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Minireview

Contribution of inflammatory pathways to Fabry disease pathogenesis

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ABSTRACT

Lysosomal storage diseases are usually considered to be pathologies in which the passive deposition of unwanted materials leads to functional changes in lysosomes. Lysosomal deposition of unmetabolized glycolipid substrates stimulates the activation of pathogenic cascades, including immunological processes, and particularly the activation of inflammation. In lysosomal storage diseases, the inflammatory response is continuously being activated because the stimulus cannot be eliminated. Consequently, inflammation becomes a chronic process. Lysosomes play a role in many steps of the immune response. Leukocyte perturbation and over-expression of immune molecules have been reported in Fabry disease. Innate immunity is activated by signals originating from dendritic cells via interactions between toll-like receptors and globotriaosylceramide (Gb3) and/or globotriaosylsphingosine (lyso-Gb3). Evidence indicates that these glycolipids can activate toll-like receptors, thus triggering inflammation and fibrosis cascades. In the kidney, Gb3 deposition is associated with the increased release of transforming growth factor beta and with epithelial-to-mesenchymal cell transition, leading to the over-expression of pro-fibrotic molecules and to renal fibrosis. Interstitial fibrosis is also a typical feature of heart involvement in Fabry disease. Endomyocardial biopsies show infiltration of lymphocytes and macrophages, suggesting a role for inflammation in causing tissue damage. Inflammation is present in all tissues and may be associated with other potentially pathologic processes such as apoptosis, impaired autophagy, and increases in pro-oxidative molecules, which could all contribute synergistically to tissue damage. In Fabry disease, the activation of chronic inflammation over time leads to organ damage. Therefore, enzyme replacement therapy must be started early, before this process becomes irreversible.

1. Introduction

Lysosomal storage disorders are a group of more than 50 monogenic disorders resulting from defects in the function of a protein essential for normal lysosome metabolism [1]. Lysosomes are membrane-bound organelles with an acidic luminal pH and are found in most animal cells [2]. The lysosome lumen contains more than 60 different hydrolytic enzymes that degrade macromolecules [3]. Defective functioning of these enzymes generally leads to the progressive accumulation of undegraded substrates inside lysosomes.

Beyond the degradation of unwanted materials, lysosomes have a greater impact than previously thought on many other cellular processes, including a central role in normal immune system functioning [4,5]. This role is played at many stages of the immune response, including antigen presentation and processing [6–8], secretion of perforins by cytotoxic T cells, phagocytosis, and release of pro-inflammatory mediators [7–9]. Pathologies altering lysosome function are therefore hypothesized to have an effect on the immune system [10,11].

Studies focusing on immune system irregularities in lysosomal storage disorders have shown that substrate deposits in lysosomes fuel multiple pathogenic cascades that ultimately lead to an inflammatory response, regardless of the specific substrate involved [10,12,13]. If the inflammation continues over a long period of time, cellular damage can increase and pathogenesis may become uncoupled from the substrate accumulation by which it was first initiated. This may be a causative

Abbreviations: CD, cluster of differentiation; CNS, central nervous system; DAMP, damage-associated molecular pattern; ERT, enzyme replacement therapy; FC, Fabry cardiomyopathy; FD, Fabry disease; Gb3, globotriaosylceramide; *GLA*, alpha-galactosidase A gene; ICAM, intercellular adhesion molecule; IL, interleukin; iNKT, invariant natural killer T cell; LVH, left ventricular hypertrophy; lyso-Gb3, globotriaosylsphingosine; MAPK, mitogen-activated protein kinase; MCP, monocyte chemoattractant protein; MHC, major histocompatibility complex; NAIP, neuronal apoptosis inhibitory protein; NF-κB, nuclear factor kappa B; NKT, natural killer T cell; NO, nitric oxide; PAMP, pathogen-associated molecular pattern; PBMC, peripheral blood mononuclear cell; sVCAM, soluble vascular cell adhesion molecule; TGF, transforming growth factor; TIA, transient ischemic attack; TLR, toll-like receptor; TNF, tumor necrosis factor; VCAM, vascular cell adhesion molecule

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factor in progression of the underlying lysosomal disorder despite initiation of therapy, and may be one possible explanation for the clinical failure of therapy in some cases. In this article, we review the current understanding of this rapidly evolving field by analyzing published data.

2. Chronic inflammation versus acute inflammation is a disease state involved in the pathogenesis of many different diseases

Inflammation is part of the complex biological response of body tissues to harmful stimuli such as pathogens, damaged cells, and irritants. It is a protective response involving immune cells, blood vessels, and molecular mediators. Understanding the type of inflammation and the mechanisms and cells involved is important for understanding its potential involvement in Fabry disease pathogenesis. Inflammation is part of an innate immune response that is generally nonspecific – one of the body's first mechanisms of defense against local infection, injury, and disease [14]. Damage-associated molecular patterns (DAMPs) are endogenous molecules released from injured or dying cells. They are recognized by pattern recognition receptors on immune cells, resulting in the initiation of an inflammatory response [15]. Physiologically, inflammation is an acute response that stops once the trigger is no longer present, and is caused by the release of cytokines and other inflammatory mediators, resulting in extravasation of leukocytes into tissues.

The main cell types in the early inflammatory response are neutrophils and macrophages. Later, lymphocytes also become involved. Acellular inflammatory mediators include several different classes of molecules (lipid mediators such as prostaglandins and leukotrienes), cytokines and chemokines, complement cascade factors, the kinin system, and the coagulation system.

Chronic systemic inflammation differs from acute inflammation because it is a disease state. If the trigger cannot be eliminated, the prolonged secretion of DAMPs results in continuous activation of inflammation and leads to self-attack of cells and tissues and, eventually, cell death. Any disease process causing tissue injury may simultaneously stimulate the inflammatory cascade. Chronic inflammation is generally a silent and slow process [16], and patients often do not realize that inflammation is present until there is irreversible damage with clinical sequelae.

Autoinflammatory disorders are caused by the recognition of “abnormal self” or DAMPs in injured cells. There is no main direct effect on lymphocyte function in autoinflammatory disorders. Instead, autoinflammatory disorders promote the release of chemokines, adhesion molecules, and other pro-inflammatory mediators that enhance tissue infiltration and eventually lead to tissue remodeling. Taking into account the molecular mechanisms fired in Fabry cells and tissues that will be described in this review, we speculate that autoinflammatory processes may contribute to the pathogenesis of Fabry disease.

3. Immune system activation via generation of an inflammatory response is observed in Fabry disease

Fabry disease is a lysosomal storage disorder caused by a deficiency of the enzyme alpha-galactosidase A, resulting from mutations in the *GLA* gene. It is characterized by accumulating levels of glycolipids, mainly globotriaosylceramide (Gb3) or globotriaosylsphingosine (lyso-Gb3), within the lysosomes of many cell types throughout the body. Early symptoms can manifest in childhood and include acroparesthesia, angiokeratoma, and anhidrosis. The disease progresses into adulthood with cardiac, renal, and cerebral complications, ultimately leading to premature death due to organ failure, peaking in the fifth decade for males and the seventh for females [17].

The high levels of glycolipids in the cells and plasma of patients with Fabry disease are not sufficient to explain the pathophysiology of this disorder. Moreover, family members with *GLA* mutations can have very different clinical presentations (intra-familial phenotypic variability) [18]. It has been suggested that the anomalous accumulation of Gb3 or lyso-Gb3 due to alpha-galactosidase A deficiency in patients with Fabry disease could trigger different cellular mechanisms that contribute to the phenotypic expression of this disease [19,20]. Lysosomal deposits may behave as DAMPs or cause DAMP production by injured cells, with subsequent pro-inflammatory activity, because it has been shown that addition of Gb3 to normal control cells induces apoptosis and cytokine secretion [21,22]. This may help explain the pathological changes seen in Fabry disease target organs, including the kidney and heart. Indeed, there is growing evidence to support the concept that different mechanisms of the immune system are activated in Fabry disease [23].

Several studies have focused on inflammation markers and leukocyte activity in Fabry disease. Leukocytes and endothelium from patients with Fabry disease show signs of inflammatory activation [24,25], characterized by increased expression of adhesion molecules, such as CD31 in CD3+ lymphocytes, monocytes, and granulocytes, when compared with healthy controls [26]. This increased surface expression of adhesion molecules could be involved in the extravasation of leukocytes into peripheral tissues.

One of the consequences of inflammation is the generation of reactive oxygen species, which were found to be produced in endothelial cells exposed to Gb3 in vitro [25]. Furthermore, Biancini and colleagues reported altered glutathione metabolism, high lipid peroxidation levels, and high levels of nitric oxide equivalents in patients with Fabry disease [27–29]. The same group also reported a significant increase in plasma carbonyl groups, indicative of oxidative protein damage [27]. Altered peptides derived from these damaged proteins may serve as neoantigens and induce autoimmune responses. Such a mechanism could potentially explain the coexistence of Fabry disease and autoimmune disorders reported in the literature, whereby the high prevalence of autoantibodies found in patients with Fabry disease may be due to glycolipids representing a constant stimulus and inducing autoimmune disorders [30,31].

If glycolipids behave as antigens, the accumulation of Gb3 or lyso-Gb3 in Fabry disease may have a direct effect on the immune system. Glycolipids are recognized as antigens when they are presented to natural killer T (NKT) cells by CD1d-bearing antigen-presenting cells [32], and CD1d moves through the endolysosomal compartments [33] where accumulation occurs. Based on this hypothesis, studies were conducted on NKT cells from patients with Fabry disease [26,34]. Although no difference was found between studies with respect to the total numbers of CD8+ NKT cells, discordant results were found between the studies in the proportion of CD8+ NKT cells: Rozenfeld et al. [26] found proportions of 26% vs 19% ($p < 0.01$) CD8+ NKT cells in samples from normal controls vs patients with Fabry disease, whereas no significant difference was found by Pereira et al. [34]. Further leukocyte perturbations were also found; specifically, reduced numbers of monocytes, CD8+ cells, and dendritic cells, and increased percentages of total lymphocytes and B cells [26]. The clinical relevance of these changes is not yet known.

Although reports of inflammatory cell infiltration into Fabry target organs are currently limited, one study reported increased levels of the macrophage-related markers CD68, CD163, and CD45 in endomyocardial biopsy samples from patients with Fabry disease [35]. This may indicate some degree of myocardial macrophage infiltration, but more evidence is required before the involvement of classical inflammatory pathways in Fabry disease can be confirmed.

4. Urinary markers of inflammation in Fabry disease have been discovered via proteomic/transcriptomic profiling

Identifying proteins differentially expressed among patients with Fabry disease compared with healthy controls could help to identify biological processes that are amplified in the disease state, and could

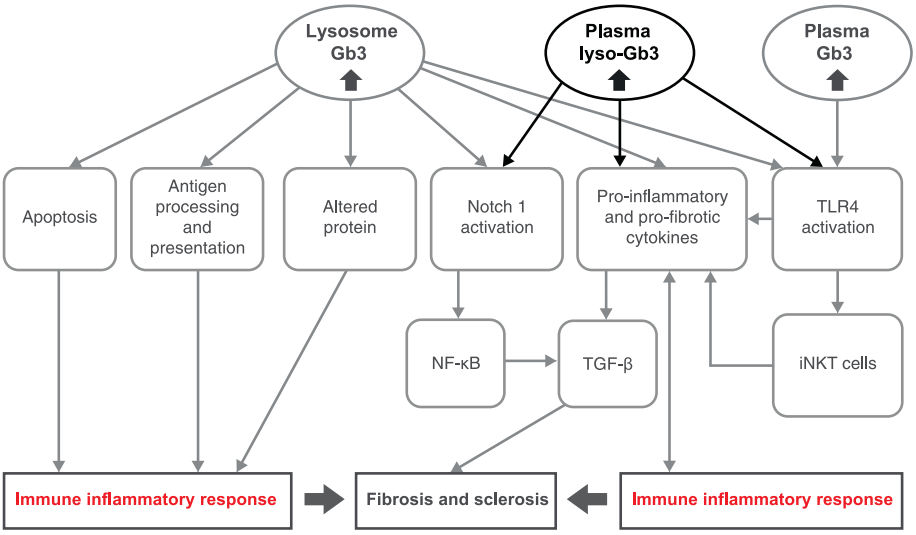

Fig. 1. Pathogenic hypothesis of tissue damage in Fabry disease. While all kinds of cells are exposed to lyso-Gb3 and Gb3, the response differs according to cell type. Apoptosis may occur in all organs, while activation of inflammation and the immune response mostly occurs in cells with exolysosomes, such as monocytes, lymphocytes, and dendritic cells. Moreover, in some parenchyma, such as the kidney, lyso-Gb3 induces the transformation of epithelial and endothelial cells into mesenchymal cells, with production of pro-inflammatory and pro-fibrotic cytokines. Gb3 = globotriaosylceramide; iNKT = invariant natural killer T cell; lyso-Gb3 = globotriaosylsphingosine; NF-κB = nuclear factor kappa B; TGF-β = transforming growth factor beta; TLR = toll-like receptor.

also help to reveal the pathophysiological mechanisms leading to organ damage. Moreover, they could serve as potential biomarkers for Fabry disease [36].

Despite the many different technologies that exist for proteomic analysis, they have not been widely applied in Fabry disease. Two studies looking at protein changes in peripheral blood mononuclear cells (PBMCs) used different methods: one looked directly at protein levels in these cells and the other looked at gene expression [37,38]. Although both studies used the same cell types, the results differed with regard to the specific proteins or genes detected; nonetheless, activation of inflammation or apoptosis was detected in both studies. The first study found higher levels of galectin-1 in PBMCs from patients with Fabry disease compared with healthy controls. This protein is expressed by many immune cell types such as neutrophils, mast cells, macrophages, T and B lymphocytes, and endothelial cells [37], and it participates in the inhibition of extravasation, mast cell degranulation, and arachidonic acid and prostaglandin E2 release by lipopolysaccharide-stimulated macrophages. Upregulation of galectin-1 could be part of the body's attempt to downregulate the inflammatory insult [37]. In a transcriptomic assay in the second study, *NAIP* (the gene for neuronal apoptosis inhibitory protein) was found to be upregulated in children with Fabry disease [38]. This gene is thought to play a role in modulating the assembly of the inflammasome, a multi-protein complex of innate immune receptors and sensors that activates inflammatory caspases in response to DAMPs or pathogen-associated molecular patterns (PAMPs) [39,40].

An interesting hypothesis is that molecular clues reflecting the kidney's lysosome-related inflammatory processes can be found in urine. A proteomic study looking for biomarkers analyzed urinary proteins from Italian patients with Fabry disease and determined that 55% of the proteins belonged to biological processes related to the immune response, inflammation, or both [41]. For example, serine-type endopeptidase inhibitor was found to be enriched in the patients analyzed. This protein is involved in the modulation of serine proteases, which are involved in blood clotting, the immune system, and inflammation [41]. Furthermore, the study revealed early urinary markers of Fabry disease-related nephropathy, showing an upregulation of some inflammatory proteins such as uromodulin and prostaglandins.

Studies have also been undertaken using tissues from Fabry knockout mice. Gene expression and protein levels of liver serum amyloid A1, S100 calcium-binding protein A8 and A9, and lipocalin 2 were significantly increased in untreated Fabry mice compared with wild-type mice [42]. Serum amyloid A is a superfamily of acute-phase proteins, the blood level of which increases in response to tissue injury and inflammation, and S100 calcium-binding proteins A8 and A9, and lipocalin 2, modulate inflammation.

5. Pro-inflammatory cytokines and apoptosis are upregulated in Fabry disease

Supported by many reports showing evidence for chronic immune system stimulation in other lysosomal storage disorders, research groups have recently focused their attention on the production of inflammatory mediators and on cellular apoptosis in Fabry disease. As part of this work, Dr. Rozenfeld's group studied apoptosis and pro-inflammatory cytokine profiles in PBMCs from patients with Fabry disease. In one study, higher levels of apoptosis were detected in cells from untreated patients compared with healthy controls, and reduced levels of apoptosis in treated patients compared with untreated patients [21]. Later, the group reported on the disease state in cultured PBMCs from patients with Fabry disease that induces constitutive secretion of pro-inflammatory cytokines tumor necrosis factor alpha (TNF-α) and interleukin (IL)-1β [22]. These cytokines secreted from Fabry PBMCs are a hallmark of autoinflammatory disorders [43].

Receptors expressed in cells of the innate immune system could be involved in the molecular mechanism by which abnormal substrate deposits in lysosomal storage disorders trigger the production of pro-inflammatory cytokines. The innate immune system recognizes danger signals through pattern recognition receptors expressed mainly on the surface of macrophages and dendritic cells; for example, toll-like receptor 4 (TLR4). TLR4 can recognize endogenous molecules exposed during cellular injury. In Fabry disease, the binding of glycolipids such as lyso-Gb3 to TLR4 may trigger Notch1 signaling, in turn activating the nuclear factor kappa B (NF-κB) pathway, resulting in the production of pro-inflammatory cytokines and giving rise to systemic and local inflammatory responses [44,45]. It has been revealed that Gb3 may also be recognized by TLR4 [22]. This receptor has been shown to participate in other lysosomal storage disorders, including mucopolysaccharidoses [46] and Niemann-Pick type C [47]. The finding that lyso-Gb3 and Gb3 are recognized by TLR4 adds to the emerging body of evidence indicating that TLRs could play a role in organ inflammation and damage (Fig. 1).

6. Inflammation plays a role in the pathogenesis of Fabry-related organ damage

Progression of this chronic disorder leads to irreversible tissue injury, resulting in fibrosis. Ultimately, these pathological changes lead to target organ failure, which may reduce life expectancy if it involves the

kidney, heart, or central nervous system (CNS) [48,49]. However, the pathogenic link between the metabolic abnormality and tissue injury is still unclear. The initial metabolic derangement may promote the production of secondary mediators of injury that lead to inflammation, parenchymal cell loss, and fibrosis.

6.1. Kidney

From a clinical point of view, Fabry disease-related nephropathy is characterized by mild proteinuria and a progressive reduction in glomerular filtration rate. As in other nephropathies, it is noteworthy that the degree of proteinuria at enzyme replacement therapy (ERT) initiation, and during treatment [50], affects the progression of renal disease [51–53]. Histologically, there is progressive glomerulosclerosis, tubular atrophy, and interstitial fibrosis associated with an interstitial inflammatory cellular infiltrate [54]. Deposition of Gb3 is mostly present in endothelial and epithelial glomerular cells. Among epithelial cells, Gb3 deposition frequently occurs in podocytes, which have shown partial resistance to ERT clearance [55]. This is most likely because podocytes have a very low turnover and are separated from blood lumina by the glomerular basement membrane. Conversely, endothelial and mesangial cells, having a higher turnover and greater exposure to blood circulation, are more accessible to Gb3 clearance by ERT [52]. The intracellular persistence of Gb3 may continuously stimulate pathogenetic processes, including inflammation, while the cellular clearance of Gb3 by ERT limits histological damage. Deposition of Gb3 in glomerular cells is followed by focal segmental glomerular sclerosis and an increase in the mesangial matrix, resulting in global glomerular sclerosis. Transforming growth factor β1 (TGF-β1), a regulatory cytokine with key functions under inflammatory conditions [56], is known to play a role in the development of nephropathy [57], and has been shown to be produced by podocytes [58]. Interestingly, it is highly expressed in kidneys from Fabry mice compared with normal controls; moreover, the exposure of bovine aortic endothelial cells to Gb3 induced TGF-β1 production [57].

Possible mediators of Fabry disease-related nephropathy were also revealed when the addition of lyso-Gb3 to in vitro cultured podocytes induced the production of TGF-β1 through Notch1 activation and the production of CD74 through macrophage migration inhibitory factor activation [58–60]. TGF-β1 promotes fibrosis in response to chronic inflammation by enhancing the synthesis of extracellular matrix in renal cells via epithelial-to-mesenchymal transition [59,61]. Indeed, blocking TGF-β1 reduced extracellular matrix protein expression, and Notch receptor inactivation prevented TGF-β1 from inducing epithelial-to-mesenchymal transition [58,59]. The activation of CD74 was followed by the release of inflammatory cytokines [60]. Further evidence to support the induction of epithelial-to-mesenchymal transition via the activation of TGF-β by lyso-Gb3 was obtained in cultured tubular cells, where blocking TGF-β also inhibited the expression of epithelial-to-mesenchymal transition markers [62].

The role of TLR4 in causing renal fibrosis has been confirmed in diabetic nephropathy, in a process by which high glucose levels stimulate TLR4, resulting in NF-κB activation and consequent fibrosis [63]. It may be possible that a similar situation occurs in Fabry disease, where continued exposure to increased levels of glycosphingolipids causes changes in inflammatory gene expression and protein production that have an impact upon the development of subsequent Fabry-related complications. This “glycolipid legacy” remains to be confirmed but, in Fabry renal disease, endogenous TLR4 ligands are indeed exposed, leading to the production of cytokines and chemokines by immune cells and intrinsic renal cells. This, in turn, is followed by leukocyte recruitment to the kidney, with consequent interstitial inflammation and interstitial fibrosis [64]. TLR4 is expressed in intrinsic and infiltrating cells in glomeruli and interstitial tissues [44]. The interaction between these cells and TLR4 determines the release of chemokines, promoting local recruitment of leukocytes and the amplification of glomerular injury [65].

A detailed description of the relationship between changes in the profile of cytokine synthesis and kidney fibrosis in Fabry nephropathy was recently reported [66]. This paper emphasizes the roles of TGF-β and the renin–angiotensin system in the progression of renal sclerosis. We believe that the perturbations in the immune response reported in Fabry disease trigger the inflammatory processes that later result in tissue fibrosis.

Proteinuria, a classic manifestation of Fabry renal disease, is itself able to stimulate interstitial inflammation and fibrosis. In the presence of proteinuria, tubular cells undergo a partial epithelial mesenchymal transdifferentiation, triggering cell-cycle arrest and promoting the release of fibrogenic cytokines [67,68].

6.2. Heart

Inflammation might play a critical role in the development of cardiac changes in Fabry disease. In end-stage cardiomyopathy in patients with Fabry disease, fibrosis in the left ventricle (but not in the right ventricle) is a common finding [69]. A study of patients with Fabry disease and cardiomyopathy revealed considerably hypertrophic and disorganized cardiomyocytes, apoptosis, expression of inducible nitric oxide synthase and nitrotyrosine, and glycosphingolipid accumulation in endomyocardial biopsies [70]. Concentric hypertrophy and extracellular matrix remodeling are associated with ischemia of the heart tissue [71,72]. Moreover, serum levels of IL-6, IL-1β, TNF-α, monocyte chemoattractant protein-1 (MCP-1), intercellular adhesion molecule-1, and soluble vascular adhesion molecule were significantly higher in patients with Fabry disease [73]. These findings indicate that pro-inflammatory cytokines might play a role in the progression of Fabry disease-related cardiomyopathy. The roles of pro-inflammatory cytokines in cardiomyopathy may differ between patients with and without Fabry disease.

Scattered apoptotic myocytes have been identified based on caspase-3-positive cytoplasmic staining in autopsy specimens from patients with Fabry disease. A mild T-lymphocyte interstitial infiltrate in the myocardium has also been demonstrated by CD3 staining [74]. Moreover, the observation that endomyocardial biopsy specimens from patients with Fabry disease were infiltrated by inflammatory macrophages suggests that these cells act as key players in myocardial injury [35].

6.3. Vascular system

Fabry disease involves smooth muscle too, although it is uncertain whether the initiating step in Fabry vasculopathy takes place in endothelial cells, with a subsequent pro-thrombotic state, or in smooth muscle cells in the arterial media layer [24,75]. It appears that lyso-Gb3 plays a major role in the pathogenesis of Fabry vasculopathy, and it has been proposed that smooth muscle cells, rather than endothelial cells, are the initial target for lyso-Gb3 accumulation [20,75,76]. Exposure of smooth muscle cells to lyso-Gb3 results in proliferation that might be associated with the hypertrophy of arterial walls [20]. Storage of lyso-Gb3 within the media layer of the arteries may also promote cell proliferation, with fibrotic remodeling of the arterial wall leading to arterial wall stiffness. The resulting shear stress may increase the expression of angiotensin 1 and 2 receptors in endothelial cells, in turn increasing reactive oxygen species, NF-κB, β-integrin, and cyclooxygenase 1 and 2 activity, and decreasing nitric oxide synthesis [75]. These factors may initiate an inflammatory cascade with pro-thrombotic and pro-inflammatory effects on leukocytes, endothelial cells, and vascular smooth muscle cells [75,77].

6.4. Central nervous system

Inflammatory processes occurring in the CNS in lysosomal storage disorders have already been reported in the literature [10]. Interest in

the CNS and lysosomal storage disorders is easy to understand when we consider that the CNS is the primary target for many lysosomal storage disorders, inasmuch as, for all its intrinsic peculiarities (highly differentiated cells with limited chances of replication), the CNS often dominates the clinical picture.

In many lysosomal storage disorders the inflammatory response in the CNS primarily involves microglial cells (resident dendritic cells) and astrocytes. Lysosomes in damaged cells release PAMPs or DAMPs that stimulate astrocytes and microglial cells, probably via TLR signaling (innate immunity), to release cytokines, causing inflammation and cellular death. The question of whether these processes are the drivers for or the consequence of tissue damage remains to be definitively clarified, and applies to all lysosomal storage disorders, including Fabry disease.

Stroke is the main symptom of CNS involvement in Fabry disease and is caused by inflammatory processes acting on vascular and cardiac pathways. The three components of the vascular pathway to stroke, which are all affected by inflammation, are endothelial cell dysfunction, impaired vessel wall structure and function, and altered blood components. The main component of the cardiac pathway is thromboembolic events, caused by arrhythmia, which is also influenced by inflammatory processes in the heart (Fig. 2).

Brain lesions are found upon magnetic resonance imaging in virtually all patients with Fabry disease who have had a stroke. Although the mechanism behind these lesions is unclear, an inflammatory component was indicated by the finding of an association between polymorphisms in genes coding for the pro-inflammatory markers IL-6, endothelial nitric oxide synthase, factor V, and protein Z and the likelihood of developing brain lesions related to small vessel disease [81].

6.5. Lungs

Fabry disease affects the lungs in all patients, characterized by moderate obstructive ventilatory disorder and associated with symptoms of dyspnea, dry cough, wheezing, and prolonged expiration. Lipid deposits are present in the vascular endothelium and bronchial smooth muscle. The sites of obstruction are the small airways. The subsequent inflammation fueled by glycolipid deposits might represent an important mechanism in the development of small airway disease [82].

7. The effect of ERT on the immune system and inflammatory processes in Fabry disease remains to be confirmed

As described earlier, Fabry disease leads to a pro-inflammatory profile in the cells of affected patients, and this immune dysregulation could be associated with the organ damage seen in patients with Fabry disease. ERT with an exogenous recombinant enzyme preparation, such as agalsidase alfa or agalsidase beta, is stabilizing because it halts the progressive accumulation of glycosphingolipids in organs and thus slows the deterioration of organ function. An important question is whether ERT also modulates the immune system to reduce the level of inflammation. Studies on pro-inflammatory cytokines have produced discordant findings in patients undergoing ERT, and direct comparisons between agalsidase alfa and beta are hindered by differences in the methodology used (Table 1).

Increased levels of pro-inflammatory cytokines and oxidative damage, along with altered antioxidant defenses, were found in patients with Fabry disease, some of whom had been treated with agalsidase alfa or agalsidase beta ERT [27–29]. However, these studies mixed agalsidase alfa and beta treatments, and also treated and untreated patients; thus, it is difficult to assess the immunomodulatory effects of ERT in these analyses. Furthermore, some of the changes in levels of pro-inflammatory cytokines described during ERT may actually be due to the effects of concomitant medications, such as non-steroidal anti-inflammatory drugs and statins.

Proteomic studies have assessed the immunomodulatory effects of ERT in animal models and human studies. One study in the Fabry mouse model showed that the expression of genes associated with inflammation and vascular and renal functions was normalized by agalsidase beta ERT [42]. In humans, one study on the urinary proteome of patients with Fabry disease showed that the production of some pro-inflammatory proteins, such as uromodulin and prostaglandins, was reduced in patients who had been treated with agalsidase beta [41]. In another study, abnormalities in urinary proteome markers from female patients with Fabry disease were corrected by ERT with agalsidase alfa (n = 11) and agalsidase beta (n = 1) [36].

One study designed to analyze the short-term effects of enzyme replacement infusions found that immune and inflammatory pathways were upregulated, on the basis of gene expression analysis, after agalsidase beta infusion. The authors observed more pronounced ERT-associated gene expression changes in male patients than female patients [83], which may have been related to the generally greater disease severity observed in male patients and the different organs involved, in turn related to lyonization and skewed X-chromosome inactivation in female patients [84].

No change in the expression of pro-inflammatory cytokines, such as IL-1β, IL-6, and TNF-α, was observed by one research group when comparing patients with Fabry disease who did not receive treatment with those who received agalsidase alfa ERT [22], whereas another group showed significant reductions in serum levels of these pro-inflammatory cytokines and markers of oxidative stress following agalsidase alfa ERT [62,73]. One limitation of the latter studies was that all the patients had the IVS4 + 919G > A cardiac variant mutation, the role of which in determining the Fabry phenotype has not yet been clearly defined [85]. However, they were comparatively large studies for this field, and the authors were able to correlate the inflammatory findings

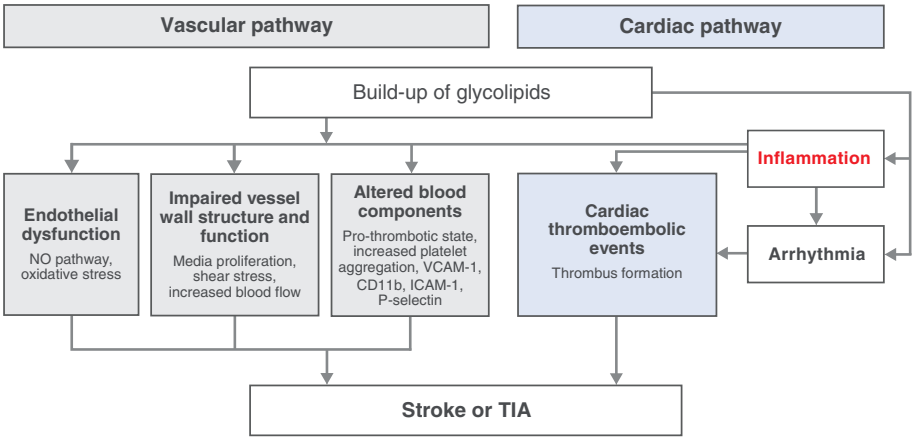

Fig. 2. Inflammation may contribute to stroke in Fabry disease. CD = cluster of differentiation; ICAM = intercellular adhesion molecule; NO = nitric oxide; TIA = transient ischemic attack; VCAM = vascular cell adhesion molecule. Figure adapted from DeGraba et al. [24], Schiffmann and Moore [78], Germain [79], Zarate and Hopkin [80].

Table 1
Studies that investigated the effect of ERT on inflammatory processes and pathological findings in Fabry disease.

Study	Subjects	Treatment	Tissue or fluid analyzed	Markers assessed	Outcome
Park et al. [42]	Male Fabry mice and wild-type mice	Agalsidase beta	Liver and kidney cells, plasma	Expression of multiple hepatic and renal genes	Expression of hepatic serum amyloid A1, S100 calcium-binding proteins A8 and A9, and lipocalin 2, which are all thought to modulate inflammation, was significantly upregulated in Fabry mice compared with wild-type mice, and was normalized by ERT
Rozenfeld et al. [26]	11 untreated and 11 ERT-treated males with FD; 22 healthy male controls	Agalsidase alfa	Peripheral blood	Leukocyte subpopulations	Compared with controls, patients with FD showed a significantly higher percentage of lymphocytes and CD19+ cells and reduced proportions of monocytes, CD8+, and myeloid dendritic cells. Expression of CD1d was significantly lower and expression of MHC II and CD31 higher
De Francesco et al. [21]	15 untreated and 17 ERT-treated patients with FD; 50 healthy controls	Agalsidase alfa	PBMCs	Apoptotic state	Apoptotic state was significantly reduced by ERT
Biancini et al. [27]	14 patients with FD receiving ERT; 14 healthy controls	Agalsidase alfa or agalsidase beta	Whole blood, plasma, urine	Glutathione, oxidative damage to lipids, TNF-α, IL-6	Glutathione levels were reduced and oxidative damage to lipids, TNF-α, and IL-6 were all increased in patients with FD vs controls
De Francesco et al. [22]	29 patients with FD, 22 receiving ERT; 15 healthy controls	Agalsidase alfa	PBMCs	Cytokine expression	No significant difference in levels of IL-1β, IL-6, IL-13, TNF-α, or IFN-γ between patients receiving ERT and those receiving no treatment
Biancini et al. [28]	10 patients with FD, 6 receiving ERT; 6 healthy controls	Agalsidase alfa or agalsidase beta	Whole blood, plasma	Oxidative DNA damage and repair	Compared with controls, patients with FD showed significantly greater levels of oxidative damage
Matafora et al. [41]	11 treatment-naïve and 12 ERT-treated patients with FD; 12 healthy controls	Agalsidase beta	Urine	Uromodulin, saposin, prostaglandin-H2 isomerase	All 3 markers, involved in inflammation and the immune response, were elevated vs normal in untreated patients with FD; levels were reduced by ERT
Biancini et al. [29]	12 patients with FD not receiving ERT, 11 patients with FD receiving ERT; 10 healthy controls	Agalsidase alfa or agalsidase beta	Whole blood, plasma, urine	Glutathione, lipid peroxidation, thiol content, nitric oxide	Glutathione levels were elevated in untreated patients vs controls. Lipid peroxidation was increased in untreated patients vs controls and decreased by ERT. No differences between groups for thiol levels. Nitric oxide levels were elevated in all patients with FD vs controls
Chen et al. [73]	25 patients with FD and cardiomyopathy receiving ERT; 25 patients without LVH not receiving ERT; 25 normal controls	Agalsidase alfa (confirmed by author)	Whole blood, peripheral blood, plasma	Multiple inflammatory markers, echocardiographic parameters	ERT reduced serum levels of IL-6, IL-2, IL-1β, TNF-α, MCP-1, ICAM-1, and sVCAM, and also reduced echocardiographic parameters: left ventricular mass, left ventricular mass index, and interventricular septal thickness at diastole
Ko et al. [83]	6 patients with FD who were receiving ERT	Agalsidase beta	Blood	Differentially expressed genes associated with inflammation	ERT was associated with upregulation of inflammation-related pathways, negative regulation of apoptosis, innate immune system, T cell receptor, P38MAPK, IL2RB, and T cell receptor signaling pathways; and with downregulation of the oxidative phosphorylation pathway
Chen et al. [62]	20 patients with FD and without LVH not receiving ERT; 22 patients with FC and receiving ERT; 20 normal controls	Agalsidase alfa	Serum	8-Hydroxy-2-deoxyguanosine	ERT reduced serum levels of oxidative stress marker 8-hydroxy-2-deoxyguanosine

CD = cluster of differentiation, ERT = enzyme replacement therapy; FC = Fabry cardiomyopathy; FD = Fabry disease; ICAM = intercellular adhesion molecule; IFN-γ = interferon gamma; IL = interleukin; IL2RB = interleukin 2 receptor subunit beta; LVH = left ventricular hypertrophy; MAPK = mitogen-activated protein kinase; MCP = monocyte chemoattractant protein; MHC = major histocompatibility complex; PBMC = peripheral blood mononuclear cell; sVCAM = soluble vascular cell adhesion molecule; TNF-α = tumor necrosis factor alpha.

with the clinical results. For example, in Chen et al. 2016 changes in left ventricular mass index correlated with changes in IL-6 and MCP-1, indicating a potential use for these cytokines as pro-inflammatory biomarkers in Fabry disease [73].

One study by Dr. Rozenfeld's group assessing the apoptotic state of PBMCs in Fabry disease found reduced levels of apoptosis in cells from patients who had received agalsidase alfa ERT compared with untreated patients [21]. Another study analyzed changes in leukocyte populations and reported higher percentages of lymphocytes and CD19+ cells, and reduced proportions of monocytes, CD8+ cells, and myeloid dendritic cells, in patients with Fabry disease compared with healthy controls. However, no significant differences in cell populations were reported between untreated patients and those who received agalsidase alfa ERT [26].

Thus, the effects of ERT on the immune system and inflammatory processes in Fabry disease need to be confirmed. Further studies are warranted to clarify whether ERT is able to modulate or reverse Fabry disease-related inflammatory responses. It is also possible that inflammatory processes are more active some days after infusion because of a reduction in enzyme activity [86], and a treatment with a longer effect might improve the outcome in patients with progressive Fabry disease despite ERT.

8. Conclusions

In recent years, it has become clear that inflammation and concomitant activation of the innate immune system are a general response in Fabry disease and are primarily caused by glycolipid accumulation (Gb3 and lyso-Gb3) and its recognition as a danger signal. Owing to continuous exposure to glycolipids, inflammation in Fabry disease is chronic, and it is possible that, after the initial inflammatory response to Gb3 deposition, tissue damage progresses independently. Chronic inflammation is a disease state and it is responsible for irreversible changes in tissues that ultimately lead to organ failure. The exact cellular and molecular mechanisms that link the intracellular accumulation of substrates like Gb3 to inflammatory processes and organ pathology are not completely understood. Mechanisms other than glycosphingolipid accumulation may also be involved. Thus, further studies investigating the pathophysiology of Fabry disease are clearly needed to help us understand the effects of specific treatments like ERT and to help developing even more targeted ones.

Competing interests

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Contribution statement

P.R. conceived the literature review. P.R. and S.F. performed the literature search. P.R. and S.F. participated in drafting the manuscript and revising it critically for important intellectual content. P.R. and S.F. read and approved the final draft.

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