Published in final edited form as:

Biol Chem. 2012 September ; 393(9): 1005–1011. doi:10.1515/hsz-2012-0192.

Adipose triglyceride lipase in immune response, inflammation, and atherosclerosis

Branislav Radovic, Elma Aflaki^a, and Dagmar Kratky^{*}

Institute of Molecular Biology and Biochemistry, Center of Molecular Medicine, Medical University of Graz, Harrachgasse 21, A-8010 Graz, Austria

Abstract

Consistent with its central importance in lipid and energy homeostasis, lipolysis occurs in essentially all tissues and cell types, including macrophages. The hydrolytic cleavage of triacylglycerol by adipose triglyceride lipase (ATGL) generates non-esterified fatty acids, which are subsequently used as essential precursors for lipid and membrane synthesis, mediators in cell signaling processes or as energy substrate in mitochondria. This review summarizes the current knowledge concerning the consequences of ATGL deficiency in macrophages with particular emphasis on macrophage (dys)-function, apoptosis, and atherosclerosis.

Keywords

apoptosis; lipolysis; lipotoxicity; macrophages; mouse model; triacylglycerol mobilization

Introduction

Fatty acids (FAs) are important metabolic substrates for energy production. In addition, FAs are precursors for numerous lipid species and important substrates for protein acylation. In contrast to these beneficial characteristics, free fatty acids (FFAs) can become deleterious for cells when present at higher concentrations. Accordingly, all eukaryotes apply biochemical strategies to detoxify FAs by esterification and storage within lipid droplets. Essentially all cells in the body generate lipid droplets and use these organelles for the production of membrane compounds, energy substrates, and signaling molecules including lipotoxic compounds (e.g., an excess of FFAs or free cholesterol) (Beckman, 2006; Martin and Parton, 2006). Macrophages have an enormous capacity to store lipids. Excess FFAs and unesterified cholesterol are stored in lipid droplets within triacylglycerol (TG) and cholesteryl esters (CEs). Accumulation of CEs after uptake of modified lipoproteins results in foam cell formation, which is the hallmark of atherosclerosis.

Atherosclerosis is an inflammatory disease and has been recognized as the leading cause of death in industrialized societies. Studies of the earliest stages of atherogenesis in humans and mouse models indicate that the key initiating step is subendothelial accumulation of apolipoprotein B (ApoB)-containing lipoproteins (Williams and Tabas, 1995). Accumulation of monocytes and monocyte-derived macrophages in the wall of large arteries leads to chronic inflammation and the development and progression of atherosclerosis. Endothelial cell activation as an early inflammatory response is enhanced by oxidative

Copyright © by Walter de Gruyter • Berlin • Boston.

^{*}Corresponding author dagmar.kratky@medunigraz.at.

^aPresent address: National Human Genome Research Institute/NIH Molecular Neurogenetics Section, 35 Convent Drive, Bethesda, MD, USA.

modification of ApoB-containing lipoproteins, concomitant upregulation of adhesion molecules and chemokine production, which mediate the recruitment of circulating monocytes (reviewed by Mestas and Ley, 2008). Driven by the macrophage colonystimulating factor, the majority of monocytes differentiate into macrophages. Owing to their heterogeneity and versatility, macrophage activation leads to either of the two extreme phenotypes: classically activated proinflammatory M1 and alternatively activated anti-inflammatory M2 macrophages (Mills et al., 2000; Mantovani et al., 2004). Reciprocal skewing of macrophage polarization between M1 and M2 states is a process modulated by diet, humoral factors, and transcription factors (reviewed by Chinetti-Gbaguidi and Staels, 2011). Mosser and Edwards (2008) suggested a more informative classification of macrophages based on their three fundamental functions that are involved in maintaining homeostasis: host defense, wound healing, and immune regulation.

Intracellular lipid catabolizing enzymes degrade CEs, TGs, and their metabolic intermediates. Mobilization of CEs and TGs from lysosomes requires the action of lysosomal acid lipase (Goldstein et al., 1975). Lipid droplet-associated CEs are hydrolyzed by a neutral CE hydrolase (Ghosh, 2011). In 2004, three groups published the discovery of an enzyme able to hydrolyze TG and named it adipose triglyceride lipase (ATGL) (Zimmermann et al., 2004), desnutrin (Villena et al., 2004), or calcium-independent phospholipase A2 (iPLA2) (Jenkins et al., 2004). ATGL selectively performs the first step in TG hydrolysis, resulting in the formation of diacylglycerol (DG) and FFAs. For maximal lipolytic activity ATGL requires CGI-58 as a coactivator (Lass et al., 2006). Recently, G0S2 (predominantly expressed in adipose tissue and liver) has been identified as a selective inhibitor of ATGL (Yang et al., 2010). ATGL exhibits 10-fold higher substrate specificity for TGs than for DGs (Zimmermann et al., 2004) and shows low transacylase and phospholipase activity (Jenkins et al., 2004). In contrast to hormone-sensitive lipase, ATGL lacks the ability to hydrolyze monoacylglycerol, CEs, or retinyl esters (Zimmermann et al., 2004).

ATGL is expressed in most tissues of the body with highest mRNA levels and enzyme activity found in white and brown adipose tissue (reviewed in Zechner et al., 2009). The important role of ATGL in lipolysis became evident from observations in *Atgl*-deficient ($-/-$) mice, which accumulate TGs in essentially all organs (Haemmerle et al., 2006). Massive TG accumulation caused by impaired TG mobilization in cardiac myocytes results in cardiac failure and premature death of *Atgl* $-/-$ mice, starting at the age of 10 weeks. Owing to the reduced availability of FFAs as energy substrate, *Atgl* $-/-$ mice show an increased utilization of carbohydrates as an energy source, leading to improved glucose tolerance and insulin sensitivity. *Atgl* $-/-$ male mice are fertile, whereas females lack the ability to suckle their offspring due to impaired milk production. In white adipose tissue, hormone-stimulated lipolysis is drastically reduced, indicating that ATGL is hormone regulated via direct or indirect mechanisms. In addition, *Atgl* $-/-$ mice exhibit defective thermogenesis in brown adipose tissue. Plasma FAs, TGs, total cholesterol (TC), high-density lipoprotein-cholesterol and ketone body concentrations are decreased in both fed and fasted *Atgl* $-/-$ mice.

ATGL in immune response

Although less than in adipose tissue, ATGL mRNA is expressed in murine peritoneal macrophages and macrophage-derived foam cells as well as in human monocyte-derived macrophages and foam cells (Chandak et al., 2010). Similar to other cells and tissues, the absence of ATGL in macrophages results in decreased TG hydrolase activity and, concomitantly, in intracellular TG accumulation. In accordance with the substrate specificity of ATGL, CE hydrolase activity is comparable in *Atgl* $-/-$ and wild-type (Wt) cells. Thus,

Atgl^{-/-} macrophages specifically accumulate TGs even in the absence of exogenous lipid loading, whereas intracellular cholesterol concentrations remain unchanged.

ATGL and macrophage phagocytosis

In contrast to extracellular lipoprotein lipase (LPL)-mediated FA mobilization (Yin et al., 1997), intracellular ATGL-mediated FA release is essential for ATP synthesis and for maximal phagocytic activity of macrophages (Chandak et al., 2010). FFAs taken up by macrophages are not directly routed to mitochondrial β -oxidation, but instead are acyl-coenzyme A (CoA) activated and subsequently oxidized or esterified to TGs (and CEs). The generation of lipolytic products such as DFAs or DG by ATGL provides ligands or ligand precursors for functional signaling by the peroxisome proliferator-activated receptor (PPAR) α -PPAR γ coactivator-1 complex, which activates mitochondrial biogenesis and oxidative phosphorylation and directs FA-CoA to oxidation in the heart (Haemmerle et al., 2011). In brown adipose tissue, ATGL-catalyzed lipolysis was suggested to activate PPAR α for maintaining the brown adipose tissue phenotype and to promote thermogenesis (Ahmadian et al., 2011). ATGL deficiency leads to decreased mitochondrial biogenesis and function and directs FA-CoA towards TG synthesis and accumulation (Chandak et al., 2010; Aflaki et al., 2011b; Haemmerle et al., 2011).

In macrophages, FFA usage as energy substrate requires ATGL hydrolysis (and the activity of neutral CE hydrolases; reviewed by Ghosh, 2011). FFAs released from intracellular TGs by ATGL are transported into the mitochondria and used for mitochondrial β -oxidation and consequently for energy production. In macrophages, activation of PPAR β/δ by very low-density lipoprotein (LDL) induces ATGL, resulting in increased FA catabolism (Lee et al., 2006). It is tempting to speculate that in the absence of ATGL in macrophages, PPAR signaling is limited and additionally accounts for the observed reduction in phagocytosis. This is the case even in conditions in which ATP concentrations are partly restored by glucose (Chandak et al., 2010). One additional mechanism that might be responsible for impaired phagocytosis of Atgl^{-/-} macrophages is defective small Rho GTPase activation (Aflaki et al., 2011a). Small Rho GTPases participate in the regulation of various biological pathways including phagocytosis (Etienne-Manneville and Hall, 2002). During phagocytosis, Cdc42 and Rac induce actin polymerization to form membrane extensions that engulf the particle. Rac also associates with a component of the NADPH oxidase enzyme complex to stimulate the production of superoxide anions as part of the bactericidal program. Thus, cellular rigidity due to defective remodeling (assembly and disassembly) of the actin cytoskeleton in Atgl^{-/-} macrophages (Aflaki et al., 2011a) might be causative for inefficient phagocytosis.

ATGL in atherosclerosis

ATGL and atherosclerotic plaque formation

In general, macrophage-derived foam cells in atherosclerotic lesions are packed with CE-rich lipid droplets. By contrast, Atgl^{-/-} macrophages accumulate TG-rich lipid droplets resulting in altered cell morphology that resemble macrophage foam cells. These alterations and functional changes strongly argue for the involvement of ATGL in atherogenesis. In fact, transplantation of Atgl^{-/-} bone marrow into γ -irradiated lowdensity lipoprotein receptor (Ldlr)^{-/-} mice resulted in highly attenuated atherosclerotic lesion formation compared with Wt bone marrow-transplanted Ldlr^{-/-} mice after feeding an atherogenic Western-type diet for 9 weeks (Lammers et al., 2011). This effect is independent of plasma lipid parameters because plasma TG, TC and FFA concentrations are comparable between Ldlr^{-/-} mice transplanted with Atgl^{-/-} or Wt bone marrow. These data indicate that in contrast to cholesterol, increased intracellular TG content in macrophages is not associated

with lesion growth. In fact, increased intracellular TG concentrations, particularly within lysosomes, correlate with reductions in CE content (Ullery-Ricewick et al., 2009). The volume of cholesterol-engorged lysosomes decreases after TG-rich particle treatment, indicating that cholesterol is more efficiently cleared.

In normal physiology, macrophage phagocytosis plays an essential role on host defense through clearance of infectious organisms and in the resolution phase of inflammation by removal of apoptotic cells (efferocytosis) and inflammatory debris (Aderem and Underhill, 1999). During atherogenesis, macrophage phagocytosis can either promote or protect against lesion progression, depending on the context (Schrijvers et al., 2007; Tabas, 2007; Thorp et al., 2008). Defects in efferocytosis are suggested to contribute to advanced lesion formation and inflammation in apolipoprotein E^{-/-} mice (Grainger et al., 2004) and other mouse models. Thus, efferocytosis of apoptotic cells in lesions reduces plaque progression by decreasing lesion cellularity, preventing post-apoptotic necrosis and promoting anti-inflammatory responses. By contrast, uptake of aggregated lipoproteins, erythrocytes, and platelets may promote foam cell formation, lesion progression, and atherogenesis (reviewed by Schrijvers et al., 2007). Thus, in addition to foam cell formation, enhanced endocytosis of modified lipoproteins is expected to contribute to plaque progression and formation of an unstable plaque phenotype due to macrophage survival. Although uptake of modified lipoproteins was slightly reduced in Atgl^{-/-} macrophages the profound decrease of phagocytosis probably contributes to attenuated lesion progression in Ldlr^{-/-} mice transplanted with Atgl^{-/-} bone marrow.

ATGL and apoptosis

The reduction in atherosclerotic lesion formation in these animals coincides with increased apoptosis (Lammers et al., 2011). The role of macrophage apoptosis in atherogenesis is dual, depending on the stage of the plaque (reviewed by Seimon and Tabas, 2009). In early lesions, apoptotic macrophages are rapidly cleared by other macrophages, thereby limiting lesion cellularity and the progression of early lesions (Babaev et al., 2008; Gautier et al., 2009). In advanced atherosclerotic lesions, however, impaired clearance of apoptotic macrophages leads to secondary necrosis and the formation of a necrotic core (Feng et al., 2003) favoring arterial wall inflammation and enhanced monocyte recruitment (Gautier et al., 2009). Unesterified cholesterol, a potent apoptosis inducer in macrophages, accumulates in advanced atherosclerotic lesions. Incubation of macrophages with unesterified cholesterol *in vitro* induces programmed cell death via activation of the mitochondrial apoptosis pathway (Yao and Tabas, 2000). A similar effect was observed in the absence of ATGL and TG accumulation in macrophages with the increase of typical markers of apoptosis, such as externalization of phosphatidylserine in the plasma membrane and caspase 3 and poly-(ADP-ribose) polymerase cleavage (Aflaki et al., 2011b). Fragmented mitochondria prior to cell death are indicative of the mitochondrial apoptosis pathway being triggered in Atgl^{-/-} macrophages. In contrast with these results, TG accumulation in macrophages was shown to be cytoprotective (Listenberger et al., 2003; Saraswathi and Hasty, 2009). This discrepancy might be explained by differences in intracellular TG concentrations between the studies or by additional mechanisms being activated in Atgl^{-/-} macrophages.

The endoplasmic reticulum (ER) is a critical organelle in the induction of apoptosis and is responsible for intracellular Ca²⁺ storage. Homeostasis of Ca²⁺ concentrations between the ER and the cytosol are essential for cell survival. Depletion of Ca²⁺ from the ER and concomitant elevated levels of cytosolic Ca²⁺ in Atgl^{-/-} macrophages indicate ER stress and the induction of the unfolded protein response (Aflaki et al., 2011b, 2012). An interaction between signals from Ca²⁺ and ceramide, which exists in various cell types, might lead to specifically increased C16:0 ceramide concentrations in Atgl^{-/-} macrophages. Accordingly, mRNA expression of ceramide synthases is markedly increased. Inhibition of C16:0

ceramide synthesis rescues $Atgl^{-/-}$ macrophages by inhibition of ceramide synthesis and protects from mitochondrial dysfunction and apoptotic cell death but fails to abolish ER stress (Aflaki et al., 2012). These results indicate that ER stress itself is not the apoptotic trigger of programmed cell death and that C16:0 ceramide is essential and sufficient for mitoapoptosis in $Atgl^{-/-}$ macrophages.

ATGL and phagocyte migration

The number of circulating white blood cells is markedly reduced in $Ldlr^{-/-}$ mice transplanted with $Atgl^{-/-}$ bone marrow (Lammers et al., 2011). Whereas the half-life of white blood cells is comparable, the LSK population (representing hematopoietic stem and multipotential progenitor cells in the bone marrow) is reduced. These data suggest that a decreased amount of circulating white blood cells in these mice is the consequence of impaired production in the bone marrow, resulting in less monocyte infiltration into the intima. In humans, it is well established that increased levels of neutrophils and monocytes induce the progression of atherosclerosis. Conversely, a reduced number of circulating monocytes inhibits the initiation and development of atherosclerotic lesions.

Movement of monocytes/macrophages to the site of atherosclerotic lesions is crucial for macrophage infiltration and requires the activation of three signaling pathways: detection of the inflammation site, polarization, and migration (Vicente-Manzanares and Sanchez-Madrid, 2004). The directed migration of leukocytes (chemotaxis) is governed by extracellular signals, such as chemoattractant gradients or adhesion signals. For active chemotaxis, the rearrangement of actin filaments is a prerequisite; leukocytes extend a front actin-rich lamellipodium (leading edge), whereas the uropod is retracted during migration. Defective activation of the Rho small GTPases RhoA, Cdc42, and Rac1 causes disturbances in actin polymerization and polarization of $Atgl^{-/-}$ macrophages, which probably account for their reduced migratory capacity. A transient phosphorylation of focal adhesion kinase (FAK) is accepted to induce cell movement (Calalb et al., 1995). In $Atgl^{-/-}$ macrophages, however, increased NADPH oxidase-mediated reactive oxygen species production, probably as a result of Rac2 activation, leads to oxidative inactivation of Src homology 2-containing phosphotyrosine phosphatase (SHP-2). This in turn results in sustained phosphorylation and activation of FAK that perturbs the disassembly of the cytoskeleton (Aflaki et al., 2011a,b). The net effect is enhanced cell spreading with concomitant impaired migration as observed in $Atgl^{-/-}$ macrophages. As a consequence and in addition to the reduced amount of white blood cells, the total number of infiltrated macrophages into the arterial wall is decreased in $Ldlr^{-/-}$ mice transplanted with $Atgl^{-/-}$ bone marrow (Lammers et al., 2011). Inhibition of the production of reactive oxygen species by both antioxidants and NADPH oxidase inhibitors restores the dynamic activation of FAK and, consequently, the migratory capacity of $Atgl^{-/-}$ macrophages (Aflaki et al., 2011a). A similar signaling pathway triggered by interactions between oxidized LDL and CD36 was demonstrated in macrophages (Park et al., 2009). The authors suggest a physical interaction between FAK/SHP-2 and CD36 that alters cytoskeletal dynamics and induces trapping of macrophages in the arterial intima. This process, however, generally promotes atherosclerosis, which is not the case in $Ldlr^{-/-}$ mice transplanted with $Atgl^{-/-}$ bone marrow (Lammers et al., 2011).

ATGL in inflammation

The proinflammatory cytokine Rantes/Ccl5 plays an active role in recruiting leukocytes to the sites of inflammation (Appay and Rowland-Jones, 2001). In addition, Rantes/Ccl5 induces lamellipodia formation (Di Marzio et al., 2005) and activates Rac1 (Weiss-Haljiti et al., 2004; Di Marzio et al., 2005). The fact that Rantes/Ccl5 mRNA expression is almost absent in $Atgl^{-/-}$ macrophages (Aflaki et al., 2011a) might contribute to their reduced migratory capacity. ATGL deficiency attenuates the mRNA expression of the

proinflammatory chemokine (C-X-C motif) ligand 1 (Gro1) and the release of interleukin (IL)-6 from macrophages. Increased secretion of anti-inflammatory IL-10 and transforming growth factor β from Atgl^{-/-} macrophages and increased mRNA expression of mannose receptor 1, arginase 1, monocyte chemoattractant protein (MCP)-2, and sphingosine kinase 1 argue for an anti-inflammatory M2-like phenotype of Atgl^{-/-} macrophages (Aflaki et al., 2011a). In line with these data, macrophage mRNA levels of acyl-CoA:diacylglycerol acyl-transferase 1, the enzyme responsible for the final step in TG biosynthesis, correlate directly with TG storage capacity and inversely with inflammatory activation by saturated FAs (Koliwad et al., 2010). Thus, increasing the capacity of macrophages for TG storage, either by DGAT1 overexpression or ATGL deficiency, protects against FA-induced inflammatory activation.

Decreased plasma concentrations of MCP-1, an important chemoattractant for mononuclear cells, might contribute to decreased inflammation in Ldlr^{-/-} mice transplanted with Atgl^{-/-} bone marrow (Lammers et al., 2011). In fact, mice lacking MCP-1 (Lu et al., 1998) or its receptor CCR2 (Boring et al., 1998) develop fewer and smaller atherosclerotic lesions compared with control mice. Reduced plaque formation in these animals is most likely the consequence of a reduction in monocyte recruitment to sites in the arterial wall, which are prone to atherosclerotic lesion development. Thus, diminished MCP1 levels probably participate in the reduced number of circulating mononuclear cells in Ldlr^{-/-} mice transplanted with Atgl^{-/-} bone marrow (Lammers et al., 2011).

Inconsistent results are published on TG accumulation in livers of Atgl^{-/-} mice with almost identical (Fuchs et al., 2012), 2.3-fold (Haemmerle et al., 2006) or at least 3-fold increased hepatic TG concentrations (Wu et al., 2011) compared with Wt livers. Intraperitoneal injections of tunicamycin induce hepatic steatosis in Atgl^{-/-} but not in Wt mice (Fuchs et al., 2012). In contrast to Atgl^{-/-} macrophages, which exhibit increased ER stress compared with Wt macrophages (Aflaki et al., 2012), livers from Atgl^{-/-} mice are protected from tunicamycin-induced hepatic ER stress and inflammation. This effect can be reversed by the enrichment of oleic acid in the hepatic TG pool of Atgl^{-/-} mice. Patients suffering from non-alcoholic fatty liver disease or non-alcoholic steatohepatitis typically display ER stress in the liver. Deficiency of liver ATGL causes progressive hepatic steatosis but inflammatory and fibrotic responses are reduced compared with those reported in other obesity models with similar degrees of steatosis (Wu et al., 2011). Further studies are necessary to elucidate whether ATGL-mediated TG hydrolysis may constitute a target for the treatment of liver diseases.

ATGL deficiency limits adipose tissue macrophage accumulation during fasting (Kosteli et al., 2010). This is an interesting finding because chronic stimulation of adipose tissue macrophages leads to local inflammation and altered metabolic function. The authors conclude that lipolysis drives the accumulation of adipose tissue macrophages and recruited macrophages might buffer the local increase in lipid concentration. Because lipolysis induces macrophage migration, it is also likely that the reduced migration rate of Atgl^{-/-} macrophages limits their infiltration into adipose tissue, which results in reduced adipose tissue inflammation.

Conclusion

Data from Atgl^{-/-} mice and humans with mutations in PNPLA2, the gene coding for ATGL, assign a central role to this enzyme for the catabolism of cellular fat stores. Do we now understand the role of ATGL in macrophages? To some extent. It is obvious that ATGL deficiency results in TG accumulation in all tissues and cells analyzed, including macrophages. A brief summary of the consequences of ATGL deficiency in macrophages is

summarized in Figure 1. Numerous issues, including how ATGL exerts mitochondrial apoptosis, remain unsettled. It is still elusive how the absence of ATGL leads to a shift in ceramide metabolism and whether these changes result in increased sphingosine formation, which is catabolized to C16:0 ceramide within mitochondria. Is ATGL deficiency in macrophages a friend or foe? Current results demonstrate both. Although lack of ATGL reduces inflammation, macrophage infiltration into the arterial wall, and early stage plaque formation, this effect might be reversed in later atherosclerosis due to reduced phagocytosis, mitochondrial dysfunction, and increased apoptosis. Additional studies are necessary to answer the question whether ATGL might be a drug target to attenuate atherosclerosis.

In summary, ATGL-mediated lipolysis is linked to macrophage function. Other previous and current discoveries reveal its involvement in the pathogenesis of lipid-associated disorders.

Acknowledgments

This work was supported by grants F30 SFB LIPOTOX, W1226 DK-MCD, P19186, and P22832, which are funded by the Austrian Science Fund FWF. This research was also supported by grant 'GOLD: Genomic Of Lipid-associated Disorders', which is part of the Austrian Genome Project 'GEN-AU: Genome Research in Austria' funded by the Austrian Ministry of Science and Research and the FFG. E.A. was financed by the PhD program 'Molecular Medicine' of the Medical University of Graz. We apologize for publications that could not be referenced owing to citation limitations.

References

- Aderem A, Underhill DM. Mechanisms of phagocytosis in macrophages. *Annu. Rev. Immunol.* 1999; 17:593–623. [PubMed: 10358769]
- Aflaki E, Balenga NA, Luschnig-Schratl P, Wolinski H, Povoden S, Chandak PG, Bogner-Strauss JG, Eder S, Konya V, Kohlwein SD, et al. Impaired Rho GTPase activation abrogates cell polarization and migration in macrophages with defective lipolysis. *Cell Mol. Life Sci.* 2011a; 68:3933–3947. [PubMed: 21533980]
- Aflaki E, Radovic B, Chandak PG, Kolb D, Eisenberg T, Ring J, Fertschai I, Uellen A, Wolinski H, Kohlwein SD, et al. Triacylglycerol accumulation activates the mitochondrial apoptosis pathway in macrophages. *J. Biol. Chem.* 2011b; 286:7418–7428. [PubMed: 21196579]
- Aflaki E, Doddapattar P, Radovic B, Povoden S, Kolb D, Vujic N, Wegscheider M, Koefeler H, Hornemann T, Graier WF, et al. C16 ceramide is crucial for triacylglycerol-induced apoptosis in macrophages. *Cell Death Dis.* 2012; 3:e280. [PubMed: 22419109]
- Ahmadian M, Abbott MJ, Tang T, Hudak CS, Kim Y, Bruss M, Hellerstein MK, Lee HY, Samuel VT, Shulman GI, et al. Desnutrin/ATGL is regulated by AMPK and is required for a brown adipose phenotype. *Cell Metab.* 2011; 13:739–748. [PubMed: 21641555]
- Appay V, Rowland-Jones SL. RANTES: a versatile and controversial chemokine. *Trends Immunol.* 2001; 22:83–87. [PubMed: 11286708]
- Babaev VR, Chew JD, Ding L, Davis S, Breyer MD, Breyer RM, Oates JA, Fazio S, Linton MF. Macrophage EP4 deficiency increases apoptosis and suppresses early atherosclerosis. *Cell Metab.* 2008; 8:492–501. [PubMed: 19041765]
- Beckman M. Cell biology. Great balls of fat. *Science.* 2006; 311:1232–1234.
- Boring L, Gosling J, Cleary M, Charo IF. Decreased lesion formation in CCR2^{-/-} mice reveals a role for chemokines in the initiation of atherosclerosis. *Nature.* 1998; 394:894–897. [PubMed: 9732872]
- Calalb MB, Polte TR, Hanks SK. Tyrosine phosphorylation of focal adhesion kinase at sites in the catalytic domain regulates kinase activity: a role for Src family kinases. *Mol. Cell Biol.* 1995; 15:954–963. [PubMed: 7529876]
- Chandak PG, Radovic B, Aflaki E, Kolb D, Buchebner M, Frohlich E, Magnes C, Sinner F, Haemmerle G, Zechner R, et al. Efficient phagocytosis requires triacylglycerol hydrolysis by adipose triglyceride lipase. *J. Biol. Chem.* 2010; 285:20192–20201. [PubMed: 20424161]
- Chinetti-Gbaguidi G, Staels B. Macrophage polarization in metabolic disorders: functions and regulation. *Curr. Opin. Lipid.* 2011; 22:365–372.

- Di Marzio P, Dai WW, Franchin G, Chan AY, Symons M, Sherry B. Role of Rho family GTPases in CCR1- and CCR5-induced actin reorganization in macrophages. *Biochem. Biophys. Res. Commun.* 2005; 331:909–916. [PubMed: 15882964]
- Etienne-Manneville S, Hall A. Rho GTPases in cell biology. *Nature.* 2002; 420:629–635. [PubMed: 12478284]
- Feng B, Zhang D, Kuriakose G, Devlin CM, Kockx M, Tabas I. Niemann-Pick C heterozygosity confers resistance to lesional necrosis and macrophage apoptosis in murine atherosclerosis. *Proc. Natl. Acad. Sci. USA.* 2003; 100:10423–10428. [PubMed: 12923293]
- Fuchs C, Claudel T, Kumari P, Haemmerle G, Pollheimer M, Stojakovic T, Scharnagl H, Halilbasic E, Gumhold J, Silbert D, et al. Absence of adipose triglyceride lipase protects from hepatic ER stress. *Hepatology.* 2012 doi: 10.1002/hep.25601.
- Gautier EL, Huby T, Witztum JL, Ouzilleau B, Miller ER, Saint-Charles F, Aucouturier P, Chapman MJ, Lesnik P. Macrophage apoptosis exerts divergent effects on atherogenesis as a function of lesion stage. *Circulation.* 2009; 119:1795–1804. [PubMed: 19307478]
- Ghosh S. Macrophage cholesterol homeostasis and metabolic diseases: critical role of cholesteryl ester mobilization. *Expert Rev. Cardiovasc. Ther.* 2011; 9:329–340. [PubMed: 21438812]
- Goldstein JL, Dana SE, Faust JR, Beaudet AL, Brown MS. Role of lysosomal acid lipase in the metabolism of plasma low density lipoprotein. Observations in cultured fibroblasts from a patient with cholesteryl ester storage disease. *J. Biol. Chem.* 1975; 250:8487–8495. [PubMed: 172501]
- Grainger DJ, Reckless J, McKilligin E. Apolipoprotein E modulates clearance of apoptotic bodies *in vitro* and *in vivo*, resulting in a systemic proinflammatory state in apolipoprotein E-deficient mice. *J. Immunol.* 2004; 173:6366–6375. [PubMed: 15528376]
- Haemmerle G, Lass A, Zimmermann R, Gorkiewicz G, Meyer C, Rozman J, Heldmaier G, Maier R, Theussl C, Eder S, et al. Defective lipolysis and altered energy metabolism in mice lacking adipose triglyceride lipase. *Science.* 2006; 312:734–737. [PubMed: 16675698]
- Haemmerle G, Moustafa T, Woelkart G, Buttner S, Schmidt A, van de Weijer T, Hesselink M, Jaeger D, Kienesberger PC, Zierler K, et al. ATGL-mediated fat catabolism regulates cardiac mitochondrial function via PPAR- α and PGC-1. *Nat. Med.* 2011; 17:1076–1085. [PubMed: 21857651]
- Jenkins CM, Mancuso DJ, Yan W, Sims HF, Gibson B, Gross RW. Identification, cloning, expression, and purification of three novel human calcium-independent phospholipase A2 family members possessing triacylglycerol lipase and acylglycerol transacylase activities. *J. Biol. Chem.* 2004; 279:48968–48975. [PubMed: 15364929]
- Koliwad SK, Streeper RS, Monetti M, Cornelissen I, Chan L, Terayama K, Naylor S, Rao M, Hubbard B, Farese RV Jr. DGAT1-dependent triacylglycerol storage by macrophages protects mice from diet-induced insulin resistance and inflammation. *J. Clin. Invest.* 2010; 120:756–767. [PubMed: 20124729]
- Kosteli A, Sugaru E, Haemmerle G, Martin JF, Lei J, Zechner R, Ferrante AW Jr. Weight loss and lipolysis promote a dynamic immune response in murine adipose tissue. *J. Clin. Invest.* 2010; 120:3466–3479. [PubMed: 20877011]
- Lammers B, Chandak PG, Aflaki E, Van Puijvelde GH, Radovic B, Hildebrand RB, Meurs I, Out R, Kuiper J, Van Berkel TJ, et al. Macrophage adipose triglyceride lipase deficiency attenuates atherosclerotic lesion development in low-density lipoprotein receptor knockout mice. *Arterioscler. Thromb. Vasc. Biol.* 2011; 31:67–73. [PubMed: 21030715]
- Lass A, Zimmermann R, Haemmerle G, Riederer M, Schoiswohl G, Schweiger M, Kienesberger P, Strauss JG, Gorkiewicz G, Zechner R. Adipose triglyceride lipase-mediated lipolysis of cellular fat stores is activated by CGI-58 and defective in Chanarin-Dorfman syndrome. *Cell Metab.* 2006; 3:309–319. [PubMed: 16679289]
- Lee CH, Kang K, Mehl IR, Nofsinger R, Alaynick WA, Chong LW, Rosenfeld JM, Evans RM. Peroxisome proliferator-activated receptor delta promotes very low-density lipoprotein-derived fatty acid catabolism in the macrophage. *Proc. Natl. Acad. Sci. USA.* 2006; 103:2434–2439. [PubMed: 16467150]

- Listenberger LL, Han X, Lewis SE, Cases S, Farese RV Jr, Ory DS, Schaffer JE. Triglyceride accumulation protects against fatty acid-induced lipotoxicity. *Proc. Natl. Acad. Sci. USA*. 2003; 100:3077–3082. [PubMed: 12629214]
- Lu B, Rutledge BJ, Gu L, Fiorillo J, Lukacs NW, Kunkel SL, North R, Gerard C, Rollins BJ. Abnormalities in monocyte recruitment and cytokine expression in monocyte chemoattractant protein 1-deficient mice. *J. Exp. Med*. 1998; 187:601–608. [PubMed: 9463410]
- Mantovani A, Sica A, Sozzani S, Allavena P, Vecchi A, Locati M. The chemokine system in diverse forms of macrophage activation and polarization. *Trends Immunol*. 2004; 25:677–686. [PubMed: 15530839]
- Martin S, Parton RG. Lipid droplets: a unified view of a dynamic organelle. *Nat. Rev. Mol. Cell Biol*. 2006; 7:373–378. [PubMed: 16550215]
- Mestas J, Ley K. Monocyte-endothelial cell interactions in the development of atherosclerosis. *Trends Cardiovasc. Med*. 2008; 18:228–232. [PubMed: 19185814]
- Mills CD, Kincaid K, Alt JM, Heilman MJ, Hill AM. M-1/M-2 macrophages and the Th1/Th2 paradigm. *J. Immunol*. 2000; 164:6166–6173. [PubMed: 10843666]
- Mosser DM, Edwards JP. Exploring the full spectrum of macrophage activation. *Nat. Rev. Immunol*. 2008; 8:958–969. [PubMed: 19029990]
- Park YM, Febbraio M, Silverstein RL. CD36 modulates migration of mouse and human macrophages in response to oxidized LDL and may contribute to macrophage trapping in the arterial intima. *J. Clin. Invest*. 2009; 119:136–145. [PubMed: 19065049]
- Saraswathi V, Hastay AH. Inhibition of long-chain acyl coenzyme A synthetases during fatty acid loading induces lipotoxicity in macrophages. *Arterioscler. Thromb. Vasc. Biol*. 2009; 29:1937–1943. [PubMed: 19679826]
- Schrijvers DM, De Meyer GR, Herman AG, Martinet W. Phagocytosis in atherosclerosis: molecular mechanisms and implications for plaque progression and stability. *Cardiovasc. Res*. 2007; 73:470–480. [PubMed: 17084825]
- Seimon T, Tabas I. Mechanisms and consequences of macrophage apoptosis in atherosclerosis. *J. Lipid Res*. 2009; 50(Suppl):S382–S387. [PubMed: 18953058]
- Tabas I. Apoptosis and efferocytosis in mouse models of atherosclerosis. *Curr. Drug Targets*. 2007; 8:1288–1296. [PubMed: 18220705]
- Thorp E, Cui D, Schrijvers DM, Kuriakose G, Tabas I. Mertk receptor mutation reduces efferocytosis efficiency and promotes apoptotic cell accumulation and plaque necrosis in atherosclerotic lesions of *apoE^{-/-}* mice. *Arterioscler. Thromb. Vasc. Biol*. 2008; 28:1421–1428. [PubMed: 18451332]
- Ullery-Ricewick JC, Cox BE, Griffin EE, Jerome WG. Triglyceride alters lysosomal cholesterol metabolism in cholesteryl ester-laden macrophage foam cells. *J. Lipid Res*. 2009; 50:2014–2026. [PubMed: 19461120]
- Vicente-Manzanares M, Sanchez-Madrid F. Role of the cytoskeleton during leukocyte responses. *Nat. Rev. Immunol*. 2004; 4:110–122. [PubMed: 15040584]
- Villena JA, Roy S, Sarkadi-Nagy E, Kim KH, Sul HS. Desnutrin, an adipocyte gene encoding a novel patatin domain-containing protein, is induced by fasting and glucocorticoids: ectopic expression of desnutrin increases triglyceride hydrolysis. *J. Biol. Chem*. 2004; 279:47066–47075. [PubMed: 15337759]
- Weiss-Haljiti C, Pasquali C, Ji H, Gillieron C, Chabert C, Curchod ML, Hirsch E, Ridley AJ, Hoof van Huijsduijnen R, Camps M, et al. Involvement of phosphoinositide 3-kinase gamma, Rac, and PAK signaling in chemokine-induced macrophage migration. *J. Biol. Chem*. 2004; 279:43273–43284. [PubMed: 15292195]
- Williams KJ, Tabas I. The response-to-retention hypothesis of early atherogenesis. *Arterioscler. Thromb. Vasc. Biol*. 1995; 15:551–561. [PubMed: 7749869]
- Wu JW, Wang SP, Alvarez F, Casavant S, Gauthier N, Abed L, Soni KG, Yang G, Mitchell GA. Deficiency of liver adipose triglyceride lipase in mice causes progressive hepatic steatosis. *Hepatology*. 2011; 54:122–132. [PubMed: 21465509]
- Yang X, Lu X, Lombes M, Rha GB, Chi YI, Guerin TM, Smart EJ, Liu J. The *G0/G1* switch gene 2 regulates adipose lipolysis through association with adipose triglyceride lipase. *Cell Metab*. 2010; 11:194–205. [PubMed: 20197052]

- Yao PM, Tabas I. Free cholesterol loading of macrophages induces apoptosis involving the fas pathway. *J. Biol. Chem.* 2000; 275:23807–23813. [PubMed: 10791964]
- Yin B, Loike JD, Kako Y, Weinstock PH, Breslow JL, Silverstein SC, Goldberg IJ. Lipoprotein lipase regulates Fc receptor-mediated phagocytosis by macrophages maintained in glucose-deficient medium. *J. Clin. Invest.* 1997; 100:649–657. [PubMed: 9239412]
- Zechner R, Kienesberger PC, Haemmerle G, Zimmermann R, Lass A. Adipose triglyceride lipase and the lipolytic catabolism of cellular fat stores. *J. Lipid Res.* 2009; 50:3–21. [PubMed: 18952573]
- Zimmermann R, Strauss JG, Haemmerle G, Schoiswohl G, Birner-Gruenberger R, Riederer M, Lass A, Neuberger G, Eisenhaber F, Hermetter A, et al. Fat mobilization in adipose tissue is promoted by adipose triglyceride lipase. *Science.* 2004; 306:1383–1386. [PubMed: 15550674]

Figure 1.

Simplified summary of the role of ATGL in macrophages.

FFAs which are taken up by macrophages are incorporated into TGs (and cholesteryl esters). LD-associated ATGL, most likely together with its activator CGI-58, hydrolyzes TGs to generate DGs and FFAs. Activated FFAs are utilized as energy substrate and PPAR signaling molecules. In the absence of ATGL, TGs accumulate within macrophages, resulting in TG-rich foam cells. Reduced FFA concentrations, due to defective TG hydrolysis, lead to decreased PPAR signaling, reduced ATP levels and impaired phagocytosis. Increased ROS production inhibits SHP-2, leading to sustained phosphorylation and activation of FAK, defective small Rho GTPase activation, and reduced migration capacity of macrophages. In addition, ATGL deficiency results in endoplasmic reticulum Ca^{2+} depletion and a concomitant increase in cytosolic Ca^{2+} , which induces the unfolded protein response. Increased ceramide C16:0 concentrations cause dysfunctional mitochondria and activate the mitochondrial apoptosis pathway. Impaired phagocytosis, reduced numbers of WBCs, decreased migration and inflammation and activation of programmed cell death lead to reduced early atherosclerosis. Data are summarized from Chandak et al. (2010), Aflaki et al. (2011a,b, 2012), Lammers et al. (2011). ATGL, adipose triglyceride lipase; CGI-58, comparative gene identification-58; DGs, diglycerides; FFAs, free fatty acids; FAK, focal adhesion kinase; HSL, hormone sensitive lipase; LD, lipid droplet; LPL, lipoprotein lipase; M, mitochondrion; MGs, monoglycerides; MGL, monoglyceride lipase; PPAR, peroxisome proliferator-activated receptor; ROS, reactive oxygen species; SHP-2, Src homology 2-containing phosphotyrosine phosphatase; TGs, triglycerides; UPR, unfolded protein response; WBCs, white blood cells.