LYSOSOMAL ACID LIPASE: FROM CELLULAR LIPID HANDLER TO IMMUNOMETABOLIC TARGET

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Abstract

Lysosomal acid lipase (LAL) hydrolyzes cholesterol esters and triglycerides to free cholesterol and fatty acids,that are then used for the metabolic purposes in the cell. The process also occurs in immune cells which adapt their metabolic machinery to cope with the different energetic requirements associated to cell activation, proliferation and/or polarization. Deficiency of LAL not only causes severe lipid accumulation, but also impacts the immunometabolic signature in animal models. In humans, LAL deficiency has been recently associated with a peculiar clinical immune phenotype, secondary hemophagocytic lymphohistiocytosis. These observations indicate that LAL represents a critical player for cellular immunometabolic modulation and the availability of an effective enzyme replacement strategy makes LAL an attractive target to rewire the immunometabolic machinery of immune cells beyond its role in controlling cellular lipid metabolism.

The evolving concept of immunometabolism and the role of sterol metabolism in immune cells

Dynamic changes in the metabolic machinery of immune cells occur during the activation, proliferation, mobilization and acquisition of effector or regulatory functions [1, 2]. The field of research which addresses these aspects is termed “immunometabolism” and specifically investigates the interplay among different metabolic pathways in supporting the anabolic versus the catabolic needs of immune cells under different conditions. While anabolic pathways provide the energy necessary for production of macromolecules, such as lipids and nucleotides, which mainly occurs in activated and proliferating cells, nutrient catabolism is directed toward the generation of energy during cell maintenance and it’s typical of quiescent, non-proliferating immune cells. Mitochondrial **tricarboxylic acid (TCA)** (see Glossary) cycle and **oxidative phosphorylation (OXPHOS)** from **fatty acid oxidation (FAO)** are thus maximized in resting conditions to generate ATP. Upon activation, the increased demand of energy and building blocks is fulfilled by the rapid increase of anaerobic glycolysis (a metabolic adaptation similar to the **Warburg effect** described for cancer cells [3]) as well as of aerobic catabolism of glucose and amino acids through OXPHOS. While *in vitro* the preference for **glycolysis** compared to OXPHOS clearly marks the functional state of activated immune cells, the picture is less clear *in vivo*. Indeed, while in *in vitro* experiments, glucose and glutamine concentrations, oxygen tension, cell density and the presence of growth factors and cytokines are strictly defined and controlled, they are extremely variable *in vivo* and may also differ among tissues of the body and be influenced by pathological conditions (i.e. cancer compared to healthy tissue). As example, while *in vitro*, regulatory T cells (Treg) engage both glycolysis and fatty acids oxidation (FAO) to support the energy demand, they are highly glycolytic when isolated from human blood [4, 5].

Nutrients are an additional factor that can profoundly influence immune functions by shaping both cellular and systemic metabolism and therefore also play a key role in tuning physiological and pathological immune responses. Among the different nutrients, cholesterol is an essential component of mammalian cells, indispensable for survival and proliferation but cytotoxic when present as unesterified form at elevated intracellular levels [6]. Cholesterol is either synthesized through the mevalonate pathway or is acquired following the uptake of **lipoproteins** (where cholesterol is mainly present in the esterified form [7]). Low levels of intracellular cholesterol activate the **sterol regulatory element binding proteins (SREBPs)**,

thus leading to transcriptional activation of genes required for cholesterol biosynthesis and uptake, such as HMGCoA reductase, the key enzyme of the mevalonate pathway and low density lipoprotein receptor (LDL-R), a key receptor involved in the uptake of lipoproteins [8]. Vice versa, when intracellular cholesterol is elevated, SREBP is retained in the endoplasmic reticulum (ER), the mevalonate pathway is inhibited, and oxidized metabolites of cholesterol, such as the oxysterols, activate the **liver X receptors (LXRs)**, thus promoting the transcription of genes involved in cholesterol efflux, such as the ATP-binding cassette transporters ABCA1 and ABCG1 [9]. Of note, esterified cholesterol (CE), derived from lipoproteins or stored in lipid droplets (LD), can be hydrolyzed mainly within the lysosomal compartment by the action of the lysosomal acid lipase (LAL) [10]. This enzyme not only provides free cholesterol (FC) but also free fatty acids (FFA), and thus potentially contribute to the immunometabolic reprogramming of immune cells. The aim of this review is to discuss recent evidence linking LAL to cholesterol and fatty acids metabolism in the context of immunometabolism.

Lysosomal acid lipase

Expression and role of LAL in cell lipid metabolism

LAL is a 378-amino acid protein which is expressed by all cell types and encoded by the *LIPA* gene on chromosome 10 (q23.2-q23.3) [11]. LAL is expressed constitutively [12] and its expression can be further increased following the activation of the transcription factor EB (TFEB), the master regulator of lysosomal biogenesis, and of the nutrient-sensitive forkhead homeobox type protein O1 (FoxO1) [13-15]. Newly synthetized LAL is transferred to the Golgi apparatus where it is glycosylated. The mannose-6-phosphate (M6P) residues allow the binding with the **M6P receptor (M6PR)** and the localization into the lysosome [16]. In the acidic environment of the lysosome, LAL dissociates from the M6P receptor and is dephosphorylated, thus generating the mature, active form of LAL. Its activity is rapidly reduced with the increase in pH, declining to zero at pH values above 4.5.

The role of LAL is to hydrolyze CE and triglycerides (TG) that reach the lysosomes as the final step of the receptor-mediated endocytosis of very low- and low- density lipoproteins (VLDL, LDL) (**Figure 1**). The products of LAL hydrolysis are either actively exported by the Niemann-Pick type C protein (FC) or likely diffuse into the cytosol (FFA) [17, 18]. FC and FFA in the cytosol repress SREBPs [10, 19], thus reducing the expression of proteins involved in

cholesterol and fatty acid biosynthesis and uptake [8], and, when oxidized, favor LXR activation and ABCA1 expression in macrophages, thus increasing cellular cholesterol efflux [20, 21].

LAL-derived FFA can be used for different purposes; in most cells FFA are directed toward the mitochondria where undergo oxidation and contribute to energy production; in addition, in hepatocytes FFA might be converted into TGs and incorporated in VLDL via MTP, while in adipocytes are released in the circulation or converted to TG and stored in LD (**Figure 1**). Moreover, while the mobilization of lipids from cytosolic LD to generate FFA has been originally attributed to neutral cytosolic or ER-associated lipases, several evidence highlighted the critical role of LAL in driving the hydrolysis of LD through the activation of **autophagy/lipophagy** [22], culminating with the formation of the autophagosome that eventually fuses with lysosomes [15, 23, 24] (**Figure 1**). The activation of lipophagy is sensitive to the nutritional status of the cell and it is now believed to play a key role in the metabolic switch related to cell differentiation process [25, 26].

When LAL is not active, CE and TG accumulate within the lysosomes; in the liver, this accumulation occurs in both hepatocytes and Kupffer cells (specialized macrophages in the liver) thus favoring **hepatic steatosis**. The decreased flux of FC and FFA to the cytosol activates SREBPs and represses LXRs. The net systemic effect on the hepatocyte is the increase of VLDL secretion [27] and the reduction of ABCA1-mediated cholesterol efflux coupled to the reduction of high density lipoproteins (HDL) biogenesis [28]. Both mechanisms contribute to the dyslipidemic profile observed in LAL defective conditions (see below).

Given the 75% identity and 95% similarity in amino acid sequences of murine and human LAL [29], the use of *lal*$^{-/-}$ mice has provided a valuable approach to study the cellular processes regulated by the enzyme. *Lal*$^{-/-}$ mice present with a massive accumulation of TG and CE in the liver, the spleen, the small intestine and the adrenals, which is associated to the loss of white (WAT) and brown adipose tissues (BAT) [30]. Despite the appearance of hepatic foamy lysosomes, *lal*$^{-/-}$ mice show an improved insulin sensitivity and glucose metabolism [31], paralleled by a shift of lipid storage from hepatocytes to Kupffer cells over time [30]. This profile mirrors the observations in hepatic biopsies of LAL deficient patients (discussed below) [11]. Moreover, hematopoietic stem cell transplantation, although limited by graft failure and severity of pre-transplant liver disease, was successfully used in patients with

Wolman disease [32, 33] to restore LAL enzymatic activity in circulating cells and in resident macrophages, thus improving growth and survival rate.

Role of LAL in immune cell maturation and function

LAL plays a crucial role in controlling cholesterol levels in immune cells. Cholesterol accumulation, as a consequence of ABCA1, ABCG1 and **apolipoprotein E (apoE)** deficiency, results in monocytosis, macrophage activation, increased **antigen presentation** [34] and adaptive immune response [35-37]. In agreement, *lal*$^{-/-}$ mice accumulate cholesterol in the lysosome and develop a myeloproliferative disorder, characterized by increased frequency of both circulating and splenic immature monocytes and neutrophils (**Figure 2a**, **Key Figure**; a simplify immune system overview is provided in **Box 1**). This is paralleled by increased infiltration of **myeloid-derived suppressive cells (MDSCs)** in several organs. Anemia, thrombocytopenia and decreased lymphopoiesis were also detected in these mice [38, 39].

LAL-dependent hydrolysis of CE in macrophages provides the substrate for the synthesis of 25-hydroxycholesterol and 27-hydroxycholesterol, both endogenous ligands of LXR, thus promoting cholesterol efflux and contributing to an efficient efferocytosis (a critical process for the phagocytosis of apoptotic cells) (**Figure 2b**) [40]. Further, the immunometabolic function of LAL also extends to the ability to contribute FFA following CE and TG hydrolysis, for energetic purposes. Indeed, in macrophages, LAL was shown to be critical in generating precursor molecules for the synthesis of lipid mediators such as eicosanoids (**Figure 2b**) [41]. Moreover, anti-inflammatory M2 macrophages rely on LAL-dependent neutral lipid lipolysis to provide FFA which are used for FAO (**Figure 2b**) [26]. These fatty acids can be either synthesized within the cells and then packaged into LD or, collected from extracellular sources such as lipoproteins via **CD36**. Of note, only LAL, and no other lipases (i.e. adipose triglyceride lipase, ATGL, or the hormone-sensitive lipase, HSL), has been implicated in the hydrolysis of endogenous and exogenous TG for energy supply purposes in macrophages [26].

In parallel with the impact on macrophage function, LAL has been shown to be crucial for the acquisition of the memory phenotype of $CD8^+$ T cells (**Figure 2c**) contributing to the rapid hydrolysis and release for mitochondrial oxidation of fatty acids *de novo* synthesized. This process has been proposed to support a **futile cycle** where lipogenesis prompted from

glycolysis-derived acetyl-CoA contributes to the storage of neutral lipids in close proximity of ER and lysosome where they are later released as free fatty acids to support cell energy demand [25]. This metabolic adaptation, where both the anabolic and catabolic machineries are active, may reflect the trait of memory cells, long-living quiescent cells that should rapidly re-activate and proliferate upon re-exposure to the antigen. By contrast, effector T cells (Teff) mainly engage fatty acid uptake [42], as demonstrated by CD36 upregulation, which might fuel a faster activation [25]. In this scenario, adipose tissue derived-FFA might potentially represent an energetic fuel of immune cells [43]; indeed, compared to T memory cells at other sites, memory pathogen-specific T cells resident in the visceral adipose tissue (VAT) possess a higher proliferative capacity which is fulfilled by increased FA uptake and mitochondrial oxidation [44]. This mechanism has also been shown to be crucial for mitochondrial oxidation of VAT resident Tregulatory cells (Treg) [45], but not for Tregs in other tissues or under pathological microenvironment, including the tumors [46], where glycolysis-driven lipogenesis appears to fuel FAO. These evidences might indicate that immune cells would shape their metabolic machinery depending on local nutrients availability thus suggesting that LAL activity might contribute to immune cells' activation. Indeed, it has been shown that obesity and lipid accumulation induce lysosome biogenesis in adipose tissue macrophages (ATM) [47] and that lysosomal-derived TG hydrolysis is essential for both adipose tissue homeostasis [48] and ATM function. Of note, deficiency of LAL associates with altered levels of Treg in lymphoid organs (**Figure 2c**) thus corroborating the crucial role of the enzyme in the maintenance of cell survival [39].

Collectively, LAL plays a key role in immune cell biology as it couples intracellular lipid metabolism to cell function. This suggests that the modulation of its activity may represent a valuable therapeutic option for the treatment of diseases characterized by dysregulated immune responses. Is this the case also in humans when LAL is not active?

Genetics of LAL deficiency

More than 50 different mutations in the *LIPA* gene affecting LAL expression or activity have been described in humans to date [11]. Mutations can span the entire gene and include point mutations and frameshifts [49]. Since two out of the three amino acid residues responsible for the enzymatic activity (Ser 153, Asp 324 and His 353) are located in the C-terminal region of the protein, almost all nonsense mutations result in complete LAL

deficiency when present on both alleles [50].According to the residual enzymatic activity, genetic LAL deficiency (LAL-D, OMIM 278000) can present with a different spectrum of severity, from lethal Wolman Disease (WD) to less severe Cholesteryl Ester Storage Disease (CESD).

WD, an extremely rare and recessive disease (1 case every 1,000,000 subjects), characterized by a neonatal onset which leads to death within the first year of life, is caused by near absence of LAL. Consequently, the massive accumulation of CE and TG in the liver, spleen, adrenal glands, bone marrow and lymph nodes cause hepatosplenomegaly, adrenal calcification, anemia and thrombocytopenia, respiratory failure, vomiting, diarrhea, cachexia and failure to thrive. Liver histology shows steatosis and fibrosis, rapidly progressing to cirrhosis. A recent analysis of 35 cases estimated a median age at death of 3.7 months and a 0.26 probability of survival past 6 months of age [51].

Cholesteryl ester storage disease (CESD) is characterized by a residual LAL activity usually within 1-12% of the normal range. About 50-60% of CESD cases are carriers of a splicing variant in the last nucleotide of exon 8 (c.894G > A, p.Ser275_Gln298del) at least on one allele. The mutation (referred to as E8SJM) causes the skipping of exon 8, generating an inactive LAL; however, a small percentage of correct splicing (<5%) still assures a residual activity. The frequency of this mutation was used to estimate the overall prevalence of CESD in the general population which is 1:200 to 1:420 in heterozygosity, while the occurrence of homozygosity/compound heterozygosity ranges between 1:40.000 to 1:175.000 [49, 52, 53]. CESD presents with a wide range of severity, with onset from infancy to adulthood. Clinically, the accumulation of lipids mainly in the liver and in macrophages throughout the body results in hepatomegaly and splenomegaly; mortality is usually due to liver failure or cardiovascular disease. Liver histology shows a peculiar microvescicular steatosis that can rapidly evolve to fibrosis and micronodular cirrhosis [54, 55]. Biochemically, almost all CESD patients present with increased plasma levels of **transaminases**, especially alanine aminotransferase, and dyslipidemia: elevated plasma levels of total and LDL-cholesterol are associated with reduced HDL-cholesterol and less frequently to hypertriglyceridemia [54-56].

Despite the reports of occurrence of anemia, thrombocytosis, and the accumulation of lipids also in the bone marrow and the lymph nodes, few data are available on the impact of LAL-D on immune response in humans. Very recently, the presence of secondary **hemophagocytic lymphohistiocytosis (HLH)** was described in WD case reports [57-63].

HLH is an immune disorder frequently associated with inborn errors of metabolism, including other lysosomal storage disorders. Since HLH phenotype is overlapping with several other conditions, the diagnosis is performed by the presence of at least 5 of the following 8 criteria: fever, splenomegaly, cytopenia, hypertriglyceridemia or hypofibrinogenemia, hyperferritinemia, reduced NK cell activity, elevated soluble CD25 and the presence of hemophagocytosis in the bone marrow (giant, lipid-laden histiocytes with cytoplasmic cellular fragments) . In one of the WD cases with secondary HLH, a 2-month-old Native American female homozygous for the c.658C>T (p.P220S) mutation in the *LIPA* gene, a further analysis of the immunophenotype was performed: a significant reduction in the absolute count of B lymphocytes, CD4+ and CD8+ T lymphocytes was detected, suggesting a potential impairment in humoral and cell-mediated adaptive immune response [63]. Although being limited to a small number of sporadic cases, these reports on such a rare disease as WD suggest that genetic LAL-D affects the immune phenotype also in humans and pave the road for further evaluations.

LAL as a therapeutic target beyond genetic LAL-D

LAL enzyme replacement therapy

In spite of the dramatic phenotypes of LAL-D patients described above, until recently no therapeutic options were available for WD patients, while CESD patients were usually treated with lipid-lowering agents to control dyslipidemia (see Box 2). The therapeutic scenario completely changed in late 2015, when sebelipase alfa, a recombinant human LAL protein (rhLAL) produced in egg whites of transgenic hens, was approved as enzyme replacement therapy (ERT) for LAL-D by the Food and Drug Administration and the European Medicines Agency [64]. As with other enzymes for ERT of lysosomal disorders, sebelipase alfa is a glycoprotein carrying M6P moieties. Since the M6P receptors are expressed on the membrane of several cell types, including hepatocytes and macrophages, sebelipase alfa is taken up by all these cells and transported to the lysosomal compartment, where it can correct the phenotype resulting from the genetic LAL-D [56]. Sebelipase alfa can be life-saving for WD, as suggested by the results of an open trial on 9 WD newborns: after an initial infusion of 0.35 mg/kg, sebelipase alfa dose was progressively increased up to 5 mg/kg once-weekly [65]. Six of the patients treated with sebelipase alfa have survived to age ≥12 months and five to ≥24 months, with a marked improvement in growth parameters and liver function.

These data are in striking contrast to an estimated 26% probability to survive past 6 months of age when left untreated (see above).

The efficacy of sebelipase alfa has been tested also in CESD patients. In the Acid Lipase Replacement Investigating Safety and Efficacy (ARISE) trial (ClinicalTrials.gov number NCT01757184), 66 patients were treated with 1.0 mg/kg of sebelipase alfa bi-weekly for 20 weeks, followed by an extension period of up to 130 weeks [56]. After 20 weeks of treatment, sebelipase reduced LDL-cholesterol by 28.4% and TG by 25.5%, with a concomitant increase of HDL-cholesterol (+19.6%). The treatment also resulted in a significant improvement in liver function: plasma alanine aminotransferase levels were reduced by up to 60% and hepatic fat by 32%, leading to a decrease of steatosis in 62% of treated patients. To what extent the treatment with sebelipase alfa could alter cardiovascular and hepatic consequences of LAL-D in the long term is presently unknown. The extension period of the ARISE trial is aimed also at investigating the effect of sebelipase alfa on fibrosis. Preliminary data by Goodman *et al* showed a regression of fibrosis of >1-stage in 12 out of 20 patients, no change in fibrosis severity in 6 patients, while worsening was observed in 2 patients [66].

Evidence for LAL replacement therapy on immune cell function

To date there are no studies which address the effect of ERT on the immune system of LAL-D patients. However, in a case of WD with secondary hemophagocytic lymphohistiocytosis, the treatment with sebelipase for 3 months resulted in the normalization of lymphocytes B cells' levels, while no changes were observed in the T cells' levels [63]. Most of the current evidence of the impact of LAL restoration on immune cells function, come from studies performed in mice. Reconstitution of hLAL activity in myeloid cells of *lal*$^{-/-}$ mice, achieved through a doxycycline-inducible transgenic system, was shown to ameliorate myelopoiesis in the bone marrow and to reduce systemic expansion of MDSCs. Myeloid hLAL expression inhibited the production of reactive oxygen species (ROS) from neutrophils and their tissue infiltration [67]. The observation that *in vitro* rhLAL treatment of *lal-/-* macrophages increased the expression of ABCA1 transporter, thus enhancing excess cholesterol efflux from the cell [21], suggests that this effect might depend on an ameliorated handling of intracellular cholesterol in immune cells. In agreement to this hypothesis, foamy macrophages were shown to take up rhLAL in the atherosclerotic plaque of *ldl-r*$^{-/-}$ treated mice, decreasing the lesion size by 50% when compared to controls [68]. Of note, mice developed anti-rhLAL

antibodies which however did not appear to inhibit LAL activity [68]; whether this immune activation might have also affected the functionality of other immune cells in the experimental setting used has not been investigated.

Taken together, these observations point to the potential role of immune cell-derived LAL in restoring intracellular cholesterol homeostasis, correcting aberrant immuno-inflammatory response thus paving the road for considering LAL as a novel immunometabolic target.

Concluding remarks and future perspectives

An intimate communication exists between cell metabolism and immune function. The regulation of cellular lipid homeostasis is achieved by several, highly controlled, steps. LAL represents a key protein controlling the availability of FC and FFA, the building and energy blocks of the cells. Thus, targeting lipid metabolism in immune cells may offer a therapeutic option not only for the treatment of metabolic disorders such as dyslipidemia, but also could potentially rewire the function of the immune system. Restoring LAL activity via ERT not only improves metabolic parameters in LAL-D patients, but is associated, at least in experimental models, with the improvement of the immuno-inflammatory responses, characterized by the decrease of myeloid cell proliferation and activation, as a consequence of increased cholesterol efflux (**Figure 3a**), by the production of pro-resolving lipid mediators ~~and~~ involved in efferocytosis (**Figure 3b**), and by the boost of Treg and $CD8^+$T memory cells oxidative metabolism (**Figure 3c**). Since the cellular uptake of sebelipase alfa requires the expression of the M6P receptor, translating this approach for immune purposes will be more effective in those cells where M6P is elevated (See Outstanding Questions). Most of the circulating leukocytes express the M6PR and, moreover, its expression has been reported to be upregulated approximately 4-fold on blood monocytes incubated with lipopolysaccharide [69] and on activated T cells [70]. These observations, together with data showing how modulation of lipid metabolism impacts on immune activities, offer the rationale to target LAL with ERT as a novel option for the treatment of immunometabolic diseases.

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Resources:

i. https://www.proteomicsdb.org/proteomicsdb/#human/proteinDetails/55301/expression

ii. https://www.proteomicsdb.org/proteomicsdb/#human/proteinDetails/P20645/expression

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TEXT BOXES

BOX 1. Immune system overview.

The immune system comprises a series of tissues, cells and molecules that protect the body from not self material and is classically divided in the innate and the adaptive arms. Granulocytes, monocytes, macrophages represent the key components of the innate arm while antigen presenting cells, B and T cells belong to the adaptive arm.

Immune cells originate from hematopoietic stem cell precursor (HSP), self-renewing stem cells that can differentiate into any blood cell type, located in perivascular bone marrow niches [71]. HSP differentiate in MPPs (multipotent progenitors), that subsequently originate the CLPs (common lymphoid progenitors) and CMPs (common myeloid progenitors) (**Figure box 1**). Whereas CLPs migrate to the thymus to complete the maturation to T cells, NK cells or B cells, CMPs further differentiate to GMP (granulocyte-macrophage progenitor) or MKEP (megakaryocyte-erythrocyte progenitor) (**Figure box 1**). While MKEPs originate

megakariocytes and erythrocytes, the GMPs differentiate to granulocytes or monocytes that are released in the circulation. Myeloproliferative disorders are characterized by the overproduction of one type of blood immune cells, such as leukemias, while anemia, identifies diseases associated to decreased blood cells production. Hemophagocytic lymphohistiocytosis (HLH) is an example of myeloproliferative diseases characterized by the production of excessive activated macrophages (known as histocytes) and lymphocytes. Patients with HLH usually develop fever, enlarged liver or spleen, cytopenia, and neurological abnormalities within the first months or years of life. HLH may be inherited in an autosomal recessive manner or can be acquired. Other abnormalities in hematopoiesis include the increase of myeloid-derived suppressor cells (MDSC) under pathological conditions, which might occur in chronic infections or cancer. MDSC, exert a strong immunosuppressive function thus leading to the inhibition of T cell proliferation and activation.

The different subsets of immune cells are recognized on the basis of the expression of peculiar patterns of superficial receptors that account for their specific functions, (**Figure box 1**). Broadly, all leucocytes express CD45, with circulating myeloid cells expressing the CD11b marker, B lymphocytes expressing CD19 and CD20 and T lymphocytes expressing CD3. CD11b+ myeloid cells are then divided in granulocytes (CD11b+/Gr-1+ in mice CD11b+/CD16+ in humans) and monocytes (CD11b+/Ly6C+ and CD11b+/CD14+ in humans), that further differentiate to macrophages (CD11b+/CD68 in humans and mice while F4/80+ only in mouse) once migrated into tissue. These cells represent the innate arm of immune response as they activate in an unspecific manner upon the encounter with any not-self antigen by its phagocytosis, cytokine and ROS (reactive oxygen species) production. By contrast, cells of the adaptive immune response need to be “instructed” by professional antigen presenting cells (as dendritic cells, characterized by CD11c expression). Lymphocyte T cells (CD3+) are further divided in CD4+ (T helper) and CD8+ (T cytotoxic). After activation, a pool of CD4+ and CD8+ T cells persists as memory cells that can be rapidly re-activated following encounter with the same not-self antigen. Paralleled to the effector arm, the adaptive immune response comprises a tolerogenic response carried by regulatory T cells (Treg), a subset of CD4+ T cells that maintain tolerance to self-antigen thus preventing autoimmunity and patrolling for exaggerated immune activation.

BOX 2. Management of CESD with lipid-lowering agents

Since CESD is characterized by hypercholesterolemia associated with low high density lipoprotein (HDL)-cholesterol and increased TG, lipid-lowering agents (mainly statins) are usually prescribed to the patients. Statins are reported to have a variable effect on total and LDL-cholesterol, with some patients responding well while others not. The average reduction for TC is around 20-30% [55]. The effect of statins on liver disease in CESD is still debated. Indeed, one would expect that the statin-mediated inhibition of cholesterol biosynthesis in the liver, which leads to the SREBP2-mediated upregulation of LDL-R expression, might contribute to an increase of hepatic uptake of LDL with a consequent worsening of hepatic steatosis. Consistently, statins were not associated with transaminases normalization in CESD patients [55].

Some reports indicate the use of ezetimibe in CESD patients, but robust data are not yet available on its lipid-lowering efficacy alone or in combination with statins [55, 72]. However, in *lal*$^{-/-}$ mice, ezetimibe significantly reduces the amount of CE sequestered in the liver and small intestine, thus improving liver steatosis and suggesting that intestinal cholesterol absorption could also play a role in cellular lipid accumulation observed in LAL-D [73]. In line with this, an amelioration of liver disease was observed in young CESD patients treated with ezetimibe alone or in association with statins [72, 74, 75].

FIGURE LEGENDS

Figure 1. Role of LAL in cell lipid metabolism

Lysosomal acid lipase (LAL) is responsible for the hydrolysis of cholesteryl esters (CE) and triglycerides (TG) carried by apoB-containing lipoproteins, as LDL and VLDL, which are internalized by receptor-mediated endocytosis. Generated free cholesterol (FC) and free fatty acids (FFA) are released into the cytosol, where their accumulation regulates their own synthesis and metabolism through the interaction with different transcription factors. The activation of autophagy also leads to the transport of lipid droplets (LD) to the lysosomes for the LAL-mediated hydrolysis and generation of FFA. This pathway is alternative to the classical mobilization of LD-stored FFA by neutral hydrolases. FFA could have different metabolic fates according to the cell type and the nutritional state. Cytosolic FFA can enter the fatty acid oxidation cascade for ATP production. In adipocytes, FFA are released in the

609 circulation or are converted to TG. In hepatocytes, TG are packed by MTP into VLDL and
610 secreted.
611 Abbreviation used: ABC, ATP-binding cassette; CE, cholesteryl esters; FAO, fatty acid
612 oxidation; FC, free cholesterol; FFA, free fatty acids; LD, lipid droplets; LDL, low density
613 lipoproteins; LDL-r, LDL-receptor; LXRs, liver X receptors; MTP, microsomal transfer protein;
614 SREBPs, sterol-regulatory element binding proteins; TG, triglycerides.
615
616 **Figure 2, Key Figure. Consequences of LAL deficiency in immune cells**
617 LAL deficiency (LAL-D) (a) promotes excessive proliferation of myeloid cells and impaired
618 maturation of monocytes and neutrophils leading to increased circulating levels of MDSC
619 (myeloid-derived suppressor cells), (b) impairs macrophage polarization toward M2,
620 efferocytosis and eicosanoids production, (c) decreases lymphopoiesis, the frequency of
621 $CD8^+$ T cells memory cells and of Tregulatory (Treg) cells in lymphoid organs.
622
623 **Figure 3. Potential effects of LAL replacement therapy on immune functions**
624 Recombinant LAL is delivered to the lysosomes via the mannose 6-phosphate receptor
625 (M6PR). Enhanced LAL activity increases the flux into the cytosol of free cholesterol (FC),
626 triggering the activation of the LXR pathway, while free fatty acids (FFA) fuel mitochondrial
627 FAO. (a) In macrophages, this results in increased cholesterol efflux, a mechanism that has
628 been shown to dampen excessive myeloid proliferation and dyslipidemia. (b) In addition, LAL-
629 dependent activation of the LXR pathway might improve efferrocytosis and promote
630 macrophage polarization toward anti-inflammatory M2 phenotype. All these mechanisms
631 could protect toward atherosclerosis development. (c) Fueling FAO in $CD8^+$ memory T cells
632 and T regulatory cell would represent a potential approach to modulate adaptive immune
633 responses in the context of auto-immune disorders and cancer.
634
635

Glossary

Antigen presentation: A process consisting in foreign antigen fragmentation and processing by phagocytes, usually macrophages and dendritic cells, followed by the binding of peptides to the major histocompatibility complex (MHC), and transport to the surface of the cell, where it can be recognized by the T cell receptor or the B cell receptor.

Apolipoprotein E (apoE): An apolipoprotein that plays a key role in cholesterol transport throughout the body. Liver-derived apoE associate to lipoproteins and promotes the catabolism of very-low density lipoprotein (VLDL) and low density lipoprotein (LDL); myeloid cell' derived apoE is involved in cholesterol efflux from the cells.

ATP-binding cassette transporters A1 and G1 (ABCA1, ABCG1): Transmembrane ATP-dependent lipid transporters which promote the efflux of cellular cholesterol and phospholipids to extracellular acceptors, as high-density lipoproteins (HDL), apolipoprotein A-I or apolipoprotein E.

Autophagy: A regulated process used by the cell for degradation and recycling of unnecessary or altered cellular components. It usually consists in the formation of double-membraned vesicle, the autophagosome, that fuses with lysosomes. Three forms of autophagy commonly exist: macroautophagy, microautophagy, and chaperone-mediated autophagy.

Cluster of differentiation 36 (CD36): A glycosylated transmembrane protein which belongs to the class B scavenger receptor family. It is expressed on the surface of several cell types and it is a multifunctional receptor, since it recognizes modified phospholipids, fatty acids and proteins containing thrombospondin-homolog domains. Oxidized LDL, which carry negatively charged phospholipids, are also recognized and internalized by CD36.

Glycolysis: A sequence of enzyme-catalyzed reactions that converts glucose into pyruvate.

Hemophagocytic lymphohistiocytosis (HLH): A rare but potentially fatal disease where phenotypically normal histiocytes and lymphocytes are overactive. This disease commonly appears in infancy and can have a genetic base or be secondary to other conditions such as LAL deficiency.

Fatty acid oxidation (FAO): The catabolic process by which fatty acids are broken down to generate acetyl-CoA.

Futile cycle: A process that occurs when two metabolic pathways run simultaneously in opposite directions and have no overall effect other than to dissipate energy in the form of heat. In the context of immune cells, this term has been used to indicate the concomitant presence of the anabolic and catabolic pathways.

Hepatic steatosis: A pathological condition characterized by excess lipid accumulation in the liver. Two types of steatosis are reported: the alcoholic liver disease and non-alcoholic fatty liver disease (NAFLD), which usually develops as a complication of diabetes and obesity.

Lipid droplets (LD): Cytosolic bodies that act as intracellular stores of fatty acids and cholesterol in the form of neutral lipids. These are directly hydrolyzed in the cytosol by neutral lipases or are routed to the lysosomes by autophagy to meet energy requirements in the cell.

Lipoproteins: Complex particles made of a central core of esterified cholesterol and triglycerides surrounded by free cholesterol, phospholipids and apolipoproteins. They allow the transport of water-insoluble lipids and are classified according to their density and composition into: chylomicrons, very-low density lipoproteins (VLDL), intermediate density lipoproteins (IDL), low density lipoproteins (LDL), high density lipoproteins (HDL) and lipoprotein (a) (Lp(a)). VLDL and LDL contain apolipoprotein B as the main protein component and their role is to deliver lipids from the liver to peripheral tissues. On the contrary, HDL, whose main protein component is apolipoprotein A-I, are the vehicles for the so-called reverse transport of cholesterol from peripheral tissues to the liver.

Liver X receptor (LXR): Nuclear receptor family of transcription factors that are important regulators of cholesterol, fatty acid, and glucose homeostasis. Two isoforms of LXR have been identified: LXRα and LXRβ. While LXRβ is ubiquitously expressed, LXRα is expressed mainly in the liver, but is also found in the kidney, intestine, fat tissue, macrophages, lung, and spleen. LXRα and LXRβ form heterodimers with the 9-cis retinoic acid receptor (RXR), following the presence of an LXR agonist (such as oxysterols). LXR controls the transcription of genes that regulate lipids and cholesterol metabolism thus pinpoint their crucial role in cell metabolism and metabolic diseases.

Lymphopoiesis: The generation of lymphocytes from a hematopoietic cell precursor. B cell lymphopoiesis is completed in the bone marrow, whereas T cell lymphopoiesis occurs in the thymus.

Lysosomal acid lipase (LAL): Enzyme responsible for the hydrolysis of cholesteryl esters and triglycerides in the lysosomes.

Mannose-6-phosphate receptor (M6PR): Member of the P-type lectin family involved in the transport of acid hydrolases from the Golgi to the lysosomes. In the Golgi apparatus, acid hydrolases are modified with the addition of mannose-6-phoshate (M6P) residues, which allow their recognition by the M6PR on the surface of lysosomes. M6PR is also expressed on the surface of several cell types, favoring the cellular uptake of proteins carrying M6P residues.

Myelopoiesis: The process of blood cells development from a myeloid progenitor cell.

Myeloid-derived suppressor cells (MDSCs): A heterogeneous population of cells defined by their myeloid origin, immature state and ability to potently suppress T cell responses.

Oxidative phosphorylation (OXPHOS): The metabolic process by which ATP is generated as the result of the transfer of electrons by a series of electron transport proteins in the mitochondria.

Sterol element-binding proteins (SREBPs): A family of transcription factors belonging to the basic-helix-loop-helix leucine zipper class and consisting of two genes, the SREBF1 and SREBF2, that encode for three different proteins: SREBP1a, SREBP1c and SREBP2. SREB proteins regulate the transcription of genes involved in cholesterol biosynthesis and uptake, and fatty acid biosynthesis.

T lymphocytes: A subset of white blood cell that play a central role in adaptive immunity. They can be differentiated into $CD4^+$ helper T cells that contribute immune response by secretion of cytokines, $CD4^+$ regulatory T cells that maintain immunological tolerance, and $CD8^+$ cytotoxic T cells that kill virus-infected and tumor cells and are also implicated in transplant rejection.

Transaminases: Hepatic enzymes that catalyze a transamination reaction between an amino acid and an α-keto acid required for amino acid synthesis. Increased transaminanses plasma levels mark liver or cardiac damage.

Tricarboxylic acid cycle (TCA): A series of chemical reactions used to generate adenosine triphosphate (ATP) via the oxidation of acetyl-CoA derived from carbohydrates, fats, and proteins.

Warburg Effect: A phenomenon characterized by increased rate of glucose uptake in which cells produce energy through increased aerobic glycolysis and preferential production of lactate, even in the presence of oxygen.