. CD40 on DCs binding to CD40L on T cells results in the activation of both cells while CD80/CD86 binding of CTLA-4 on T cells results in inhibition of T cell activation. Binding of CD80/CD86 with CD28 leads to T cell proliferation and increased secretion of IL-2 and is essential for preventing T cell apoptosis (187,188).

Effects of Mtb and HIV on antigen presentation

Both Mtb and HIV can inhibit antigen presentation in order to persist inside the host. HIV can down-modulate the surface expression of CD80 and CD86 on APCs through Nef (189), and inhibit up-regulation of CD40L on T cells and CD80 on APCs through gp120, thus inhibiting T cell activation (190). HIV also causes a reduction in IL-12 and an increase in IL-10 production (191), which reduces induction of Th1 responses. HIV infection in dendritic cells promotes upregulation of inhibitory molecules such as PD-1 and CTLA-4 on T cells, which suppresses T cells and decrease their proliferation (188). Mtb also has strategies to evade T cell activation. As mentioned earlier, Mtb can inhibit phagosomal maturation and autophagic flux, thereby preventing antigen processing and sequentially antigen presentation. Mtb can also export their antigens from infected dendritic cells in order to reduce their presentation and activation of T cells. Although these exported antigens can be presented by uninfected cells the protective T cell response remains limited (192). Furthermore, the Mtb lipoprotein LpqH can inhibit MHC II expression in macrophages through TLR2 signaling and interfere with the peptide loading to MHC II and the subsequent trafficking to the cell surface. The inhibition of antigen presentation promotes Mtb survival in APCs and enables persistence without the recognition of CD4 T cells (91,193). Mtb can also disrupt presentation of Mtb antigens in DCs by for example promoting DC maturation and MHC expression before antigen processing and loading occurs (71). Conversely, mycobacteria-induced TLR2 and TLR4 signaling promote antigen presentation, through stimulation of DC maturation including increased expression of MHC class II along with the co-stimulatory molecules CD80 and CD86. This mycobacteria-induced maturation also leads to the activation of Th1 and Th17 cells (194,195).

The activation of CD8 T cells during Mtb infection has mainly been associated with cross-presentation since Mtb primarily is confined within endosomal compartments, but through

phagosomal rupture antigens can gain access to the cytosol where they can be processed by the proteasome and loaded onto MHC I (196). Mycobacterial antigens from apoptotic cells can also be presented through cross-presentation. The released apoptotic vesicles from the infected cells can be taken up and processed by DCs which in turn can present the antigens to CD8 T cells through MHC class I. By enhancing apoptosis of Mtb infected cells a higher CD8 T cells response can be mounted (197). However, as mentioned earlier virulent mycobacteria inhibit apoptosis and induce necrosis (178) and can thereby prevent cross-presentation to CD8 T cells. By a mechanism similar to the cross-presentation of antigens from apoptotic cells, Mtb lipids could also be accessed by this route, and be presented by CD1 on DCs to Natural killer T cells (NKT cells) which upon activation produce large amounts of IFN- γ (196). However, this lipid presentation can also be inhibited by Mtb by similar mechanisms already described for antigen presentation.

T cell responses

The adaptive immune responses against Mtb, mainly consisting of antigen specific T cell responses, are not detected until several weeks after infection but plays an important part in the outcome of the disease (184). CD4 T cells are essential in the immune response against TB for controlling the infection. Naïve CD4 T cells can differentiate into different subsets depending on the cytokines produced by the APC during antigen presentation and T cell activation. The CD4 T cell subset T helper type 1 (Th1) cells have typically been considered as one of the most important during Mtb infection, but in recent years the importance of Th17 cells has also been emphasized. Th17 cell polarization is induced by IL-6, IL-23, IL-1 β , IL-21 and TGF- β which activates STAT3 that induces the expression of the transcription factor ROR- γ t, leading to the production of IL-17, IL-22, IL-26 and GM-CSF. These cytokines mediate pleiotropic activities such as induction of pro-inflammatory genes and antimicrobial peptides along with stimulation of granulopoiesis and neutrophil recruitment and activation. Th17 cells have mainly been associated with clearance of extracellular pathogens, but they are also involved in protection against some intracellular pathogens. Their role during TB infection is still controversial, but some studies suggest a protective role in combination with Th1 responses. Th1 cell polarization is induced by IL-12 or INF- γ and upon activation, these cells mainly secrete IFN- γ . When IL-12 binds to its receptor on T cells, STAT4 is activated which induces the transcription factor T-bet which in turn binds to many Th1 specific genes and induces their expression. Th1 cells with the production of INF- γ are important in the activation of macrophages for antimicrobial defense and for promotion of MHC II expression on macrophages (198). This cytokine can act on both infected and uninfected macrophages and thus when evaluating antigen presentation the MHC expression on APCs may be a poor indication since not all cells contain antigens. Additionally, antigens also needs to be sufficiently processed to be presented and therefore functional studies (of T cell activation) are preferred in order to properly evaluate antigen presentation (199).

HIV infection is characterized by the progressive loss of CD4 T cells, causing an immunodeficiency that leaves the host more vulnerable to other infections. It has also been

shown that during a co-infection, HIV preferentially infects and causes depletion of Mtb-specific CD4 T cells (49). Furthermore, PBMCs from coinfecting individuals were shown to proliferate less, release less IFN- γ and express less IL-2 and IL-12 when stimulated with heat-killed Mtb, compared to PBMCs from TB only infected individuals (200). Apart from HIV's manipulation of the immune response, Mtb can by manipulating DCs accelerate the progression of HIV. Mtb can promote *trans* infection, when DCs do not become infected with HIV but deliver the virus directly to T cells across an infectious synapse, leading to infection of the T cell. Furthermore Mtb can reduce the processing of HIV and inhibit antigen presentation of HIV through MHC II, leaving the virus undetected by CD4 T cells (201).

Another subset of CD4 T cells are regulatory T cells (Tregs), which are important in the control of the immune response and in preventing autoimmunity. However during TB the Tregs dampen the immunity and function of effector T cells and may promote disease progression (202). During active disease an increased number of Tregs have been found in both blood and at the site of infection. These cells suppressed the Mtb-specific immunity by reducing IFN- γ and IL-10 production (202).

CD8 T cells (CD8 cytotoxic T lymphocytes (CTL)) can upon activation produce IFN- γ , lyse the infected antigen presenting cell, and mediate an antimicrobial activity through secreted granulysin and pore-forming perforin, thereby contributing to the reduction in Mtb burden (203). CD8 T cells also play a major role during virus infections, since viral antigens are mainly presented by MHC I molecules. CD8 T cells have been shown to be able to control HIV during early infection but later fail to prevent replication of the virus (204). This was due to the viral Nef protein, which blocks MHC I expression on the surface of the infected cell in a PI3K-dependent manner, and thus inhibits CTL-mediated lysis of the HIV infected CD4 T cells (205,206). Nef is an important HIV protein, contributing to pathogenicity, promoting virus replication and is known to also downregulate CD4 of infected cells (207,208) thereby preventing superinfection (209). HIV also avoids T cell responses by upregulating Fas on CD8 T cells, leading to apoptosis of these cells upon interaction with FasL on APCs (210). Furthermore, HIV causes an up-regulation of PD-1 on CD4 and CD8 T cells which impairs their proliferation and response (211).

AIMS

The overall aim was to investigate how Mtb and HIV can impair the protective immune system, and how it can be boosted in order to enhance the innate immune response against Mtb during HIV coinfection.

Paper I

The aim was to investigate if autophagy induction could impair Mtb replication in HIV coinfecting macrophages. More specifically if the autophagy induction would cause an increased acidification in Mtb phagosomes when the macrophages were coinfecting with Mtb and HIV.

Paper II

The aim was to investigate how HIV affects processing and presentation of Mtb antigen in coinfecting dendritic cells, focusing on the expression of MHC II and co-stimulatory molecules as well as the Mtb-specific CD4 T cell response.

Paper III

The aim was to investigate how apoptotic neutrophils could augment the antimicrobial response against Mtb in HIV coinfecting macrophages through efferocytosis, focusing on the role of ROS and myeloperoxidase.

RESULTS

Paper I

Autophagy induction targeting mTORC1 enhances Mycobacterium tuberculosis replication in HIV co-infected human macrophages

In this paper the effect of autophagy induction on Mtb was studied in HIV coinfecting macrophages. Initially an increased LC3 formation and co-localization to Mtb was observed in HIV coinfecting cells compared to Mtb single infected cells. Unexpectedly, when inducing autophagy with the well-known inducer rapamycin an increased Mtb replication was detected in both Mtb single and HIV coinfecting cells, while autophagy inhibition with 3-Methyladenine (3-MA) had no effect. The autophagy inducer and inhibitor had no direct effect on Mtb (in the absence of macrophages), confirming that they only target processes inside the macrophages. The increased Mtb replication after autophagy induction, lead us to try yet another autophagy inducer, Torin1, which also inhibits the mTORC1 pathway. Previous studies have shown that this inducer is more efficient than rapamycin (212) which we confirmed by western blot. With increasing concentrations of Torin1 and rapamycin an increased autophagic flux was observed, leading to degradation of the autophagy proteins LC3 and SQSTM1 (also called p62). To confirm an inhibition in the mTORC1 pathway, the phosphorylated downstream targets S6 and 4EBP1 was also detected through western blot, showing a reduction with increasing concentrations of the inhibitors. These effects were especially prominent with Torin1, which lead us to investigate its effect on Mtb replication. Using a low MOI (MOI=1) of Mtb, a concentration dependent increase in replication upon Torin1 treatment was observed, although abolished when cells were infected with an MOI of 5 or 10. Thus, during a low grade infection the induction of autophagy can promote Mtb replication in single and coinfecting macrophages.

The reason why the bacteria replicated could be due to inhibition of flux. Therefore acidification of the Mtb phagosomes was studied. Using the probe LysoTracker to visualize acidic compartments in the cells, co-localization to Mtb phagosomes was studied through microscopy. Yeast particles were used as a positive control and bafilomycin was added as a negative control, since this compound inhibits the V-ATPase and thereby prevents autophagic flux and autophagosome-lysosome fusion (135,136). When single and coinfecting macrophages were treated with Torin1, a decreased co-localization between LysoTracker and Mtb phagosomes was detected. A decrease in acidification can lead to an increased replication of Mtb, as seen when treating infected macrophages with bafilomycin. Mtb can also inhibit phagosome maturation and autophagic flux in order to survive in the cells (213). Previous studies have shown that this block can be overcome by inducing autophagy, which increases the flux in the cells (143,144,214). Our study demonstrates that the autophagic flux is cellular (seen by western blot) and not specifically localized to the Mtb phagosomes (as seen by microscopy). Hence, we hypothesize that Mtb, especially during HIV coinfection, benefits from decreased phagosomal acidification and increased cellular flux, providing nutrients to the pathogen, which leads to increased replication. Further evidence for this was

the build-up of SQSTM1 in Mtb phagosomes upon Torin1 treatment and the increase in autophagosomes (LC3+ Mtb phagosomes) without an increase in autophagolysosomes (LC3+LysoTracker+ Mtb phagosomes). These findings stresses the importance of microscopy studies of the Mtb phagosomes as preferred to looking at the cell as a whole by western blot.

In summary, autophagy induction through mTORC1 inhibition during a low grade Mtb infection causes a cellular autophagic flux that is not localized to the Mtb phagosomes. This autophagy induction causes a disruption in the growth equilibrium of Mtb, leading to increased replication caused by the decreased phagosomal acidification, especially in HIV coinfectd macrophages. Therefore autophagy induction through mTORC1 inhibition does not seem to be a good treatment option during Mtb and HIV coinfection.

Figure 5: Schematic summary of Paper I. Autophagy in macrophages infected with yeast leads to degradation through autophagic flux, while Mtb can inhibit this acidification. Upon autophagy induction with Torin1 in Mtb and HIV co-infected cells, the acidification is further reduced leading to Mtb replication.

Paper II

HIV interferes with Mycobacterium tuberculosis antigen presentation in human dendritic cells

In this paper antigen presentation by Mtb and HIV coinfecting dendritic cells and their subsequent activation of Mtb antigen-specific (Ag-specific) CD4 T cells were studied, focusing on the role of HIV. During antigen presentation, three signals are required in order to mount a T cell response; the binding of MHC II to CD4, the subsequent binding of co-stimulatory molecules with receptors on T cells, and cytokine secretion from APCs (186). Therefore we studied these signals before measuring the T cell response.

Surface expression of MHC class II (HLA-DR), co-stimulatory molecules (CD40, CD80, CD83 and CD86) and CCR7 was analyzed through flow cytometry in coinfecting DCs. There were no significant differences in the percentage of marker positive DCs, but the total surface expression on DCs revealed an increase of co-stimulatory molecules and HLA-DR in Mtb single infected cells, with exception of CCR7. The Mtb-induced increase was inhibited in HIV coinfecting DCs, with a reduction in the expression of CD40, CD80, CD86, HLA-DR and slightly of CD83. As the third signal of antigen presentation includes the secretion of cytokines (186), the production of both pro- and anti-inflammatory cytokines were measured. Mtb induced the production of IL-6, IL-1 β and TNF- α from dendritic cells, while HIV coinfecting cells had reduced levels of these cytokines in comparison to Mtb single infected cells. Production of IL-12p70, IFN- α/β , IFN- γ and IFN- γ inducible protein 10 (IP-10) which is a surrogate cytokine for type I IFNs, were low in both single and coinfecting cells. The pathogens did not induce significant levels of IL-10 either.

These initial experiments were performed to get an indication of how HIV interferes with antigen presentation, but in order to confirm its impact on T cell activation, functional studies are needed. Mtb Ag-specific CD4 T cells were therefore generated and co-cultured with Mtb and HIV coinfecting autologous dendritic cells. The readout for T cell activation was production of IFN- γ . Two populations of CD4 T cells were generated, which were specific to different Mtb antigens, namely PPD and Ag85B. Both types of Mtb Ag-specific CD4 T cells exhibited a decrease in IFN- γ production after stimulation with HIV coinfecting DCs compared to Mtb single infected cells. Also, PPD stimulation as replacement for Mtb displayed the same reduction of IFN- γ production upon HIV infection of DCs. To reveal if HIV reduced antigen presentation and subsequent T cell activation through decreasing antigen processing, DCs were pulsed with Ag85B peptides and co-cultured with the Mtb Ag-specific T cells. HIV did not affect the IFN- γ production of the T cells, which indicates that HIV affects the antigen presentation by disrupting antigen processing. An important antigen processing pathway is autophagy (215). To investigate if this pathway was affected by the pathogen, the levels of LC3 and SQSTM1 was quantified through western blot. Bafilomycin was used to measure autophagic flux, where a high flux would be recognized by increased accumulation of the autophagic protein upon bafilomycin treatment. Mtb infected DCs displayed an increase in autophagosome formation, and blocked acidification. HIV co-infected DCs however showed

a decreased autophagosome formation in addition to decreased accumulation of autophagy proteins. These findings indicate that HIV infection could lead to disruption in the autophagy-dependent processing of Mtb antigens. This suppression of autophagy was however not achieved through inhibition of the cGAS-STING pathway, which has been shown to induce autophagy upon DNA sensing (216,217).

In summary, HIV reduces Mtb antigen presentation and subsequent Mtb Ag-specific CD4 T cell activation and response by decreasing the Mtb-induced expression of HLA-DR and co-stimulatory molecules on DCs, and through suppression of antigen processing by autophagy. Impaired Mtb antigen presentation would allow the bacteria to remain undetected by the adaptive immunity, accelerating the pathogenesis of the Mtb/HIV coinfection.

Figure 6: Schematic summary of Paper II. HIV inhibits antigen presentation by suppressing autophagy of Mtb, decreasing expression of HLA-DR and co-stimulatory molecules on coinfecting DCs and decreasing the release of cytokines. This leads to inhibited activation of Mtb specific T cells, with decreased release of IFN- γ .

Paper III

The enhanced control of Mycobacterium tuberculosis in HIV coinfecting macrophages by apoptotic neutrophils is myeloperoxidase dependent.

In this paper the effect of efferocytosis of apoptotic neutrophils by Mtb and HIV coinfecting macrophages was investigated. Previous studies have shown a beneficial role of efferocytosis in combating mycobacteria infection (78,175,218), and our group has previously shown that apoptotic neutrophils can induce a pro-inflammatory response and enhanced control of Mtb in infected macrophages (174). The aim was to explore this further, and see if apoptotic neutrophils also could inhibit Mtb growth during coinfection. Therefore human macrophages were infected with HIV prior to Mtb infection and stimulation with apoptotic neutrophils. After 5 days the growth of Mtb was significantly decreased after efferocytosis, both in single and HIV coinfecting macrophages. To confirm uptake of apoptotic neutrophils, we performed kinetics experiments. A time-dependent uptake was observed, with 50% of macrophages having ingested apoptotic neutrophils after 1h of stimulation, increasing to almost 100% after 24h. Moreover, there was a time-dependent increase of Mtb co-localization with apoptotic neutrophils. Additionally, flow cytometry experiments revealed an increase in phagocytosis of Mtb by macrophages containing apoptotic neutrophils.

To investigate if Mtb growth inhibition was caused by autophagy induction triggered by the apoptotic neutrophils, western blot was performed. Studying the levels of SQSTM1 and LC3 II, no increased autophagic flux upon apoptotic neutrophil stimulation could be observed. Furthermore, the results point towards a decrease of autophagosome formation upon stimulation with apoptotic neutrophils. Since Paper I showed that western blot analysis is not reliable for studying the environment in the phagosome, also microscopy studies were performed, using LysoTracker as a marker for acidic compartments. LysoTracker co-localized with apoptotic neutrophils, but to a lesser degree with Mtb, indicating a decreased flux/acidification in the Mtb phagosomes. Although flow cytometry data showed a general increase in LysoTracker signal in cells containing apoptotic neutrophils, the microscopy results clearly show that this acidification is concentrated to apoptotic neutrophils and did not localize to Mtb. Therefore, other mechanisms by which the apoptotic neutrophils exert their effects must be involved.

Since a previous study has shown that the granules from neutrophils can be utilized by macrophages for antimicrobial defense (78), we studied the putative role of neutrophil granules and ROS. The most abundant granule protein of neutrophils is myeloperoxidase (MPO) (219). Immunostaining showed that MPO was present in macrophages that had ingested apoptotic neutrophils. With increasing uptake of apoptotic neutrophils, the presence of MPO was also increased until 6h, then a decrease of the protein was observed. The distribution of MPO was dispersed within the macrophage and very little co-localized to Mtb. Using luminol-enhanced chemiluminescence we observed that PMA stimulated ROS production in apoptotic neutrophils. The irreversible MPO inhibitor ABAH (4-Aminobenzoic acid hydrazide) inhibited chemiluminescence in a dose-dependent manner, proving that MPO and the NADPH oxidase (NOX2) are active in apoptotic neutrophils. ROS production was also

measured in macrophages after efferocytosis. Apoptotic neutrophils increased the ROS production in infected or PMA stimulated macrophages. Furthermore, the inhibited Mtb growth caused by apoptotic neutrophils was dependent on MPO, since apoptotic neutrophils treated with ABAH abolished the suppressed bacterial growth in coinfecting macrophages.

In summary, this paper shows that efferocytosis of apoptotic neutrophils enhance the control of Mtb growth in Mtb and HIV coinfecting macrophages. This effect was not dependent on autophagy or increased phagosome acidification, but on MPO and ROS from apoptotic neutrophils.

Figure 7: Schematic summary of Paper III. Uptake of apoptotic neutrophils by Mtb and HIV coinfecting macrophages leads to increased ROS production. Upon efferocytosis of apoptotic neutrophils MPO spreads out in the macrophage and mediates inhibition of Mtb growth.

DISCUSSION

Since a common scenario during a TB and HIV coinfection is a preceding HIV infection, which makes the patient more susceptible to secondary infections, we established an HIV infection in macrophages prior to Mtb infection. Macrophages are the main cells to be infected by Mtb, and we have therefore primarily used them for our *in vitro* experiments to study if their antimicrobial defense could be enhanced by different means. Dendritic cells on the other hand are superior cells for antigen presentation, and we used these cells to study how HIV can manipulate this process, in addition to Mtb Ag-specific CD4 T cells. The focus of this thesis is on the innate immune reactions, but we have also studied the link to the adaptive immunity.

Autophagy is believed to be an important antimicrobial defense mechanism affecting both Mtb viability and antigen processing (215). Therefore we investigated (i) the antimycobacterial capacity in HIV coinfecting macrophages (**Paper I**), (ii) if HIV affected autophagy and antigen processing in Mtb co-infected dendritic cells (**Paper II**) and (iii) if autophagy could be enhanced by efferocytosis of apoptotic neutrophils (**Paper III**).

In coinfecting dendritic cells, HIV is able to inhibit autophagy and flux and thereby decrease Mtb antigen processing (**Paper II**), making autophagy an important part in activating the adaptive immunity. However, in macrophages we surprisingly found that during a low grade infection, induction of autophagy through inhibition of mTOR results in an *increased* replication of Mtb (**Paper I**). This was however not observed with higher MOIs. We also detected a general increase in autophagic flux with increased acidification which was not localized to the Mtb phagosomes, showing how resistant and competent this pathogen is in escaping and battling defense mechanisms of the cells. We hypothesized that other ways of inducing autophagy than through mTOR inhibition might be more beneficial and we therefore investigated if efferocytosis of apoptotic neutrophils could induce this process in infected macrophages (**Paper III**). We did not detect an increased autophagic flux upon uptake of apoptotic neutrophils, but enhanced antimicrobial activity mediated through MPO and ROS.

Cells have a basal level of autophagy to maintain homeostasis and if this process is inhibited, it can lead to cell death, which is another innate defense mechanism against Mtb (220,221). When investigating autophagy during infection it is important to differentiate between basal autophagy and xenophagy (autophagy of intracellular pathogens) (221). Therefore, microscopy studies in addition to western blot analysis is preferred to detect *phagosomal* flux. Chandra et al (222) has also used this approach to study the difference in autophagy in macrophages infected with either H37Ra or H37Rv. They showed that both the virulent and the nonvirulent strain induced similar levels of overall autophagic flux (western blot). Microscopic examination however showed that H37Rv inhibited maturation of the autophagosomes that harbored the bacteria (xenophagosomes), but allowed maturation of other autophagosomes. This selective inhibition was caused by the virulence factors PhoP and ESAT-6, and could therefore not be accomplished by the nonvirulent H37Ra (222). This and our study (**Paper I**) stress the importance of studying the actual autophagosomes wherein the bacteria reside, in order to determine any effects of autophagy on the bacteria. To conclude,

virulent Mtb inhibits maturation of xenophagosomes but not autophagosomes, thereby promoting Mtb survival and preventing cell death (221). Killing of Mtb will therefore depend on the induction of xenophagosome maturation and not only the induction of general autophagy.

Rapamycin stimulation of dendritic cells infected with virulent Mtb was able to override the inhibition of autophagic flux (xenophagic flux), and thereby increase cytokine release to stimulate a Th1 response. The autophagy induction did however not decrease Mtb viability, but merely promoted Mtb infected DCs to stimulate a Th1 response (141). Although other studies have shown that autophagy induction with rapamycin decreases the viability of Mtb (143,147), the T cell response with production of IFN- γ might be more efficient in promoting Mtb killing. Along with inducing autophagy, IFN- γ also increases other antimicrobial activities in macrophages (223). Furthermore, Mtb-specific T cells have been shown to restore the inhibited autophagic flux and increase Mtb killing, although independently of IFN- γ (224). Thus, a T cell response may be crucial for control of Mtb. The stimulation of such a response is however inhibited in HIV coinfecting DCs (**Paper II**), decreasing the immunity against Mtb. HIV inhibits the Mtb specific T cell response through inhibition of antigen presentation at the level of autophagy inhibition and reduction in cytokine secretion and expression of HLA-DR and co-stimulatory molecules. By inducing autophagy the processing of antigens might be increased, which can promote a protective T cell response. How autophagy is induced might however be important. For example by inducing autophagy with Earle's balanced salts solution (EBSS) in BCG infected DCs, cytokine production was promoted and expression of HLA-DR was increased, while treatment with rapamycin had the opposite effect (225). Since rapamycin is a rather poor inducer of autophagy in mammalian cells with its effect depending on cell type (212), we additionally used Torin1 in our experiments (**Paper I**). Torin1 is an ATP-competitive inhibitor that suppresses both mTORC1 and mTORC2 and is more potent and selective than rapamycin (212). Despite of this we found that also Torin1 increased the replication of Mtb, indicating that inhibition of mTOR might not be a beneficial way of inducing autophagy during HIV coinfection.

We further explored the possibility of autophagy induction in macrophages by efferocytosis of apoptotic neutrophils (**Paper III**), but did not find such an effect. Still, efferocytosis has been shown to be able to promote a T cell response. Dendritic cells that phagocytose apoptotic macrophages containing Mtb antigens could through cross-presentation activate CD8 T cells for IFN- γ production (226,227). The efferocytosis, promoted MHC-I upregulation along with increased expression of CD40, CD80 and CD86 and increased cytokine production (226). Furthermore Mtb infected neutrophils provided antigens to DCs upon efferocytosis and facilitated activation of CD4 T cells (228), while Mtb-inhibited neutrophil apoptosis lead to delayed activation of T cell responses (229). These findings suggest a beneficial role of efferocytosis for activation of the adaptive immunity during Mtb infection. However during HIV coinfection the activation of the adaptive immunity is impaired (**Paper II**), and the well-known decrease in CD4 T cells creates a severe immunodeficiency. Therefore we instead investigated if efferocytosis of apoptotic neutrophils could boost the *innate* immune response against Mtb in macrophages (**Paper III**).

Most studies investigating efferocytosis have focused on the antibacterial effect of uptake of infected apoptotic cells by uninfected cells. However since our group has previously found that uninfected apoptotic neutrophils promote an antibacterial response in Mtb infected macrophages (79), we explored this further in a HIV coinfection model (**Paper III**). We found that efferocytosis of apoptotic neutrophils promoted ROS production in infected macrophages. The role of ROS in defense against Mtb is controversial and the bacteria has several ways to counteract redox stress. However we hypothesize that neutrophils which are able to mount a strong oxidative burst can aid macrophages in their ROS production upon uptake of apoptotic neutrophils and their granules. This increased ROS production might override the bacterial defense and cause decreased replication. We found not only increased ROS production but also MPO after efferocytosis, which indicates a role for neutrophils which harbor abundant amounts of MPO in their granules. Neutrophils are the biggest cell source of MPO since 5% of their dry weight consist of it, while monocytes and macrophages have considerably less (219). MPO is responsible for the production of the radical HOCl which is not produced in the absence of the enzyme (230). Although this radical and other oxidative metabolites can be bactericidal for some pathogens, we do not exclude the possibility of other ROS-mediated effects in addition to a direct effect of ROS or MPO on Mtb. ROS can promote cell signaling and production of cytokines (155,230), and since we have previously seen an increased IL-1 β production upon efferocytosis of apoptotic neutrophils (79) it might be mediated by ROS. We used ABAH to specifically inhibit MPO in neutrophils and thereby confirmed that decreased Mtb growth was caused by this enzyme, derived from the neutrophils. Although MPO was able to boost the immunity against Mtb in macrophages (**Paper III**), the enzyme has been shown to have a suppressive effect on the adaptive immunity (231). MPO released in lymph nodes by neutrophils inhibited DC activation and caused a decreased T cell response (231). Upon infection however, neutrophil-derived MPO mediates a bactericidal effect in macrophages (**Paper III**), which are the primary host cells of Mtb.

Boosting of the innate immunity might therefore be a good strategy to compensate for the adaptive immunity, which is normally delayed during Mtb infection (184), and severely deficient during HIV coinfection. Along with our observed impaired CD4 T cell response during coinfection (**Paper II**), the CD8 T cell response has also been shown to be affected. TB and HIV coinfecting patients display an absence of co-stimulatory molecules and cytokine secretion in CD8 T cells, along with diminished levels of intracellular IFN- γ , perforin and granzyme B in HIV specific CD8 T cells (232). Thus, HIV does not only impair the CD4 T cell response against Mtb, but also during coinfection reduces the CD8 T cell response against the virus. The HIV-mediated T cell suppression by secretion of IDO and upregulation of PD-1 and CTLA-4 on T cells, further decrease the responses against both Mtb and HIV, and contributes to disease progression (52,211).

Previous work have shown that HIV preferentially infects and depletes Mtb Ag-specific T cells and thereby decrease the immune response against TB (49). We found that HIV also interfered with the response of Mtb Ag-specific T cells upon antigen presentation by HIV coinfecting dendritic cells (**Paper II**). In order to create Mtb Ag-specific T cells we used the Mtb antigens Ag85B and PPD. The Ag85 complex (Ag85A, Ag85B, Ag85C) is one of the major

secretory antigens of Mtb, with Ag85B as the most abundant one, which is able to induce a Th1 response with production of IFN- γ (233,234). After confirming the specificity of the T cells, we observed an increased IFN- γ production upon Mtb infection and antigen presentation by dendritic cells. However, HIV inhibited Mtb antigen presentation and IFN- γ production from the Mtb Ag-specific CD4 T cells. Furthermore, our group has recently shown that HIV coinfecting dendritic cells induce increased expression of co-inhibitory molecules on Mtb Ag-specific T cells, along with increased production of IL-10 and TGF- β . These suppressive Mtb Ag-specific T cells failed to control Mtb in macrophages (235). Without the activation of T cells and their production of IFN- γ , the enhancement of macrophage immunity against Mtb is impaired, and need to be activated in other ways during an HIV coinfection. A boosting of macrophages through cooperation with neutrophils (**Paper III**) could therefore compensate for the deficient adaptive immune response against Mtb during HIV infection.

GENERAL CONCLUSIONS

In conclusion this thesis demonstrates that autophagy induction through mTOR inhibition by Torin1 caused an increased replication of Mtb along with decreased acidification of Mtb phagosomes in HIV coinfecting macrophages. In contrast to the belief that autophagy is an important defense mechanism against Mtb, mTOR inhibition might play a detrimental role during an HIV coinfection scenario.

Furthermore HIV can inhibit autophagy and antigen processing and prevent Mtb Ag-specific T cell responses through decreasing cytokine production and expression of HLA-DR and costimulatory molecules on coinfecting dendritic cells. This inhibited antigen presentation and T cell activation explains the impaired immune defense against Mtb during an HIV coinfection.

Boosting the innate immunity was attempted by adding apoptotic neutrophils to coinfecting macrophages. The macrophages became more capable of controlling Mtb infection since they acquired MPO. MPO or ROS could either have a direct bactericidal effect on Mtb or mediate it through cell signaling and cytokine production in the infected macrophages.

Stimulation of an increased uptake of apoptotic neutrophils or MPO could arm the macrophages in the battle against Mtb, and possibly compensate for the inhibited T cell response during HIV coinfection.

REFERENCES

1. Daniel TM. The history of tuberculosis. *Respir Med.* 2006 Nov;100(11):1862–70.
2. World Health Organization. Global Tuberculosis report 2018 [Internet]. 2018. Available from: http://www.who.int/tb/publications/global_report/en/
3. Gagneux S. Ecology and evolution of *Mycobacterium tuberculosis*. *Nat Rev Microbiol.* 2018 Feb 19;16(4):202–13.
4. Ducati RG, Ruffino-Netto A, Basso LA, Santos DS. The resumption of consumption -- a review on tuberculosis. *Mem Inst Oswaldo Cruz.* 2006 Nov;101(7):697–714.
5. Guenin-Macé L, Siméone R, Demangel C. Lipids of pathogenic *Mycobacteria*: contributions to virulence and host immune suppression. *Transbound Emerg Dis.* 2009 Aug;56(6–7):255–68.
6. Quigley J, Hughitt VK, Velikovskiy CA, Mariuzza RA, El-Sayed NM, Briken V. The cell wall lipid PDIM contributes to phagosomal escape and host cell exit of *Mycobacterium tuberculosis*. *MBio.* 2017;8(2).
7. Simeone R, Bottai D, Brosch R. ESX/type VII secretion systems and their role in host-pathogen interaction. *Curr Opin Microbiol.* 2009 Feb;12(1):4–10.
8. Wong K-W. The Role of ESX-1 in *Mycobacterium tuberculosis* Pathogenesis. *Microbiol Spectr.* 2017 Jun 8;5(3).
9. Philips JA, Ernst JD. Tuberculosis Pathogenesis and Immunity. *Annu Rev Pathol Mech Dis.* 2012 Feb 28;7:353–84.
10. Ernst JD. Macrophage receptors for *Mycobacterium tuberculosis*. *Infect Immun.* 1998 Apr;66(4):1277–81.
11. Gengenbacher M, Kaufmann SHE. *Mycobacterium tuberculosis*: success through dormancy. *FEMS Microbiol Rev.* 2012 May 1;36(3):514–32.
12. Cadena AM, Fortune SM, Flynn JL. Heterogeneity in tuberculosis. *Nat Rev Immunol.* 2017 Nov 24;17(11):691–702.
13. Lay G, Poquet Y, Salek-Peyron P, Puissegur M-P, Botanch C, Bon H, et al. Langhans giant cells from *M. tuberculosis*-induced human granulomas cannot mediate mycobacterial uptake. *J Pathol.* 2007 Jan 1;211(1):76–85.
14. Peyron P, Vaubourgeix J, Poquet Y, Levillain F, Botanch C, Bardou F, et al. Foamy Macrophages from Tuberculous Patients' Granulomas Constitute a Nutrient-Rich Reservoir for *M. tuberculosis* Persistence. Bishai W, editor. *PLoS Pathog.* 2008 Nov 11;4(11):e1000204.
15. Silva Miranda M, Breiman A, Allain S, Deknuydt F, Altare F. The Tuberculous Granuloma: An Unsuccessful Host Defence Mechanism Providing a Safety Shelter for the Bacteria? *Clin Dev Immunol.* 2012 Jul 3;2012.
16. Rubin EJ. The Granuloma in Tuberculosis — Friend or Foe? *N Engl J Med.* 2009 Jun 4;360(23):2471–3.

17. Xie Z, Siddiqi N, Rubin EJ. Differential antibiotic susceptibilities of starved *Mycobacterium tuberculosis* isolates. *Antimicrob Agents Chemother*. 2005 Nov 1;49(11):4778–80.
18. Lyon SM, Rossman MD. Pulmonary Tuberculosis. *Microbiol Spectr*. 2017 Feb 1;5(1).
19. Yang H, Kruh-Garcia NA, Dobos KM. Purified protein derivatives of tuberculin—past, present, and future. *FEMS Immunol Med Microbiol*. 2012 Dec;66(3):273–80.
20. Lalvani A, Pareek M. Interferon gamma release assays: principles and practice. *Enferm Infecc Microbiol Clin*. 2010 Apr;28(4):245–52.
21. Tiberi S, du Plessis N, Walzl G, Vjecha MJ, Rao M, Ntoumi F, et al. Tuberculosis: progress and advances in development of new drugs, treatment regimens, and host-directed therapies. *Lancet Infect Dis*. 2018 Jul;18(7):e183–98.
22. Delogu G, Sali M, Fadda G. The biology of mycobacterium tuberculosis infection. *Mediterr J Hematol Infect Dis*. 2013 Nov 16;5(1):e2013070.
23. Nusrath Unissa A, Hanna LE. Molecular mechanisms of action, resistance, detection to the first-line anti tuberculosis drugs: Rifampicin and pyrazinamide in the post whole genome sequencing era. *Tuberculosis*. 2017 Jul;105:96–107.
24. Palucci I, Delogu G. Host Directed Therapies for Tuberculosis: Futures Strategies for an Ancient Disease. *Chemotherapy*. 2018;63(3):172–80.
25. Kolloli A, Subbian S. Host-Directed Therapeutic Strategies for Tuberculosis. *Front Med*. 2017 Oct 18;4:171.
26. Barré-Sinoussi F, Chermann JC, Rey F, Nugeyre MT, Chamaret S, Gruest J, et al. Isolation of a T-lymphotropic retrovirus from a patient at risk for acquired immune deficiency syndrome (AIDS). *Science*. 1983 May 20;220(4599):868–71.
27. Fauci AS. 25 years of HIV. *Nature*. 2008;453(7193):289–90.
28. Parekh BS, Ou C-Y, Fonjungo PN, Kalou MB, Rottinghaus E, Puren A, et al. Diagnosis of Human Immunodeficiency Virus Infection. *Clin Microbiol Rev*. 2018 Nov 28;32(1).
29. Shaw GM, Hunter E. HIV Transmission. *Cold Spring Harb Perspect Med*. 2012 Nov 1;2(11):a006965.
30. German Advisory Committee Blood (Arbeitskreis Blut) S 'Assessment of PT by B. Human Immunodeficiency Virus (HIV). *Transfus Med Hemother*. 2016 May;43(3):203–22.
31. Clapham PR, McKnight A. HIV-1 receptors and cell tropism. *Br Med Bull*. 2001;58:43–59.
32. Hladik F, McElrath MJ. Setting the stage: host invasion by HIV. *Nat Rev Immunol*. 2008 Jun 1;8(6):447–57.
33. de Goede AL, Vulto AG, Osterhaus ADME, Gruters RA. Understanding HIV infection for the design of a therapeutic vaccine. Part I: Epidemiology and pathogenesis of HIV infection. *Ann Pharm Françaises*. 2015 Mar;73(2):87–99.

34. Coffin JM, Fan H. The Discovery of Reverse Transcriptase. *Annu Rev Virol*. 2016 Sep 29;3(1):29–51.
35. Wilen CB, Tilton JC, Doms RW. HIV: cell binding and entry. *Cold Spring Harb Perspect Med*. 2012 Aug 1;2(8):a006866.
36. Freed EO. HIV-1 replication. *Somat Cell Mol Genet*. 2001 Nov;26(1–6):13–33.
37. Maartens G, Celum C, Lewin SR. HIV infection: epidemiology, pathogenesis, treatment, and prevention. *Lancet*. 2014 Jul 19;384(9939):258–71.
38. World Health Organization. Updated recommendations on first-line and second-line antiretroviral regimens and post-exposure prophylaxis and recommendations on early infant diagnosis of HIV: interim guidelines. 2018.
39. World Health Organization. WHO | Data and statistics. WHO [Internet]. 2018 [cited 2018 Dec 28]; Available from: <https://www.who.int/hiv/data/en/>
40. World Health Organization. Consolidated guidelines on the use of antiretroviral drugs for treating and preventing HIV infection: recommendations for a public health approach – 2nd ed [Internet]. 2016 [cited 2018 Dec 28]. Available from: http://apps.who.int/iris/bitstream/handle/10665/208825/9789241549684_eng.pdf?sequence=1
41. Selwyn PA, Sckell BM, Alcabes P, Friedland GH, Klein RS, Schoenbaum EE. High risk of active tuberculosis in HIV-infected drug users with cutaneous anergy. *JAMA*. 1992;268(4):504–9.
42. Whalen CC, Nsubuga P, Okwera A, Johnson JL, Hom DL, Michael NL, et al. Impact of pulmonary tuberculosis on survival of HIV-infected adults: a prospective epidemiologic study in Uganda. *AIDS*. 2000 Jun 16;14(9):1219–28.
43. Daley CL, Small PM, Schecter GF, Schoolnik GK, McAdam RA, Jacobs WR, et al. An outbreak of tuberculosis with accelerated progression among persons infected with the human immunodeficiency virus. An analysis using restriction-fragment-length polymorphisms. *N Engl J Med*. 1992 Jan 23;326(4):231–5.
44. de Noronha AL, Báfica A, Nogueira L, Barral A, Barral-Netto M. Lung granulomas from *Mycobacterium tuberculosis*/HIV-1 co-infected patients display decreased in situ TNF production. *Pathol Res Pract*. 2008;204(3):155–61.
45. Goletti D, Weissman D, Jackson RW, Collins F, Kinter A, Fauci AS. The in vitro induction of human immunodeficiency virus (HIV) replication in purified protein derivative-positive HIV-infected persons by recall antigen response to *Mycobacterium tuberculosis* is the result of a balance of the effects of endogenous interleukin. *J Infect Dis*. 1998 May;177(5):1332–8.
46. Lawn SD, Pisell TL, Hirsch CS, Wu M, Butera ST, Toossi Z. Anatomically compartmentalized human immunodeficiency virus replication in HLA-DR+ cells and CD14+ macrophages at the site of pleural tuberculosis coinfection. *J Infect Dis*. 2001 Nov 1;184(9):1127–33.
47. Toossi Z, Johnson JL, Kanost RA, Wu M, Luzze H, Peters P, et al. Increased replication

- of HIV-1 at sites of *Mycobacterium tuberculosis* infection: potential mechanisms of viral activation. *J Acquir Immune Defic Syndr*. 2001 Sep 1;28(1):1–8.
48. Nakata K, Rom WN, Honda Y, Condos R, Kanegasaki S, Cao Y, et al. *Mycobacterium tuberculosis* enhances human immunodeficiency virus-1 replication in the lung. *Am J Respir Crit Care Med*. 1997 Mar;155(3):996–1003.
 49. Geldmacher C, Ngwenyama N, Schuetz A, Petrovas C, Reither K, Heeregrave EJ, et al. Preferential infection and depletion of *Mycobacterium tuberculosis*-specific CD4 T cells after HIV-1 infection. *J Exp Med*. 2010;207(13):2869–81.
 50. Wilkinson KA, Seldon R, Meintjes G, Rangaka MX, Hanekom WA, Maartens G, et al. Dissection of regenerating T-Cell responses against tuberculosis in HIV-infected adults sensitized by *Mycobacterium tuberculosis*. *Am J Respir Crit Care Med*. 2009 Oct 1;180(7):674–83.
 51. Meintjes G, Rabie H, Wilkinson RJ, Cotton MF. Tuberculosis-associated Immune Reconstitution Inflammatory Syndrome and Unmasking of Tuberculosis by Antiretroviral Therapy. *Clin Chest Med*. 2009 Dec;30(4):797–810.
 52. Shankar EM, Vignesh R, Ellegård R, Barathan M, Chong YK, Bador MK, et al. HIV-*Mycobacterium tuberculosis* co-infection: a “danger-couple model” of disease pathogenesis. *Pathog Dis*. 2014 Mar;70(2):110–8.
 53. Jambo KC, Banda DH, Kankwatira AM, Sukumar N, Allain TJ, Heyderman RS, et al. Small alveolar macrophages are infected preferentially by HIV and exhibit impaired phagocytic function. *Mucosal Immunol*. 2014 Sep 29;7(5):1116–26.
 54. Mazzolini J, Herit F, Bouchet J, Benmerah A, Benichou S, Niedergang F. Inhibition of phagocytosis in HIV-1-infected macrophages relies on Nef-dependent alteration of focal delivery of recycling compartments. *Blood*. 2010 May 27;115(21):4226–36.
 55. Dumas A, Lê-Bury G, Marie-Anaïs F, Herit F, Mazzolini J, Guilbert T, et al. The HIV-1 protein Vpr impairs phagosome maturation by controlling microtubule-dependent trafficking. *J Cell Biol*. 2015;211(2):359–72.
 56. Pathak S, Wentzel-Larsen T, Asjo B. Effects of in vitro HIV-1 infection on mycobacterial growth in peripheral blood monocyte-derived macrophages. *Infect Immun*. 2010 Sep 1;78(9):4022–32.
 57. Kumawat K, Pathak SK, Spetz A-L, Kundu M, Basu J. Exogenous Nef is an inhibitor of *Mycobacterium tuberculosis*-induced tumor necrosis factor- α production and macrophage apoptosis. *J Biol Chem*. 2010 Apr 23;285(17):12629–37.
 58. Tomlinson GS, Bell LCK, Walker NF, Tsang J, Brown JS, Breen R, et al. HIV-1 infection of macrophages dysregulates innate immune responses to *Mycobacterium tuberculosis* by inhibition of interleukin-10. *J Infect Dis*. 2014 Apr 1;209(7):1055–65.
 59. Bell LCK, Noursadeghi M. Pathogenesis of HIV-1 and *Mycobacterium tuberculosis* co-infection. *Nat Rev Microbiol*. 2018 Feb;16(2):80–90.
 60. Poli G, Bressler P, Kinter A, Duh E, Timmer WC, Rabson A, et al. Interleukin 6 induces human immunodeficiency virus expression in infected monocytic cells alone and in

- synergy with tumor necrosis factor alpha by transcriptional and post-transcriptional mechanisms. *J Exp Med.* 1990 Jul 1;172(1):151–8.
61. Hoshino Y, Tse DB, Rochford G, Prabhakar S, Hoshino S, Chitkara N, et al. Mycobacterium tuberculosis-induced CXCR4 and chemokine expression leads to preferential X4 HIV-1 replication in human macrophages. *J Immunol.* 2004 May 15;172(10):6251–8.
 62. Juffermans NP, Speelman P, Verbon A, Veenstra J, Jie C, van Deventer SJH, et al. Patients with active tuberculosis have increased expression of HIV coreceptors CXCR4 and CCR5 on CD4+ T cells. *Clin Infect Dis.* 2001 Feb 15;32(4):650–2.
 63. Schorey JS, Schlesinger LS. Innate Immune Responses to Tuberculosis. *Microbiol Spectr.* 2016;4(6).
 64. Cohen SB, Gern BH, Delahaye JL, Adams KN, Plumlee CR, Winkler JK, et al. Alveolar Macrophages Provide an Early Mycobacterium tuberculosis Niche and Initiate Dissemination. *Cell Host Microbe.* 2018 Sep 12;24(3):439–446.e4.
 65. Hussell T, Bell TJ. Alveolar macrophages: plasticity in a tissue-specific context. *Nat Rev Immunol.* 2014 Feb 21;14(2):81–93.
 66. Liu CH, Liu H, Ge B. Innate immunity in tuberculosis: Host defense vs pathogen evasion. *Cell Mol Immunol.* 2017;14(12):963–75.
 67. Schreiber T, Ehlers S, Heitmann L, Rausch A, Mages J, Murray PJ, et al. Autocrine IL-10 induces hallmarks of alternative activation in macrophages and suppresses antituberculosis effector mechanisms without compromising T cell immunity. *J Immunol.* 2009 Jul 15;183(2):1301–12.
 68. Singh V, Kaur C, Chaudhary VK, Rao KVS, Chatterjee S. M. tuberculosis Secretory Protein ESAT-6 Induces Metabolic Flux Perturbations to Drive Foamy Macrophage Differentiation. *Sci Rep.* 2015 Aug 7;5:12906.
 69. Mihret A. The role of dendritic cells in Mycobacterium tuberculosis infection. *Virulence.* 2012 Nov 15;3(7):654–9.
 70. Hanekom WA, Mendillo M, Manca C, Haslett PAJ, Siddiqui MR, Barry C, et al. Mycobacterium tuberculosis inhibits maturation of human monocyte-derived dendritic cells in vitro. *J Infect Dis.* 2003 Jul 15;188(2):257–66.
 71. Hava DL, Wel N van der, Cohen N, Dascher CC, Houben D, León L, et al. Evasion of peptide, but not lipid antigen presentation, through pathogen-induced dendritic cell maturation. *Proc Natl Acad Sci U S A.* 2008 Aug 12;105(32):11281–6.
 72. Mayadas TN, Cullere X, Lowell CA. The multifaceted functions of neutrophils. *Annu Rev Pathol.* 2014 Jan 24;9:181–218.
 73. Warren E, Teskey G, Venketaraman V. Effector Mechanisms of Neutrophils within the Innate Immune System in Response to Mycobacterium tuberculosis Infection. *J Clin Med.* 2017 Feb 7;6(2):E15.
 74. Martineau AR, Newton SM, Wilkinson KA, Kampmann B, Hall BM, Nawroly N, et al.

- Neutrophil-mediated innate immune resistance to mycobacteria. *J Clin Invest*. 2007 Jul 2;117(7):1988–94.
75. Alemán M, García A, Saab MA, de la Barrera SS, Finiasz M, Abbate E, et al. Mycobacterium tuberculosis-induced activation accelerates apoptosis in peripheral blood neutrophils from patients with active tuberculosis. *Am J Respir Cell Mol Biol*. 2002 Nov;27(5):583–92.
 76. Perskvist N, Long M, Stendahl O, Zheng L. Mycobacterium tuberculosis promotes apoptosis in human neutrophils by activating caspase-3 and altering expression of Bax/Bcl-xL via an oxygen-dependent pathway. *J Immunol*. 2002 Jun 15;168(12):6358–65.
 77. Dallenga T, Repnik U, Corleis B, Eich J, Reimer R, Griffiths GW, et al. M. tuberculosis-Induced Necrosis of Infected Neutrophils Promotes Bacterial Growth Following Phagocytosis by Macrophages. *Cell Host Microbe*. 2017 Oct 11;22(4):519–530.e3.
 78. Tan BH, Meinken C, Bastian M, Bruns H, Legaspi A, Ochoa MT, et al. Macrophages acquire neutrophil granules for antimicrobial activity against intracellular pathogens. *J Immunol*. 2006;177(3):1864–71.
 79. Andersson H, Andersson B, Eklund D, Ngoh E, Persson A, Svensson K, et al. Apoptotic neutrophils augment the inflammatory response to Mycobacterium tuberculosis infection in human macrophages. Rottenberg ME, editor. *PLoS One*. 2014 Jul 7;9(7):e101514.
 80. Francis RJ, Butler RE, Stewart GR. Mycobacterium tuberculosis ESAT-6 is a leukocidin causing Ca²⁺ influx, necrosis and neutrophil extracellular trap formation. *Cell Death Dis*. 2014 Oct 16;5:e1474.
 81. Björnsdóttir H, Welin A, Michaëlsson E, Osla V, Berg S, Christenson K, et al. Neutrophil NET formation is regulated from the inside by myeloperoxidase-processed reactive oxygen species. *Free Radic Biol Med*. 2015 Dec;89:1024–35.
 82. Braia C, Hoge V, Stendahl O. Mycobacterium tuberculosis- induced neutrophil extracellular traps activate human macrophages. *J Innate Immun*. 2013;5(6):591–602.
 83. Tailleux L, Schwartz O, Herrmann J-L, Pivert E, Jackson M, Amara A, et al. DC-SIGN is the major Mycobacterium tuberculosis receptor on human dendritic cells. *J Exp Med*. 2003 Jan 6;197(1):121–7.
 84. Stamm CE, Collins AC, Shiloh MU. Sensing of Mycobacterium tuberculosis and consequences to both host and bacillus. *Immunol Rev*. 2015;264(1):204–19.
 85. Geijtenbeek TBH, Van Vliet SJ, Koppel EA, Sanchez-Hernandez M, Vandenbroucke-Grauls CMJE, Appelmek B, et al. Mycobacteria target DC-SIGN to suppress dendritic cell function. *J Exp Med*. 2003 Jan 6;197(1):7–17.
 86. Zenaro E, Donini M, Dusi S. Induction of Th1/Th17 immune response by Mycobacterium tuberculosis: role of dectin-1, mannose receptor, and DC-SIGN. *J Leukoc Biol*. 2009 Dec 1;86(6):1393–401.
 87. Schoenen H, Bodendorfer B, Hitchens K, Manzanero S, Werninghaus K, Nimmerjahn F,

- et al. Cutting edge: Mincle is essential for recognition and adjuvanticity of the mycobacterial cord factor and its synthetic analog trehalose-dibehenate. *J Immunol*. 2010 Mar 15;184(6):2756–60.
88. Franchi L, Warner N, Viani K, Nuñez G. Function of Nod-like receptors in microbial recognition and host defense. *Immunol Rev*. 2009 Jan;227(1):106–28.
 89. Pecora ND, Gehring AJ, Canaday DH, Boom WH, Harding C V. Mycobacterium tuberculosis LprA is a lipoprotein agonist of TLR2 that regulates innate immunity and APC function. *J Immunol*. 2006 Jul 1;177(1):422–9.
 90. Fremont CM, Yeremeev V, Nicolle DM, Jacobs M, Quesniaux VF, Ryffel B. Fatal Mycobacterium tuberculosis infection despite adaptive immune response in the absence of MyD88. *J Clin Invest*. 2004 Dec 15;114(12):1790–9.
 91. Noss EH, Pai RK, Sellati TJ, Radolf JD, Belisle J, Golenbock DT, et al. Toll-like receptor 2-dependent inhibition of macrophage class II MHC expression and antigen processing by 19-kDa lipoprotein of Mycobacterium tuberculosis. *J Immunol*. 2001 Jul 15;167(2):910–8.
 92. Drennan MB, Nicolle D, Quesniaux VJF, Jacobs M, Allie N, Mpagi J, et al. Toll-Like Receptor 2-Deficient Mice Succumb to Mycobacterium tuberculosis Infection. *Am J Pathol*. 2004 Jan 1;164(1):49–57.
 93. Gehring AJ, Dobos KM, Belisle JT, Harding C V, Boom WH. Mycobacterium tuberculosis LprG (Rv1411c): a novel TLR-2 ligand that inhibits human macrophage class II MHC antigen processing. *J Immunol*. 2004 Aug 15;173(4):2660–8.
 94. Domingo-Gonzalez R, Prince O, Cooper A, Khader SA. Cytokines and Chemokines in Mycobacterium tuberculosis Infection. *Microbiol Spectr*. 2016;4(5).
 95. Imperiali FG, Zaninoni A, La Maestra L, Tarsia P, Blasi F, Barcellini W. Increased Mycobacterium tuberculosis growth in HIV-1-infected human macrophages: role of tumour necrosis factor- α . *Clin Exp Immunol*. 2001 Mar;123(3):435–42.
 96. Herbst S, Schaible UE, Schneider BE. Interferon gamma activated macrophages kill mycobacteria by nitric oxide induced apoptosis. Tailleux L, editor. *PLoS One*. 2011 May 2;6(5):e19105.
 97. Via LE, Fratti RA, McFalone M, Pegán-Ramos E, Deretic D, Deretic V. Effects of cytokines on mycobacterial phagosome maturation. *J Cell Sci*. 1998;905(111):897–905.
 98. Travar M, Petkovic M, Verhaz A. Type I, II, and III Interferons: Regulating Immunity to Mycobacterium tuberculosis Infection. *Arch Immunol Ther Exp (Warsz)*. 2016 Feb 11;64(1):19–31.
 99. Wong K-W, Jacobs WR. Mycobacterium tuberculosis exploits human interferon γ to stimulate macrophage extracellular trap formation and necrosis. *J Infect Dis*. 2013 Jul 1;208(1):109–19.
 100. Altfeld M, Gale Jr M. Innate immunity against HIV-1 infection. *Nat Immunol*. 2015 Jun;16(6):554–62.

101. Watson RO, Bell SL, MacDuff DA, Kimmey JM, Diner EJ, Olivas J, et al. The Cytosolic Sensor cGAS Detects Mycobacterium tuberculosis DNA to Induce Type I Interferons and Activate Autophagy. *Cell Host Microbe*. 2015 Jun 10;17(6):811–9.
102. Lee C, Tomkowicz B, Freedman BD, Collman RG. HIV-1 gp120-induced TNF- α production by primary human macrophages is mediated by phosphatidylinositol-3 (PI-3) kinase and mitogen-activated protein (MAP) kinase pathways. *J Leukoc Biol*. 2005 Oct;78(4):1016–23.
103. Duh EJ, Maury WJ, Folks TM, Fauci AS, Rabson AB. Tumor necrosis factor alpha activates human immunodeficiency virus type 1 through induction of nuclear factor binding to the NF-kappa B sites in the long terminal repeat. *Proc Natl Acad Sci U S A*. 1989 Aug;86(15):5974–8.
104. Sawaya BE, Khalili K, Gordon J, Taube R, Amini S. Cooperative interaction between HIV-1 regulatory proteins Tat and Vpr modulates transcription of the viral genome. *J Biol Chem*. 2000 Nov 10;275(45):35209–14.
105. Varin A, Manna SK, Quivy V, Decrion A-Z, Van Lint C, Herbein G, et al. Exogenous Nef protein activates NF-kappa B, AP-1, and c-Jun N-terminal kinase and stimulates HIV transcription in promonocytic cells. Role in AIDS pathogenesis. *J Biol Chem*. 2003 Jan 24;278(4):2219–27.
106. Li W, Xie J. Role of mycobacteria effectors in phagosome maturation blockage and new drug targets discovery. *J Cell Biochem*. 2011;112(10):2688–93.
107. Rink J, Ghigo E, Kalaidzidis Y, Zerial M. Rab conversion as a mechanism of progression from early to late endosomes. *Cell*. 2005;122(5):735–49.
108. Desjardins M, Huber LA, Parton RG, Griffiths G. Biogenesis of phagolysosomes proceeds through a sequential series of interactions with the endocytic apparatus. *J Cell Biol*. 1994;124(5):677–88.
109. Armstrong JA, Hart PD. Response of cultured macrophages to Mycobacterium tuberculosis, with observations on fusion of lysosomes with phagosomes. *J Exp Med*. 1971;134(3).
110. Rajaram MVS, Arnett E, Azad AK, Guirado E, Ni B, Gerberick AD, et al. M. tuberculosis-Initiated Human Mannose Receptor Signaling Regulates Macrophage Recognition and Vesicle Trafficking by FcR γ -Chain, Grb2, and SHP-1. *Cell Rep*. 2017;21(1):126–40.
111. Kang PB, Azad AK, Torrelles JB, Kaufman TM, Beharka A, Tibesar E, et al. The human macrophage mannose receptor directs Mycobacterium tuberculosis lipoarabinomannan-mediated phagosome biogenesis. *J Exp Med*. 2005;202(7):987–99.
112. Ferrari G, Langen H, Naito M, Pieters J. A Coat Protein on Phagosomes Involved in the Intracellular Survival of Mycobacteria. *Cell*. 1999;97(4):435–47.
113. Deghmane A-E, Soualhine H, Bach H, Sendide K, Itoh S, Tam A, et al. Lipoamide dehydrogenase mediates retention of coronin-1 on BCG vacuoles, leading to arrest in phagosome maturation. *J Cell Sci*. 2007;120(16):2796–806.

114. Anand PK, Kaul D. Vitamin D3-dependent pathway regulates TACO gene transcription. *Biochem Biophys Res Commun.* 2003;310(3):876–7.
115. Queval CJ, Song OR, Carralot JP, Saliou JM, Bongiovanni A, Deloison G, et al. *Mycobacterium tuberculosis* Controls Phagosomal Acidification by Targeting CISH-Mediated Signaling. *Cell Rep.* 2017;20(13):3188–98.
116. Pelchen-Matthews A, Kramer B, Marsh M. Infectious HIV-1 assembles in late endosomes in primary macrophages. *J Cell Biol.* 2003;162(3):443–55.
117. Sherer NM, Lehmann MJ, Jimenez-Soto LF, Ingmundson A, Horner SM, Cicchetti G, et al. Visualization of retroviral replication in living cells reveals budding into multivesicular bodies. *Traffic.* 2003;4(11):785–801.
118. Upadhyay S, Mittal E, Philips JA. Tuberculosis and the art of macrophage manipulation. *Pathog Dis.* 2018;76(4).
119. Simeone R, Sayes F, Song O, Gröschel MI, Brodin P, Brosch R, et al. Cytosolic Access of *Mycobacterium tuberculosis*: Critical Impact of Phagosomal Acidification Control and Demonstration of Occurrence In Vivo. *PLoS Pathog.* 2015;11(2):e1004650.
120. de Duve C. The lysosome. *Sci Am.* 1963;208:64–72.
121. Ohsumi Y. Historical landmarks of autophagy research. *Cell Res.* 2014;24(1):9–23.
122. Baba M, Takeshige K, Baba N, Ohsumi Y. Ultrastructural analysis of the autophagic process in yeast: Detection of autophagosomes and their characterization. *J Cell Biol.* 1994;124(6):903–13.
123. Xie Z, Klionsky DJ. Autophagosome formation: Core machinery and adaptations. *Nat Cell Biol.* 2007;9(10):1102–9.
124. Eskelinen EL. Maturation of autophagic vacuoles in Mammalian cells. *Autophagy.* 2005;1(1):1–10.
125. Kang R, Zeh HJ, Lotze MT, Tang D. The Beclin 1 network regulates autophagy and apoptosis. *Cell Death Differ.* 2011;18(4):571–80.
126. Takeshige K, Baba M, Tsuboi S, Noda T, Ohsumi Y. Autophagy in Yeast Demonstrated with Proteinase-deficient Mutants and Conditions for its Induction. *J Cell Biol.* 1992;73(6):346–8.
127. Mizushima N, Noda T, Yoshimori T, Tanaka Y, Ishii T, George MD, et al. A protein conjugation system essential for autophagy. *Nature.* 1998;395(6700):395–8.
128. Klionsky DJ. Look people, “Atg” is an abbreviation for “autophagy-related.” That’s it. *Autophagy.* 2012;105(1):13–4.
129. Mizushima N, Yoshimori T, Ohsumi Y. The Role of Atg Proteins in Autophagosome Formation. *Annu Rev Cell Dev Biol.* 2011;27(1):107–32.
130. Russell RC, Tian Y, Yuan H, Park HW, Chang YY, Kim J, et al. ULK1 induces autophagy by phosphorylating Beclin-1 and activating VPS34 lipid kinase. *Nat Cell Biol.* 2013;15(7):741–50.

131. Mizushima N, Yamamoto A, Hatano M, Kobayashi Y, Kabeya Y, Suzuki K, et al. Dissection of autophagosome formation using Apg5-deficient mouse embryonic stem cells. *J Cell Biol.* 2001;152(4):657–67.
132. Kabeya Y, Mizushima N, Ueno T, Yamamoto A, Kirisako T, Noda T, et al. LC3, a mammalian homologue of yeast Apg8p, is localized in autophagosome membranes after processing. *EMBO J.* 2000;19(21).
133. Mizushima N, Yoshimori T. How to interpret LC3 immunoblotting. *Autophagy.* 2007;3(6):542–5.
134. Klionsky DJ, Abdalla FC, Abeliovich H, Abraham RT, Acevedo-Arozena A, Adeli K, Agholme L, Agnello M, Agostinis P, Aguirre-Ghiso JA, Ahn HJ, Ait-Mohamed O, Ait-Si-Ali S, Akematsu T, Akira S, Al-Younes HM, Al-Zeer MA, Albert ML, Albin RL, Alegre-Abarategui ZB. Guidelines for the use and interpretation of assays for monitoring autophagy. *Autophagy.* 2012;(April):445–544.
135. Yamamoto A, Tagawa Y, Yoshimori T, Moriyama Y, Masaki R, Tashiro Y. Bafilomycin A1 prevents maturation of autophagic vacuoles by inhibiting fusion between autophagosomes and lysosomes in rat hepatoma cell line, H-4-II-E cells. *Cell Struct Funct.* 1998;
136. Mauvezin C, Neufeld TP. Bafilomycin A1 disrupts autophagic flux by inhibiting both V-ATPase-dependent acidification and Ca-P60A/SERCA-dependent autophagosome-lysosome fusion. *Autophagy.* 2015;(August):1437–8.
137. Pankiv S, Clausen TH, Lamark T, Brech A, Bruun JA, Outzen H, et al. p62/SQSTM1 binds directly to Atg8/LC3 to facilitate degradation of ubiquitinated protein aggregates by autophagy. *J Biol Chem.* 2007;282(33):24131–45.
138. Zheng YT, Shahnazari S, Brech A, Lamark T, Johansen T, Brumell JH. The Adaptor Protein p62/SQSTM1 Targets Invading Bacteria to the Autophagy Pathway. *J Immunol.* 2009;183(9):5909–16.
139. Ponpuak M, Davis AS, Roberts EA, Delgado MA, Dinkins C, Zhao Z, et al. Delivery of Cytosolic Components by Autophagic Adapter Protein p62 Endows Autophagosomes with Unique Anti-Microbial Properties. *Immunity.* 2010;32(3):329–41.
140. Kyei GB, Dinkins C, Davis AS, Roberts E, Singh SB, Dong C, et al. Autophagy pathway intersects with HIV-1 biosynthesis and regulates viral yields in macrophages. *J Cell Biol.* 2009;186(2):255–68.
141. Romagnoli A, Etna MP, Giacomini E, Pardini M, Remoli ME, Corazzari M, et al. ESX-1 dependent impairment of autophagic flux by Mycobacterium tuberculosis in human dendritic cells. *Autophagy.* 2012;8(9):1357–70.
142. Duan L, Yi M, Chen J, Li S, Chen W. Mycobacterium tuberculosis EIS gene inhibits macrophage autophagy through up-regulation of IL-10 by increasing the acetylation of histone H3. *Biochem Biophys Res Commun.* 2016;473(4):1229–34.
143. Gutierrez MG, Master SS, Singh SB, Taylor GA, Colombo MI, Deretic V. Autophagy is a defense mechanism inhibiting BCG and Mycobacterium tuberculosis survival in infected macrophages. *Cell.* 2004;119(6):753–66.

144. Campbell GR, Spector SA. Vitamin D inhibits human immunodeficiency virus type 1 and *Mycobacterium tuberculosis* infection in macrophages through the induction of autophagy. *PLoS Pathog.* 2012;8(5):e1002689.
145. Dutta RK, Kathania M, Raje M, Majumdar S. IL-6 inhibits IFN- γ induced autophagy in *Mycobacterium tuberculosis* H37Rv infected macrophages. *Int J Biochem Cell Biol.* 2012 Jun;44(6):942–54.
146. Jagannath C, Lindsey DR, Dhandayuthapani S, Xu Y, Hunter RL, Eissa NT. Autophagy enhances the efficacy of BCG vaccine by increasing peptide presentation in mouse dendritic cells. *Nat Med.* 2009 Mar 1;15(3):267–76.
147. Gupta A, Sharma D, Meena J, Pandya S, Sachan M, Kumar S, et al. Preparation and Preclinical Evaluation of Inhalable Particles Containing Rapamycin and Anti-Tuberculosis Agents for Induction of Autophagy. *Pharm Res.* 2016 Aug 19;33(8):1899–912.
148. Rekha RS, Rao Muvva SJ, Wan M, Raqib R, Bergman P, Brighenti S, et al. Phenylbutyrate induces LL-37-dependent autophagy and intracellular killing of *Mycobacterium tuberculosis* in human macrophages. *Autophagy.* 2015 Sep 2;11(9):1688–99.
149. Mily A, Rekha RS, Kamal SMM, Akhtar E, Sarker P, Rahim Z, et al. Oral intake of phenylbutyrate with or without vitamin D3 upregulates the cathelicidin LL-37 in human macrophages: a dose finding study for treatment of tuberculosis. *BMC Pulm Med.* 2013 Dec 16;13:23.
150. Yuk J-M, Shin D-M, Lee H-M, Yang C-S, Jin HS, Kim K-K, et al. Vitamin D3 Induces Autophagy in Human Monocytes/Macrophages via Cathelicidin. *Cell Host Microbe.* 2009 Sep 17;6(3):231–43.
151. Eklund D, Persson HL, Larsson M, Welin A, Idh J, Paues J, et al. Vitamin D enhances IL-1 β secretion and restricts growth of *Mycobacterium tuberculosis* in macrophages from TB patients. *Int J Mycobacteriology.* 2013 Mar;2(1):18–25.
152. Paik S, Kim JK, Chung C, Jo E-K. Autophagy: A new strategy for host-directed therapy of tuberculosis. *Virulence.* 2018;1–12.
153. Wejse C, Gomes VF, Rabna P, Gustafson P, Aaby P, Lisse IM, et al. Vitamin D as supplementary treatment for tuberculosis: a double-blind, randomized, placebo-controlled trial. *Am J Respir Crit Care Med.* 2009 May 20;179(9):843–50.
154. Mily A, Rekha RS, Kamal SMM, Arifuzzaman ASM, Rahim Z, Khan L, et al. Significant Effects of Oral Phenylbutyrate and Vitamin D3 Adjunctive Therapy in Pulmonary Tuberculosis: A Randomized Controlled Trial. *PLoS One.* 2015 Sep 22;10(9):e0138340.
155. Winterbourn CC. Reconciling the chemistry and biology of reactive oxygen species. *Nat Chem Biol.* 2008 May 1;4(5):278–86.
156. Dickinson BC, Chang CJ. Chemistry and biology of reactive oxygen species in signaling or stress responses. *Nat Chem Biol.* 2011 Aug 1;7(8):504–11.
157. Bedard K, Krause K-H. The NOX Family of ROS-Generating NADPH Oxidases:

- Physiology and Pathophysiology. *Physiol Rev.* 2007 Jan;87(1):245–313.
158. Savina A, Jancic C, Hugues S, Guermontprez P, Vargas P, Moura IC, et al. NOX2 Controls Phagosomal pH to Regulate Antigen Processing during Crosspresentation by Dendritic Cells. *Cell.* 2006;126(1):205–18.
 159. Deffert C, Cachat J, Krause K-H. Phagocyte NADPH oxidase, chronic granulomatous disease and mycobacterial infections. *Cell Microbiol.* 2014 Aug;16(8):1168–78.
 160. Shastri MD, Shukla SD, Chong WC, Dua K, Peterson GM, Patel RP, et al. Role of Oxidative Stress in the Pathology and Management of Human Tuberculosis. *Oxid Med Cell Longev.* 2018 Oct 11;2018:7695364.
 161. Ng VH, Cox JS, Sousa AO, MacMicking JD, McKinney JD. Role of KatG catalase-peroxidase in mycobacterial pathogenesis: Countering the phagocyte oxidative burst. *Mol Microbiol.* 2004;52(5):1291–302.
 162. Tyagi P, Dharmaraja AT, Bhaskar A, Chakrapani H, Singh A. Mycobacterium tuberculosis has diminished capacity to counteract redox stress induced by elevated levels of endogenous superoxide. *Free Radic Biol Med.* 2015;84:344–54.
 163. Voskuil MI, Bartek IL, Visconti K, Schoolnik GK. The response of mycobacterium tuberculosis to reactive oxygen and nitrogen species. *Front Microbiol.* 2011;2:105.
 164. Miller JL, Velmurugan K, Cowan MJ, Briken V. The type I NADH dehydrogenase of Mycobacterium tuberculosis counters phagosomal NOX2 activity to inhibit TNF-alpha-mediated host cell apoptosis. Deretic V, editor. *PLoS Pathog.* 2010 Apr 22;6(4):e1000864.
 165. de Quay B, Malinverni R, Lauterburg BH. Glutathione depletion in HIV-infected patients: role of cysteine deficiency and effect of oral N-acetylcysteine. *AIDS.* 1992 Aug;6(8):815–9.
 166. Treitinger A, Spada C, Verdi JC, Miranda AF, Oliveira O V, Silveira M V, et al. Decreased antioxidant defence in individuals infected by the human immunodeficiency virus. *Eur J Clin Invest.* 2000 May;30(5):454–9.
 167. Elbim C, Pillet S, Prevost MH, Preira A, Girard PM, Rogine N, et al. Redox and activation status of monocytes from human immunodeficiency virus-infected patients: relationship with viral load. *J Virol.* 1999 Jun;73(6):4561–6.
 168. Couret J, Chang TL. Reactive Oxygen Species in HIV Infection. *EC Microbiol.* 2016;3(6):597–604.
 169. Zhang H-S, Sang W-W, Ruan Z, Wang Y-O. Akt/Nox2/NF-κB signaling pathway is involved in Tat-induced HIV-1 long terminal repeat (LTR) transactivation. *Arch Biochem Biophys.* 2011 Jan 15;505(2):266–72.
 170. Kurata S i. Sensitization of the HIV-1-LTR upon long term low dose oxidative stress. *J Biol Chem.* 1996 Sep 6;271(36):21798–802.
 171. Ho W-Z, Douglas SD. Glutathione and N-Acetylcysteine Suppression of Human Immunodeficiency Virus Replication in Human Monocyte/Macrophages In Vitro. *AIDS*

- Res Hum Retroviruses. 1992 Jul;8(7):1249–53.
172. Duprez L, Wirawan E, Vanden Berghe T, Vandenabeele P. Major cell death pathways at a glance. *Microbes Infect.* 2009 Nov;11(13):1050–62.
 173. Huynh M-LN, Fadok VA, Henson PM. Phosphatidylserine-dependent ingestion of apoptotic cells promotes TGF-beta1 secretion and the resolution of inflammation. *J Clin Invest.* 2002 Jan 1;109(1):41–50.
 174. Andersson H, Andersson B, Eklund D, Ngoh E, Persson A, Svensson K, et al. Apoptotic neutrophils augment the inflammatory response to *Mycobacterium tuberculosis* infection in human macrophages. *PLoS One.* 2014;9(7):e101514.
 175. 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(3):289–300.
 176. Lee J, Hartman M, Kornfeld H. Macrophage apoptosis in tuberculosis. *Yonsei Med J.* 2009 Feb 28;50(1):1–11.
 177. Molloy A, Laochumroonvorapong P, Kaplan G. Apoptosis, but not necrosis, of infected monocytes is coupled with killing of intracellular bacillus Calmette-Guérin. *J Exp Med.* 1994 Oct 1;180(4):1499–509.
 178. Chen M, Gan H, Remold HG. A mechanism of virulence: virulent *Mycobacterium tuberculosis* strain H37Rv, but not attenuated H37Ra, causes significant mitochondrial inner membrane disruption in macrophages leading to necrosis. *J Immunol.* 2006 Mar 15;176(6):3707–16.
 179. Keane J, Balcewicz-Sablinska MK, Remold HG, Chupp GL, Meek BB, Fenton MJ, et al. Infection by *Mycobacterium tuberculosis* promotes human alveolar macrophage apoptosis. *Infect Immun.* 1997 Jan;65(1):298–304.
 180. Balcewicz-Sablinska MK, Keane J, Kornfeld H, Remold HG. Pathogenic *Mycobacterium tuberculosis* evades apoptosis of host macrophages by release of TNF-R2, resulting in inactivation of TNF-alpha. *J Immunol.* 1998 Sep 1;161(5):2636–41.
 181. Oddo M, Renno T, Attinger A, Bakker T, MacDonald HR, Meylan PR. Fas ligand-induced apoptosis of infected human macrophages reduces the viability of intracellular *Mycobacterium tuberculosis*. *J Immunol.* 1998 Jun 1;160(11):5448–54.
 182. Sly LM, Hingley-Wilson SM, Reiner NE, McMaster WR. Survival of *Mycobacterium tuberculosis* in host macrophages involves resistance to apoptosis dependent upon induction of antiapoptotic Bcl-2 family member Mcl-1. *J Immunol.* 2003 Jan 1;170(1):430–7.
 183. Timilsina U, Gaur R. Modulation of apoptosis and viral latency – an axis to be well understood for successful cure of human immunodeficiency virus. *J Gen Virol.* 2016 Apr;97(4):813–24.
 184. Jasenosky LD, Scriba TJ, Hanekom WA, Goldfeld AE. T cells and adaptive immunity to *Mycobacterium tuberculosis* in humans. *Immunol Rev.* 2015 Mar 1;264(1):74–87.

185. Neefjes J, Jongsma MLM, Paul P, Bakke O. Towards a systems understanding of MHC class I and MHC class II antigen presentation. *Nat Rev Immunol*. 2011 Dec 11;11(12):823–36.
186. Kapsenberg ML. Dendritic-cell control of pathogen-driven T-cell polarization. *Nat Rev Immunol*. 2003 Dec 1;3(12):984–93.
187. Khan N, Gowthaman U, Pahari S, Agrewala JN. Manipulation of costimulatory molecules by intracellular pathogens: veni, vidi, vici!! Hobman TC, editor. *PLoS Pathog*. 2012;8(6):e1002676.
188. Larsson M, Shankar EM, Che KF, Saeidi A, Ellegård R, Barathan M, et al. Molecular signatures of T-cell inhibition in HIV-1 infection. *Retrovirology*. 2013 Mar 20;10:31.
189. Chaudhry A, Das SR, Hussain A, Mayor S, George A, Bal V, et al. The Nef protein of HIV-1 induces loss of cell surface costimulatory molecules CD80 and CD86 in APCs. *J Immunol*. 2005 Oct 1;175(7):4566–74.
190. Chirmule N, McCloskey TW, Hu R, Kalyanaraman VS, Pahwa S. HIV gp120 inhibits T cell activation by interfering with expression of costimulatory molecules CD40 ligand and CD80 (B71). *J Immunol*. 1995 Jul 15;155(2):917–24.
191. Chougnet C, Wynn TA, Clerici M, Landay AL, Kessler HA, Rusnak J, et al. Molecular analysis of decreased interleukin-12 production in persons infected with human immunodeficiency virus. *J Infect Dis*. 1996 Jul;174(1):46–53.
192. Srivastava S, Grace PS, Ernst JD. Antigen Export Reduces Antigen Presentation and Limits T Cell Control of *M. tuberculosis*. *Cell Host Microbe*. 2016 Jan 13;19(1):44–54.
193. Pai RK, Convery M, Hamilton TA, Boom WH, Harding C V. Inhibition of IFN-gamma-induced class II transactivator expression by a 19-kDa lipoprotein from *Mycobacterium tuberculosis*: a potential mechanism for immune evasion. *J Immunol*. 2003 Jul 1;171(1):175–84.
194. Choi H-G, Kim WS, Back YW, Kim H, Kwon KW, Kim J-S, et al. *Mycobacterium tuberculosis* RpfE promotes simultaneous Th1- and Th17-type T-cell immunity via TLR4-dependent maturation of dendritic cells. *Eur J Immunol*. 2015 Jul 1;45(7):1957–71.
195. Jiang L, Liu G, Ni W, Zhang N, Jie J, Xie F, et al. The Combination of MBP and BCG-Induced Dendritic Cell Maturation through TLR2/TLR4 Promotes Th1 Activation In Vitro and Vivo. *Mediators Inflamm*. 2017 Feb 15;2017:1953680.
196. Baena A, Porcelli SA. Evasion and subversion of antigen presentation by *Mycobacterium tuberculosis*. *Tissue Antigens*. 2009 Sep 1;74(3):189–204.
197. Hinchey J, Lee S, Jeon BY, Basaraba RJ, Venkataswamy MM, Chen B, et al. Enhanced priming of adaptive immunity by a proapoptotic mutant of *Mycobacterium tuberculosis*. *J Clin Invest*. 2007 Aug 1;117(8):2279–88.
198. Lyadova I V., Panteleev A V. Th1 and Th17 Cells in Tuberculosis: Protection, Pathology, and Biomarkers. *Mediators Inflamm*. 2015 Nov 10;2015:854507.

199. Harding C V., Boom WH. Regulation of antigen presentation by *Mycobacterium tuberculosis*: a role for Toll-like receptors. *Nat Rev Microbiol.* 2010 Apr 1;8(4):296–307.
200. Zhang M, Gong J, Iyer D V, Jones BE, Modlin RL, Barnes PF. T cell cytokine responses in persons with tuberculosis and human immunodeficiency virus infection. *J Clin Invest.* 1994 Dec 1;94(6):2435–42.
201. Reuter MA, Pecora ND, Harding C V, Canaday DH, McDonald D. *Mycobacterium tuberculosis* promotes HIV trans-infection and suppresses major histocompatibility complex class II antigen processing by dendritic cells. *J Virol.* 2010 Sep 1;84(17):8549–60.
202. Chen X, Zhou B, Li M, Deng Q, Wu X, Le X, et al. CD4+CD25+FoxP3+ regulatory T cells suppress *Mycobacterium tuberculosis* immunity in patients with active disease. *Clin Immunol.* 2007 Apr 1;123(1):50–9.
203. Cho S, Mehra V, Thoma-Uszynski S, Stenger S, Serbina N, Mazzaccaro RJ, et al. Antimicrobial activity of MHC class I-restricted CD8+ T cells in human tuberculosis. *Proc Natl Acad Sci U S A.* 2000 Dec 21;97(22):12210–5.
204. Koup RA, Safrit JT, Cao Y, Andrews CA, McLeod G, Borkowsky W, et al. Temporal association of cellular immune responses with the initial control of viremia in primary human immunodeficiency virus type 1 syndrome. *J Virol.* 1994 Jul;68(7):4650–5.
205. Tomiyama H, Akari H, Adachi A, Takiguchi M. Different effects of Nef-mediated HLA class I down-regulation on human immunodeficiency virus type 1-specific CD8(+) T-cell cytolytic activity and cytokine production. *J Virol.* 2002 Aug 1;76(15):7535–43.
206. Swann SA, Williams M, Story CM, Bobbitt KR, Fleis R, Collins KL. HIV-1 Nef blocks transport of MHC class I molecules to the cell surface via a PI 3-kinase-dependent pathway. *Virology.* 2001 Apr 10;282(2):267–77.
207. Lundquist CA, Tobiume M, Zhou J, Unutmaz D, Aiken C. Nef-mediated downregulation of CD4 enhances human immunodeficiency virus type 1 replication in primary T lymphocytes. *J Virol.* 2002 May 1;76(9):4625–33.
208. Miller MD, Warmerdam MT, Gaston I, Greene WC, Feinberg MB. The human immunodeficiency virus-1 nef gene product: a positive factor for viral infection and replication in primary lymphocytes and macrophages. *J Exp Med.* 1994 Jan 1;179(1):101–13.
209. Benson RE, Sanfridson A, Ottinger JS, Doyle C, Cullen BR. Downregulation of cell-surface CD4 expression by simian immunodeficiency virus Nef prevents viral super infection. *J Exp Med.* 1993 Jun 1;177(6):1561–6.
210. Boudet F, Lecoœur H, Gougeon ML. Apoptosis associated with ex vivo down-regulation of Bcl-2 and up-regulation of Fas in potential cytotoxic CD8+ T lymphocytes during HIV infection. *J Immunol.* 1996 Mar 15;156(6):2282–93.
211. Day CL, Kaufmann DE, Kiepiela P, Brown JA, Moodley ES, Reddy S, et al. PD-1 expression on HIV-specific T cells is associated with T-cell exhaustion and disease progression. *Nature.* 2006 Sep 20;443(7109):350–4.

212. Thoreen CC, Kang SA, Chang JW, Liu Q, Zhang J, Gao Y, et al. An ATP-competitive mammalian target of rapamycin inhibitor reveals rapamycin-resistant functions of mTORC1. *J Biol Chem*. 2009 Mar 20;284(12):8023–32.
213. Zulauf KE, Sullivan JT, Braunstein M. The SecA2 pathway of *Mycobacterium tuberculosis* exports effectors that work in concert to arrest phagosome and autophagosome maturation. Behr MA, editor. *PLOS Pathog*. 2018 Apr 30;14(4):e1007011.
214. Romagnoli A, Etna MP, Giacomini E, Pardini M, Remoli ME, Corazzari M, et al. ESX-1 dependent impairment of autophagic flux by *Mycobacterium tuberculosis* in human dendritic cells. *Autophagy*. 2012 Sep 14;8(9):1357–70.
215. Deretic V. Autophagy in innate and adaptive immunity. *Trends Immunol*. 2005 Oct;26(10):523–8.
216. Liu D, Wu H, Wang C, Li Y, Tian H, Siraj S, et al. STING directly activates autophagy to tune the innate immune response. *Cell Death Differ*. 2018 Dec 19;
217. Liang Q, Seo GJ, Choi YJ, Kwak M-J, Ge J, Rodgers MA, et al. Crosstalk between the cGAS DNA sensor and Beclin-1 autophagy protein shapes innate antimicrobial immune responses. *Cell Host Microbe*. 2014 Feb 12;15(2):228–38.
218. Hartman ML, Kornfeld H. Interactions between naïve and infected macrophages reduce *Mycobacterium tuberculosis* viability. *PLoS One*. 2011;6(11):e27972.
219. Odobasic D, Kitching AR, Holdsworth SR. Neutrophil-Mediated Regulation of Innate and Adaptive Immunity: The Role of Myeloperoxidase. *J Immunol Res*. 2016;2016:2349817.
220. Maiuri MC, Zalckvar E, Kimchi A, Kroemer G. Self-eating and self-killing: crosstalk between autophagy and apoptosis. *Nat Rev Mol Cell Biol*. 2007 Sep 1;8(9):741–52.
221. Chandra P, Kumar D. Selective autophagy gets more selective: Uncoupling of autophagy flux and xenophagy flux in *Mycobacterium tuberculosis*-infected macrophages. *Autophagy*. 2016 Mar 3;12(3):608–9.
222. Chandra P, Ghanwat S, Matta SK, Yadav SS, Mehta M, Siddiqui Z, et al. *Mycobacterium tuberculosis* Inhibits RAB7 Recruitment to Selectively Modulate Autophagy Flux in Macrophages. *Sci Rep*. 2015 Nov 6;5:16320.
223. Fabri M, Stenger S, Shin D-M, Yuk J-M, Liu PT, Realegeno S, et al. Vitamin D is required for IFN-gamma-mediated antimicrobial activity of human macrophages. *Sci Transl Med*. 2011 Oct 12;3(104):104ra102.
224. Petruccioli E, Romagnoli A, Corazzari M, Coccia EM, Butera O, Delogu G, et al. Specific T Cells Restore the Autophagic Flux Inhibited by *Mycobacterium tuberculosis* in Human Primary Macrophages. *J Infect Dis*. 2012 May 1;205(9):1425–35.
225. Min Y, Xu W, Liu D, Shen H, Xu Y, Zhang S, et al. Earle's balanced salts solution and rapamycin differentially regulate the *Bacillus Calmette-Guerin*-induced maturation of human dendritic cells. *Acta Biochim Biophys Sin (Shanghai)*. 2013 Mar 1;45(3):162–9.

226. Espinosa-Cueto P, Magallanes-Puebla A, Castellanos C, Mancilla R. Dendritic cells that phagocytose apoptotic macrophages loaded with mycobacterial antigens activate CD8 T cells via cross-presentation. Eugenin EA, editor. PLoS One. 2017 Aug 2;12(8):e0182126.
227. Schaible UE, Winau F, Sieling PA, Fischer K, Collins HL, Hagens K, et al. Apoptosis facilitates antigen presentation to T lymphocytes through MHC-I and CD1 in tuberculosis. Nat Med. 2003 Aug 20;9(8):1039–46.
228. Blomgran R, Ernst JD. Lung neutrophils facilitate activation of naive antigen-specific CD4+ T cells during Mycobacterium tuberculosis infection. J Immunol. 2011;186(12):7110–9.
229. Blomgran R, Desvignes L, Briken V, Ernst JD. Mycobacterium tuberculosis inhibits neutrophil apoptosis, leading to delayed activation of naive CD4 T cells. Cell Host Microbe. 2012 Jan 19;11(1):81–90.
230. Aratani Y. Myeloperoxidase: Its role for host defense, inflammation, and neutrophil function. Arch Biochem Biophys. 2018;640:47–52.
231. Odobasic D, Kitching AR, Yang Y, O’Sullivan KM, Muljadi RCM, Edgton KL, et al. Neutrophil myeloperoxidase regulates T-cell-driven tissue inflammation in mice by inhibiting dendritic cell function. Blood. 2013 May 16;121(20):4195–204.
232. Saeidi A, Chong YK, Yong YK, Tan HY, Barathan M, Rajarajeswaran J, et al. Concurrent loss of co-stimulatory molecules and functional cytokine secretion attributes leads to proliferative senescence of CD8+ T cells in HIV/TB co-infection. Cell Immunol. 2015 Sep;297(1):19–32.
233. Palma C, Iona E, Giannoni F, Pardini M, Brunori L, Orefici G, et al. The Ag85B protein of Mycobacterium tuberculosis may turn a protective immune response induced by Ag85B-DNA vaccine into a potent but non-protective Th1 immune response in mice. Cell Microbiol. 2007 Jun;9(6):1455–65.
234. Karbalaee Zadeh Babaki M, Soleimanpour S, Rezaee SA. Antigen 85 complex as a powerful Mycobacterium tuberculosis immunogene: Biology, immune-pathogenicity, applications in diagnosis, and vaccine design. Microb Pathog. 2017 Nov;112:20–9.
235. Singh SK, Larsson M, Schön T, Stendahl O, Blomgran R. HIV Interferes with the Dendritic Cell–T Cell Axis of Macrophage Activation by Shifting Mycobacterium tuberculosis–Specific CD4 T Cells into a Dysfunctional Phenotype. J Immunol. 2019 Feb 1;202(3):816–26.

ACKNOWLEDGEMENTS

First of all I would like to thank my main supervisor **Robert Blomgran** for taking me in as a master student and for giving me the opportunity to start a PhD. I have learned a lot during these years and you have helped me to grow as a scientist.

My co-supervisor **Marie Larsson**, thank you for agreeing to stand in as my main supervisor in the beginning of my PhD and giving me the chance to start earlier. Your advice and input during these years have been much appreciated.

My co-supervisor **Olle Stendahl**, thank you for all the discussions and advice during my PhD and for taking time to read manuscripts and giving valuable comments.

I'd like to give a great thanks to all the group members throughout the years:

Christoffer, I'm happy to have worked together with you when I first came to the lab as a student. We were a great team and I thank you for your help in reaching me stuff from the high shelves. **Naomi**, you were truly a cheerful companion to work with. **Susmita**, thank you for being such a positive and patient lab partner. I enjoyed working together with you and I think we both learned a lot during this experience. **Giggil**, you were one of the first master students I supervised, and I learned a lot from that experience. **Shambhuprasad**, thank you for sharing fun stories about India and Paris as well as for scientific discussions.

I would also like to thank **Thomas, Mickan**, and **Maria** together with group members for good discussions during journal clubs and meetings. **Blanka**, I'm so happy I got to share office with you throughout my PhD. Your hard work as a manager of the TB lab and your willingness to help others is admirable. Thank you for all the discussions and laughs. **Johanna**, thank you for always, with a smile on your face, been willing to give advice, or help me to solve problems. **Danne**, thank you for first introducing me to microscopy. **Sadaf**, especially thanks for helping me figure out all the paperwork needed for defending, but also for all the discussions and laughs. **Ramana** and **Deepti** you always had a big scientific interest and I thank you for all the discussions. **Clara**, thank you for always being happy to help even with the small things. **Jyotirmoy**, thanks for all the help with the IncuCyte and for patiently explaining bioinformatics at journal clubs, I understood some of it. **Hansi**, thank you for all the nice discussions. **Nina**, your clinical point of view has been refreshing and I always enjoy your company and talking about science, life and everything in between. Also, great thanks for making me discover that running is actually fun! We'll see about orientation...

While working in the TB lab, we are often taking help from the clinical lab for autoclaving etc. and I'd like to send a big thanks to all of the staff (**Maria, Vita, Sara, Erika, Martin, Malin** and **Mickan**) for their patience and kindness.

During my PhD I also got the opportunity to teach different student groups about microbiology lab work and there are several people I would like to thank for being there with me:

Tony, I'm very happy I met you and got the chance to learn more about different bacteria and how to teach from you. I think your commitment inspired all of us! **Elena**, we have held several

labs together and I think we have had a great team-work both in planning, preparations and during the labs, thanks for making it a fun experience. Even after repeating a lab seven times in a row, it was still fun to do it together with you **Peter**, keep it up!

There are many people at Medical Microbiology I would like to thank for making it a nice and enjoyable work place, but most of all thanks to **Mary**. What would the department be without you? Thank you **Lena, Martin, Felipe** and **Amaia** for always spreading positive energy around you. Thank you **Vesa** for teaching me more about microscopy. And a great thanks to all the others for nice conversations at fika, lunch and afterworks.

On the side of my PhD studies I have also been studying Spanish for a couple of years. And I'd like to thank my teachers for my progress but most of all I'm grateful to my fellow student **Richard** for helping me, motivating me and always convincing me to continue although it at times felt a bit overwhelming. ¡Muchas gracias!

I would like to thank all my friends who have been beside me during these years (or longer) and supported me and helped me enjoy the simple things in life outside of research. **Tanja**, du är min äldsta vän och jag är så tacksam att ha någon som dig i mitt liv. Jag uppskattar att jag kan dela allt med dig, stort som smått och att du alltid finns där för mig. **Viktoria**, I'm so happy we quickly became friends during the master and afterwards became travel buddies. I really enjoyed all of our adventures together and sharing PhD experiences. I have no doubt you will rock your thesis defense! **Conny**, thank you for being such a good friend and always want to listen and share experiences about research and life. And of course thanks to both you and **Sofia** for all the times of delicious and creative baking, it's always great fun. **Diana** and **Andrea**, it's so nice to spend time with you both and I have enjoyed all the brunches, lunches, movies and travels together. I wish you both the best in life. **Adine**, tack för alla promenader och långa samtal, det är alltid lika roligt och uppfriskande.

Tack till min familj som alltid funnits där för att lyssna, stötta mig, och tro på att jag kan klara allt jag tar mig för. Det känns alltid skönt att ta en paus och kunna komma hem-hem för en helg och bara slappna av.

To **Alejandro**, I never thought I would be lucky enough to find (and keep!) such a wonderful and caring partner as you. I'm so very grateful for all your love, support and advice during this journey. Now I'm finishing one chapter in my life but I know that you and I have many more to look forward to. Te quiero <3

Papers

The papers associated with this thesis have been removed for copyright reasons. For more details about these see:

<http://urn.kb.se/resolve?urn=urn:nbn:se:liu:diva-154605>

FACULTY OF MEDICINE AND HEALTH SCIENCES

Linköping University Medical Dissertation No. 1659, 2019
Department of Clinical and Experimental Medicine

Linköping University
SE-581 83 Linköping, Sweden

www.liu.se