Investigations on the role of impaired lysosomes of macrophages in disease

Lienden, M.J.C. van der

General introduction

Lysosome function and macrophage lysosomes

The lysosome is a membrane enclosed organelle that contains at least 60 hydrolases and mediates degradation of macromolecules. Macromolecules are delivered to lysosomes through endocytosis, macropinocytosis, phagocytosis and autophagy. Hydrolysis of macromolecules is generally facilitated by low pH. Consistently, the luminal pH of the lysosome ranges between 4.0 and 5.0 and is generated at the expense of ATP via the lysosomal proton pump. This allows for optimal activity of lysosomal hydrolases. The lysosomal enzymes are in turn subjected to proteolytic degradation in lysosomes. Their ongoing synthesis and delivery to lysosomes is therefore crucial. Upon synthesis and N-glycosylation in the endoplasmic reticulum (ER), lysosomal enzymes traverse the Golgi apparatus and are sorted to lysosomes by shuttling protein receptors that recognize lysosomal sorting motifs. The major sorting mechanism for soluble acid hydrolases is via mannose-6-phosphate receptors, which recognize unique mannose-6-phosphate moieties on lysosomal glycoproteins attached in the Golgi system. In specific cells alternative lysosomal routing occurs, for example via progranulin and sortillin.[1] A fraction of most soluble acid hydrolases is secreted by cells and may subsequently be delivered to lysosomes by receptor-mediated endocytosis. The mannose-6-phosphate receptor and additional receptors can mediate this re-uptake.[1]

Glucocerebosidase (GBA)

One of the lysosomal hydrolases is the retaining beta-glucosidase named glucocerebrosidase, encoded by the *GBA* gene. GBA catalyses the fragmentation of the ubiquitous glycosphingolipid glucosylceramide (GlcCer) in lysosomes (**Figure 1**). The enzyme is assisted in its activity by saposin C, which promotes activity towards lipid substrate. GBA is uniquely sorted to lysosomes by binding to the membrane protein LIMP2 in the ER. Together, these proteins are routed to lysosomes where the acidic environment triggers dissociation of the complex. GBA can be detected and visualized by means of its enzymatic activity towards fluorogenic substrates. GBA-specific antisera also available. Relatively novel detection methods are fluorescent activity-based probes (ABPs) that allow selective labelling and visualization of catalytically active GBA molecules in cells and organisms.[2–5] These ABPs are based on a cyclophellitol scaffold to which a fluorophore is covalently attached. Cyclophellitol, and the related conduritol B epoxide, are known suicide inhibitors of GBA that covalently link to its catalytic nucleophile, glutamate 340, in mechanism-based manner. Modified cyclophellitols with a hydrophobic extension at C6 have been designed as suicide inhibitors, allowing generation of enzyme deficiency on demand.[6]

Lysosomal storage disease (LSD)

Deficiency of GBA due to mutations in the encoding gene forms the molecular basis of Gaucher disease (GD).[7] In rare cases, GBA activity is reduced due to inherited defects in LIMP2 (Action Myoclonus Renal Failure Syndrome; AMRF), or in saposin C or its precursor protein prosaposin.[8–10] The clinical presentation of GBA deficiency in GD patients is remarkably heterogeneous and ranges from neonatal lethality due to skin barrier abnormalities to a virtual asymptomatic course of disease.[11,12] The GBA genotype and concomitant residual enzyme activity correlates to some extent with severity of disease but is only partly predictive in milder cases that do not develop neurological complications, the so-called type 1 variant of GD.[13,14] A hallmark of GD is accumulation

of GlcCer in storage tubules inside lysosomes of tissue macrophages that transform into swollen Gaucher cells. These lipid-laden macrophages are viable and show features of alternatively activated macrophages.[15] Gaucher cells are thought to underly typical GD symptoms such as hepatosplenomegaly and hematologic abnormalities, but the pathophysiological basis for other symptoms (e.g. neurological complications and osteoporosis) is still enigmatic. Adaptive metabolism of GlcCer during GBA deficiency is considered to produce toxic factors such as glucosylsphingosine.[5,16]

Inherited deficiencies of other lysosomal enzymes also occur and lead to lysosomal accumulation of their corresponding substrates in one or many cell types. These disorders, more than 60 discrete entities, are collectively named lysosomal storage diseases (LSDs) and constitute a major part of the inherited metabolic disorders.[17,18] Lysosomal storage may also be caused by defects in lysosomal proteins that are responsible for metabolite export or by defects in accessory proteins that assist lysosomal hydrolases in their activity or stability. One example of this is Niemann Pick disease type C (NPC), which is caused by inherited defects in either the lysosomal transporter NPC1 or NPC2, proteins involved in the export of cholesterol from the lysosome to the ER.[19,20] NPC cells show a lysosomal accumulation of cholesterol that is accompanied by a secondary, partial deficiency of GBA.[21]

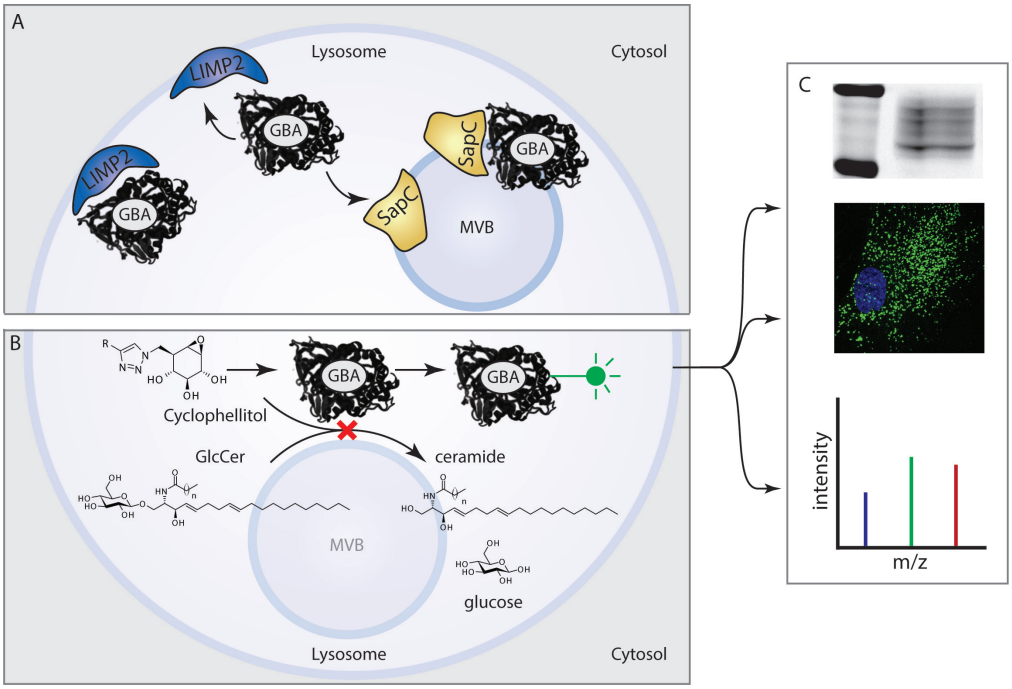

***Figure 1. Localisation and function of GBA in the lysosome**; **(A)** Lysosomal targeting of GBA is dependent on LIMP2. Luminal pH facilitates dissociation of the GBA-LIMP2 complex. GBA is assisted by Saposin C in its hydrolytic function at intralumenal membranes. **(B)** GBA catalyses the hydrolysis of glucosylceramide into ceramide and glucose. Cyclophellitol competes with the endogenous substrate of GBA and binds irreversibly in the active site. To visualize or isolate active GBA, cyclophellitol can be attached via a linker (R) to a detection group such as a fluorophore or a biotin. **(C)** Properties of GBA can be analysed through several methods including SDS-PAGE, fluorescence microscopy*

and LC-MS/MS (depicted from top to bottom); GBA, glucocerebrosidase; LIMP2, lysosomal integral protein 2; SapC, saposin C; GlcCer, glucosylceramide, MVB, multivesicular body.

Regulation of lysosomes

Cells contain on average 100-200 lysosomes which can change in composition and position.[22] A fraction of a cell's lysosomes is found in the periphery and controlled movement towards the nucleus can occur along microtubules. Vice versa, the large proportion of perinuclear lysosomes reside near the microtubule-organising centre (MTOC) and may move outward. Autophagosomes and endosomes also move along the same microtubules and can peripherally exchange content through organelle fusion.[23] The morphology of individual lysosomes is influenced by their actual content, which includes membranes and macromolecules that are destined for degradation.[22] In addition, external factors impact on lysosomes. One example of this is the major effect of the buffer compound HEPES in cell culture medium (see chapter 1 and 2).[24]

Functions of lysosomes in signalling

The importance of the lysosome in cellular metabolism is illustrated by the progressive pathology that is observed in inherited LSDs.[25,26] Of note, extracellular functions for lysosomes and their hydrolases have recently been discovered and are subjected to specific regulatory processes. Secreted lysosomal hydrolases exhibit physiological functions in certain biological processes such bone remodelling (Cathepsin K) and lipid barrier formation in the stratum corneum (GBA).[27,28] The discovery of protein complexes at the lysosome membranes has revealed a nutrient sensing mechanism that impacts on the metabolic state of the cell. A key role is fulfilled in this respect by the mechanistic target of rapamycin complex 1 (mTORC1). Its activity is regulated by the combined presence of lysosomal nutrients and cytosolic signalling as triggered by growth factors.[29] For example, the reciprocal presence of AMPK at the surface of lysosomes during nutrient shortage provides the cell with a switch for anabolic and catabolic processes, respectively.[30] A general mode of action for mTORC1 has been proposed. Upon nutrient rich conditions, active mTORC1 localizes to the lysosomal membrane. In this state mTORC1 promotes cell growth and protein synthesis. It simultaneously inhibits transcriptional processes that promote catabolism, such as autophagy and lysosomal biogenesis.[29] Nutrient poor conditions inhibit mTORC1 and allow a transcriptionally regulated switch towards degradation of macromolecules.

Transcriptional control of lysosomal apparatus

Identification of a common sequence upstream of lysosomal genes named Coordinated Lysosomal Expression and Regulation (CLEAR) element, led to the discovery of Transcription factor EB (TFEB) as master regulator of lysosomal biogenesis.[31] TFEB is a basic helix-loop-helix (bHLH) transcription factor that belongs to the microphthalmia–transcription factor E (MiT/TFE) subfamily.[32] The family furthermore contains the transcription factors MITF, TFE3 and TFEC. The subfamily classification is based on exclusive heterodimerization among MiT/TFE members, but not with other bHLH transcription factors.[32] It has become apparent that the status of lysosomes has impact on transcription factors that regulate expression of genes encoding lysosomal proteins and essential components of autophagy (**Figure 2**). These transcription factors (TFs) couple

lysosome status and the related ability to supply nutrients with lysosome biogenesis and autophagy.[33,34] Under nutrient-rich conditions, serine phosphorylation of TFEB prevents its translocation to the nucleus.[34,35] A homologous serine residue is found in MITF, which renders cytosolic retention in phosphorylated state.[36] TFEB, MITF and TFE3 are sequestered in the cytosol by Rag GTPases and 14-3-3 proteins and drive lysosomal biogenesis upon nuclear translocation.[34,37–39] Upon perturbation of lysosomal function by increasing lysosomal pH, employing the lysosomotropic compound chloroquine, or by depletion of amino acids, TFEB is dephosphorylated and translocates into the nucleus.[34,40] Similarly, exposure of cells to chloroquine induces translocation of MITF and TFE3 into the nucleus.[34] The transcriptional activity of various MiT/TFE transcription factors is highly cell type and context dependent.[41] Of note, TFEC is assumed to counteract transcription due to a lack of an activation domain, but its exact function remains enigmatic.[42] MTORC1 has been shown to regulate MiT/TFE factors, providing a direct link between lysosomal nutrient status and the transcription of lysosome genes.[43,44] In addition, alternative regulation of MiT/TFE members exists and involves signaling proteins such as MAPK, PKC and AKT.[45] Complicating the regulation of lysosomal biogenesis, additional transcription factors that have been found to modulate expression of lysosomal and autophagy genes, such as MYC, STAT3 and zinc finger with KRAB and SCAN domains 3 (ZKSCAN3).[46–49]

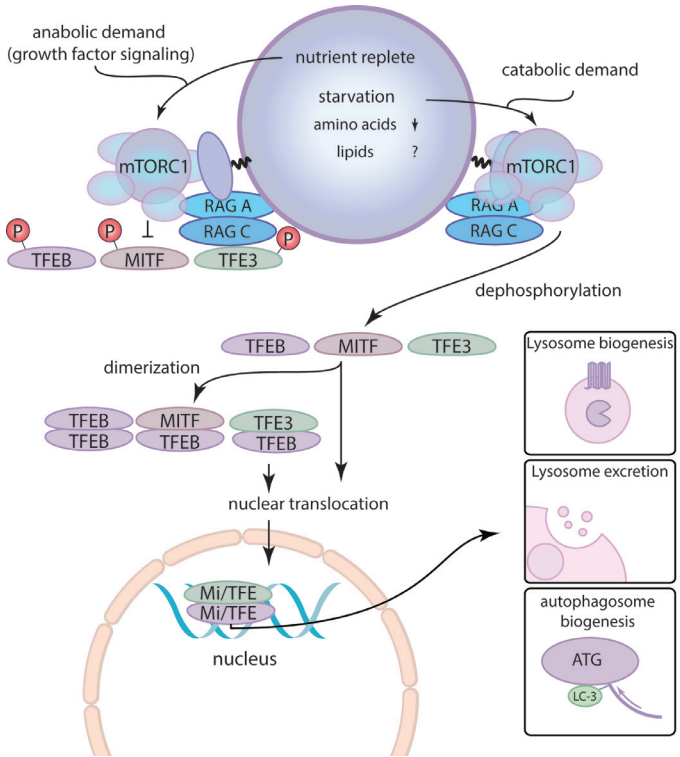

Figure 2. The role of the lysosome in regulating transcription of lysosome -and autophagy genes*; Upon sufficient nutrient availability mTORC1 is sequestered at the cytosolic side of the lysosomal membrane by Rag GTPases. This location favours the catalytic activity of mTORC1. MiT/TFE members are likewise sequestered in the cytosol by Rag GTPases and close proximity to active*

mTORC1 renders MiT/TFE members in an inactive, phosphorylated state. Upon high catabolic demand or reduced lysosomal amino acid content, mTORC1 is inactivated. Dephosphorylated MiT/TFE members may homo- or heterodimerize and translocate into the nucleus to initiate transcription of lysosome and autophagy genes and lysosome excretion. Mechanisms through which alternative metabolites such as lipids and sugars influence mTORC1 activity are less characterized.

Role of lysosomes in lipid metabolism.

Although *de novo* synthesis and lipid-driven ATP-production (through beta-oxidation) takes place in other cellular compartments, lysosomes are important in releasing free fatty acids and cholesterol from esterified lipid substrates. The sources of lysosomal lipid substrates can be endogenous (being delivered by autophagy processes) or exogenous (being delivered by endocytosis). Lysosome mediated degradation of lipids is highly dynamic.[50] In view of this, it is not surprising that the transcription factor TFEB can trigger transcription of proliferator-activated receptor α (PPARα) as well as PPAR-γ Coactivator 1 Alpha (PGC1α), key regulators of lipid catabolism.[51] The role of lysosomes in fragmentation of various types of complex lipids, including sphingolipids and cholesterol, warrants further introduction.

Sphingolipids

Sphingolipids are a class of lipids characterized by a ceramide lipid moiety existing of a variable fatty acyl moiety linked via an amide bound to a C18 sphingosine. A prominent and ubiquitous sphingolipid is sphingomyelin (SM) in which a phosphorylcholine moiety is linked to ceramide. Glycosphingolipids (GSLs) are a class of sphingolipids in which sugars are attached to the ceramide backbone. Numerous distinct glycosphingolipids exist due to variations in the sugar moiety. The simplest GSLs are galactosylceramide (GalCer) and glucosylceramide (GlcCer) with a single sugar beta-linked to ceramide. Complex GSLs have additional sugars (or sulfates) attached to the primary sugar. Sphingolipids are synthesized by multi-step formation of ceramide from serine and palmitoyl CoA followed by sequential attachments of moieties to generate the head groups.[52,53] Together with cholesterol, SM and GSLs are major components of lipid rafts, highly specialized compartments in the cell membrane that facilitate cell signalling, polarization and movement.[54] Glycan composition on sphingolipids determines protein-lipid and lipid-lipid interactions at the plasma membrane as well as on intracellular membrane structures such as the Golgi-network.[54–56] Knock-out models lacking enzymes that synthesize specific subsets of glycosphingolipids revealed their importance in a wide variety of processes such as neuronal development and function, skin permeability, immune function, pathogen-host interactions and intracellular protein trafficking.[53,55,57–62] GSLs and SM are ongoingly synthesized and fragmented in lysosomes. The lysosomal degradation involves stepwise removal of terminal moieties (phosphorylcholine, sulfate, sugars) rendering ceramide that is cleaved into a fatty acid and sphingosine. The lysosomal degradation products are exported to the cytosol and may be re-used or enter re-synthesis routes for other purposes. Deficiencies in sphingomyelinase and GSL degrading glycosidases give rise to sphingolipidoses (diseases characterized by lysosomal storage of sphingolipids).[17,25] The glycosphingolipidoses such as Gaucher disease, are the most prevalent among the LSDs.[63–66] Besides the turnover of various cellular membranes via autophagy pathways, the recycling of plasma membrane and uptake of lipoproteins

via endocytosis provides lysosomes with sphingolipid substrates for fragmentation.

Cholesterol

Cholesterol is a major constituent of cellular membranes. Excessive cholesterol in cells is converted to cholesterol-ester that is relatively inert and accumulates in lipid droplets (LD). Lipoproteins, particularly low-density lipoproteins (LDL), are rich in cholesterol ester. Other important membrane lipids are the diglyceride-based structures such as the various phospholipids that contain distinct polar head group composition. Attachment of another fatty acyl to diglyceride results in triglyceride that similarly to cholesterol ester does not incorporate in membranes but in LDs. Cholesterol-esters and diglyceride-based lipids undergo lysosomal fragmentation by lipase activity, releasing free fatty acids that are subsequently exported to the cytosol. Other degradation products (monoacylglycerol, cholesterol) are similarly exported from lysosomes. Lysosomal acid lipase (LAL) hydrolyses triglycerides and cholesterol-esters.[67,68] Deficiency in LAL results in two lysosomal storage disorders (LSDs): Cholesteryl Ester Storage Disease (CESD) is characterized by hypercholesterolemia and high blood LDL, whereas Wolman Disease (WD) is characterized by dramatic increases in triglycerides and cholesteryl esters. CESD is a heterogenous disease with predominant hepatic complications, whereas WD is lethal within the first five years due to massive lipid storage in most organs.[69] Defects in the export of cholesterol due to deficiencies in NPC1 or NPC2 cause endolysosomal cholesterol accumulations, as well as dramatic secondary elevations in neutral and acidic glycosphingolipids.[19,20,70] Recently, the lysosomal membrane protein LIMP-2 has been reported to also facilitate cholesterol transport, but this pathway is apparently insufficient to compensate NPC1-mediated sterol export.[71]

Endocytosis of lipoproteins supplies lysosomes with cholesterol-ester and diglyceride-based lipids destined for fragmentation.[68] Importantly, lipophagy, a specialized form of autophagy, mediates the transfer of LDs to lysosomes.[72] LDs are specialized structures that contain triacylglycerols and sterol esters and are surrounded by a phospholipid monolayer containing membrane proteins such as perilipins (PLIN), lipid synthesizing proteins, lipid degrading proteins (lipases) and proteins involved in vesicular transport.[73] The degree of lipid storage and composition of LDs depends on cell type, cellular metabolic status and whole-body metabolism. Primary LD storing cells include hepatocytes and adipocytes and serve as crucial regulators of lipid storage in other organs.[50,74]

Lipotoxicity

The toxicity of excessive fatty acids has been appreciated for a long time and has led to the concept of lipotoxicity, initially formulated by Unger.[75,76] During the last decades, several lipids have been proposed to play a pivotal role in obesity-induced insulin resistance, including excessive diacylglycerol (DAG), ceramide and acyl carnitines.[77] Impaired insulin sensitivity has indeed been linked to elevation of DAG species and their ability to activate protein kinase C subvariants.[77] Moreover, excessive ceramide has been shown to be a major cause for insulin sensitivity in various cell types and tissues, an effect ascribed to the sphingolipid's interaction with atypical PKCζ and AKT.[78,79] Levels of glycosphingolipids are similarly increased in obese individuals and rodents. Reduction of glycosphingolipid levels through inhibition of glucosylceramide synthase, the enzymatic reaction that

catalyses the formation of glucosylceramide from ceramide, has been found to markedly improve insulin sensitivity in obese rodents.[80] These findings are consistent with other observations regarding detrimental effects of excessive gangliosides, in particular GM3, on insulin sensitivity.[81] This effect is ascribed to disturbed lipid rafts in which the insulin receptor preferentially resides.[5] Of note, it has been proposed that in some cell types (such as muscle) excessive ceramide is the primary cause for insulin resistance, whereas in adipocytes glycosphingolipids are the major underlying cause.[82]

Macrophages

The primary function of the tissue resident macrophage is to maintain tissue homeostasis and the basal physiological state of these macrophages will therefore predominantly be of an anti-inflammatory nature. Hence, gut, adipose tissue, and spleen resident macrophages show anti-inflammatory expression profiles. Interference with, or change in their function, results in widespread (low grade) inflammation and auto-immune reactions.[83–85] These tissue-associated macrophages are generally termed alternatively activated macrophages or M2-macrophages, as opposed to pro-inflammatory, classically activated M1-macrophages. The dichotomous M1/M2-classification may however be too simplified, as differentiated macrophages subsets have been suggested to exist that exhibit varying stages of inflammatory states. A main distinction between different populations of tissue resident macrophages is between those that differentiate from hematopoietic progenitor cells that reside in the yolk sac or foetal liver and infiltrate the tissue during development (e.g. microglia and hepatocytes), and those derived from circulating monocytes that are recruited into the tissue after development (Figure 3).[86,87,88,89] Intrinsic and environmental factors, such as a bidirectional interaction with tissue, further trigger epigenetic changes and determine the chromatin status of macrophages.[88] A recent study by Glass and co-workers revealed that following acute depletion of Kupffer cells, peripheral monocytes are recruited to the liver where they can differentiate in response to local stimuli into Kupffer cell-like macrophages.[90] Transcriptome profiling and single-cell sequencing techniques have indeed unravelled a highly diverse mix of distinct macrophage populations within tissues, occuring in a spectrum of inflammatory states rather than a dichotomy among macrophage subtypes (Figure 3).[87,91,92] These populations respond differently to external stimuli such as pathogens, tissue damage and metabolic stress, and contribute differently to disorders in which the macrophage (or microglia) are known to play a crucial role, such as cancer, neurodegeneration and diabetes type 2.[93,94] Despite these phenotypical differences, the macrophage is in general a professional phagocyte, which clears pathogens and removes apoptotic cells through phagocytosis and efferocytosis respectively.[95] These processes occur at steady state under physiological circumstances and rapidly increase upon tissue damage or exposure to pathogen associated molecules.[96]

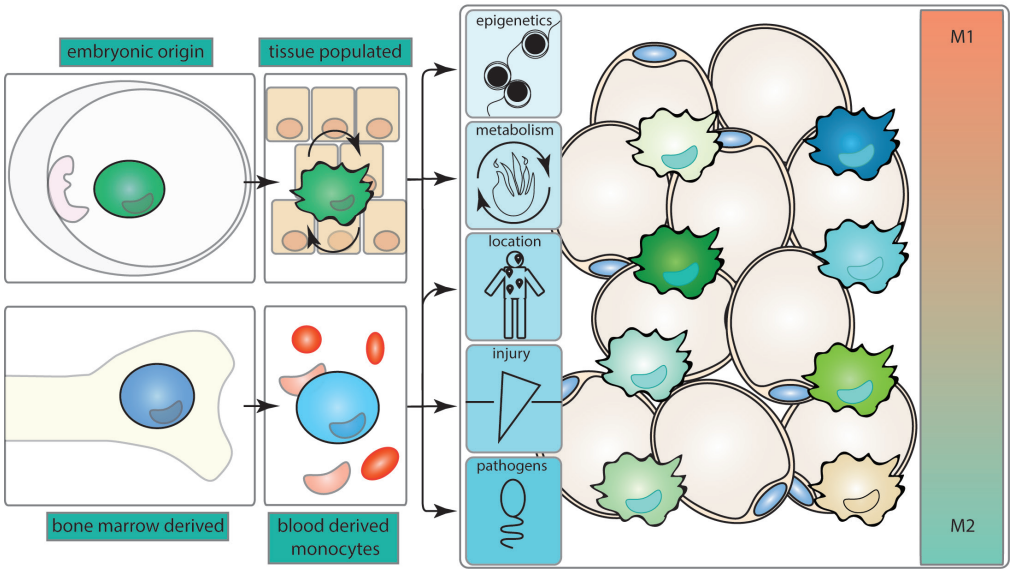

Figure 3. The ontology and heterogeneity of tissue resident macrophages*; Embryonic hematopoietic cells from the yolk sac and foetal liver give rise to a macrophage population that infiltrate the developing tissue and self-sustain locally throughout life. Adult hematopoietic stem cells that reside in the bone marrow differentiate into circulating monocytes which upon recruitment into the tissue give rise to a distinct pool of tissue resident macrophages. Both groups of macrophages are subjected to a variety of intrinsic and environmental factors such as epigenetics, tissue type, metabolism, injury, pathogens that determines the function of each macrophage within the tissue, giving rise to a high heterogeneity. This function can be placed within the spectrum between pro-inflammatory M1-macrophages and anti-inflammatory M2-macrophages.*

The macrophage plays an important role in lysosomal degradation of lipids. This is best illustrated by the occurrence of lipid-laden macrophages during deficiencies of lysosomal hydrolases and the spectacular clinical improvements upon correction of these cells.[70,95] One example offers WD, where bone marrow transplantation, or macrophage specific re-introduction of LIPA [98–100], renders major improvements. Another example is Gaucher disease where macrophage targeted enzyme replacement therapy (ERT) rapidly corrects hepatosplenomegaly and hematologic abnormalities such as anaemia and thrombocytopenia.[11,66] Immunological characterization of a Gaucher patients' spleen identified the lipid-laden macrophage (Gaucher cell) to be predominantly of a M2-nature.[15] Strikingly, Gaucher cells were found to be surrounded by pro-inflammatory M1-macrophages without an overt storage phenotype. Circulating pro-inflammatory factors are likely derived from M1-inflammatory macrophages. In a proposed model, a systemic low-grade inflammatory state is sustained through a vicious cycle in which tissue damage recruits lytic, pro-inflammatory macrophages that cause further tissue damage.

Gaucher cells are viable macrophages that, in response to their lysosomal distress, produce and secrete unique proteins. These include chitotriosidase, CCL18 and GPNMB (described extensively in chapter 4). The markedly elevated plasma chitotriosidase, CCL18 and GPNMB in GD patients are exploited to monitor disease progression and correction following ERT and substrate reduction therapy (SRT) with small compound

inhibitors of glucosylceramide synthase.[101-103] Elevated plasma levels of macrophage-derived chitotriosidase are documented for various lysosomal lipid storage disorders, that in addition to glycosphingolipidoses also include Wolman disease and NPC.[104] This illustrates the common occurrence of lysosomal distress in macrophages in disorders with quite distinct primary defects.

In adaptation to GlcCer accumulation in lysosomes of Gaucher cells, excessive glycosphingolipids are de-acylated by acid ceramidase, thereby producing glucosylsphingosine. This bioactive sphingoid base can leave storage cells and it is more than hundred-fold elevated in plasma of symptomatic GD patients. It is assumed that the chronic increase in glucosylsphingosine contributes to specific symptoms of Gaucher disease.[5] In the acid sphingomyelinase deficiencies Niemann Pick types A and B (NPA, NPB), lipid-laden macrophages are also a hallmark of pathology. The so-called Pick cells resemble Gaucher cells both morphologically and molecularly, and excessively produce chitotriosidase and CCL18, which can be exploited for diagnostic and monitoring purposes.[105] Moreover, acid ceramidase converts accumulating SM in lysosomes to its deacylated form, phosphocholinesphingosine, which is dramatically elevated in the patient's plasma. The same phenomenon is observed with other sphingolipidoses like Fabry disease and Krabbe disease. Toxic deacylated forms of accumulating lipids are generated in these disorders and are utilized as biomarkers.[5]

Moreover, lipid-laden macrophages are present in spleen and liver of NPC patients. The primary storage lipid is considered to be cholesterol, but profound accumulation of (glyco)sphingolipids occurs concomitantly. This is attributed to secondary perturbation of lysosomal hydrolase function due to the NPC1-deficiency. Apparently, the aberrant lysosomal lipid composition exerts pleiotropic effects within the lysosome, which causes multiple deficiencies to develop simultaneously. This may explain some similarities among NPC, other inherited sphingolipidoses and possibly acquired metabolic disorders with respect to biomarker and clinical representation.[106]

Acquired metabolic disorders

A group of macrophages that is typically exposed to high lipid load are adipose tissue macrophages (ATMs). These ATMs can acquire a storage phenotype during obesity when the adipose tissue (AT) hypertrophies and adipocytes become dysfunctional. A systemic low-grade inflammation accompanies this AT change, which contributes to impaired insulin sensitivity in adipocytes and other organs. Consequently, glucose uptake by AT and muscle is impaired, along with insulin mediated suppression of glucose secretion by the liver. Blood glucose levels progressively increase during the development of obesity. Insulin resistance (IR) is a major risk factor for the development of type 2 diabetes mellitus and cardiovascular complications.[107,108] These acquired metabolic disorders pose an increasing threat to global health. Estimates of the world health organisation indicated that 39% (1.9 billion) of adults were overweight in 2016, and 13% (650 million) of the global population was obese.[109] The prevalence of diabetes has similarly increased in the last 20 years, and has entered the top ten of global causes of deaths.[110]

For a long time, a low-grade inflammatory state has been associated with perturbed whole-body metabolism as during obesity. Early work revealed a causal relationship between TNFα, then alternatively called cachectin, and insulin homeostasis.[111,112] Administration of neutralizing antibodies against TNFα was employed to improve

metabolic stress as a consequence of acute elevations of TNFα and of chronic levels as induced by obesity.[113,114] Reduction of elevated circulating TNFα in obese mice restores insulin sensitivity, demonstrating a relationship between insulin resistance and inflammation.[114,115] In obese AT, the amount of macrophages is dramatically increased and apoptotic adipocytes are surrounded by aggregates of macrophages, termed crown-like structures.[116–119] Indeed, the ATM was identified as the primary source of TNFα during obesity.[85] Upon excessive nutritional intake, the properties of ATMs change from an anti-inflammatory (M2-like) to a pro-inflammatory (M1-like) profile.[120] The bidirectional communication between macrophages and adipocytes is a major determinant in the phenotypic switch that macrophages undergo, and many stimuli have been identified, including hormones, cytokines, chemokines as well as lipid mediators released from hypertrophied adipocytes.[121] For example, adipocytes communicate by secreting the hormone adiponectin, which acts as potent systemic insulin sensitizer and reducer of inflammation.[122] Conceivably, obese ATMs are stressed by lipids and undergo differentiation to a specific phenotype. Recent data indicated a foamy appearance of obese ATMs on an ultrastructural level, which was associated with an increased lipid degradation by a lysosome driven program.[123] Moreover, GPNMB was recently identified as a potentially novel biomarker of these obese ATMs.[119] The storage cell marker has been found to be under transcriptional control of MiT/TFE subfamily of transcription factors and expression could be potently induced *in vitro* upon application of lysosomal stress through palmitate feeding or chloroquine exposure.[124] These data suggest that lysosomal storage in macrophages is a hallmark of obesity and that adaptation to a storage phenotype is at least partly mediated by MiT/TFE family members.

Atherosclerosis is another pathology that is characterized by lipid laden macrophages. Incomplete clearance of oxidized LDL-apolipoprotein B particles gives rise to a storage phenotype.[125] These storage cells contribute to the chronic, non-resolving inflammation that occurs in the intima and are assumed to be crucial in the progression of the lesion. Expression of lipid-laden macrophage markers are strongly elevated in atherosclerotic lesion macrophages and include chitotriosidase and GPNMB.[126–128] Additional similarities can be found in lipid laden microglia, which are considered to contribute to progression of neurodegenerative diseases.[129,130] Again, typical storage markers identified in LSDs such as GPNMB are elevated in these lipid-filled microglia.

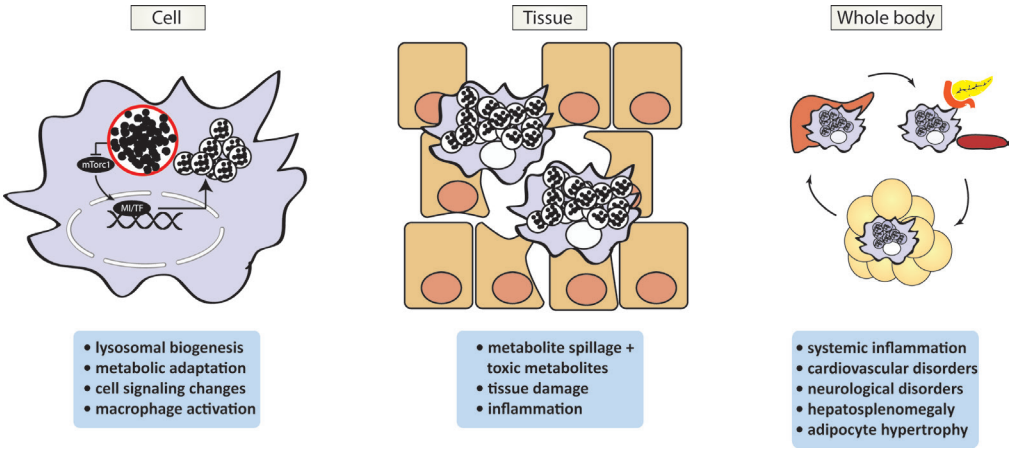

Figure 4. Proposed impact of lysosomal stress at a molecular level, within the tissue and on the whole body*. On a cellular level lysosomal stress triggers a molecular response, involving increased lysosomal biogenesis and autophagy through transcription factor family MiT/TFE. In macrophages this induces a differentiation associated with the nature of metabolic and inflammatory triggers. In organs containing cells with lysosomal stress, storage cells drive disease progression through recruitment of lytic immune cells causing further damage to the affected tissue. Systemically, a low-grade inflammation can arise in pathologies characterized by lipid-laden macrophages, which drives chronic multi-organ pathology.*

Relevance of lysosomal biogenesis for disease

Perturbation of the lysosome can lead to a coordinated signalling cascade that changes metabolism and signalling in cells, tissues and whole body, as is illustrated by the lysosomal accumulation of lipids in inborn errors of lysosomal lipid metabolism (**Figure 4**). Prominent in these diseases are lipid-laden macrophages, which secrete markers that are connected to disease burden and could be exploited as biomarkers. Importantly, storage cells have emerged as a hallmark in acquired metabolic pathologies such as obesity and atherosclerosis. It remains largely unclear however, how canonical regulators of lysosome biogenesis, the family of MiT/TFE, contribute to the storage-cell phenotype in response to lipid-laden lysosomes. Moreover, it is of therapeutic relevance to study whether increased lysosomal biogenesis in storage cells is beneficial to the cell and to its surrounding tissue.

Scope of thesis

This thesis aims to explore the pathophysiological role of the lysosome with a focus on the macrophage. The studies employ an integrated approach that involves biochemical and transcriptional analysis to combine data on hydrolytic capacity with lysosomal signalling. In addition, ultrasensitive visualization by activity-based probe labelling enabled in depth studies on glucocerebrosidase in controlled cellular models as well as in metabolic pathologies.

Chapter 1 characterizes the lysosome integrity and the following cellular response in cultured cells. It identifies HEPES as a potent inducer of a MiT/TFE mediated transcriptional program in cultured macrophage-like cells that affects lysosomal function, as well as the immunological phenotype of the macrophage.

Chapter 2 characterizes the maturation of acid hydrolase in cultured cells. It identifies a perturbed maturation of glucocerebrosidase (GBA) by use of the zwitterionic buffers HEPES and illustrates the importance of culture conditions when using cultured cells for diagnosis.

Chapter 3 investigates the localization of lysosomal enzymes and delivery of recombinant lysosomal enzyme by combining ultrasensitive activity-based probe labeling (ABP) with the high spatial resolution of electron microscopy through correlative light electron microscopy (CLEM).

Chapter 4 explores the recent developments in storage cell characterization. The potential is discussed of a recently identified transmembrane protein glycoprotein non metastatic protein B (GPNMB) as biomarker in lipid laden macrophage associated disorders.

Chapter 5 identifies tissue specific adaptation upon lysosomal deficiency in cholesterol efflux transporter NPC1 in mice. A hepatocyte specific upregulation of LIMP2 is identified without concomitant upregulation of known NPC biomarkers, as opposed to biomarkers that are upregulated in lipid laden Kupffer cells.

Chapter 6 characterizes the contribution of lysosome signalling in lipid-laden macrophages in the adipose tissue present during obesity, an acquired metabolic disorder. Glucan encapsulated particles (Gerps) were employed to perform an siRNA mediated knock down of MiT/TFE genes in adipose tissue macrophages.

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