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Opinion

Is the endotoxin–complement cascade the major driver in lipedema?

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Lipedema is a poorly understood disorder of adipose tissue characterized by abnormal but symmetrical deposition of subcutaneous white adipose tissue (WAT) in proximal extremities. Here, we propose that the underlying cause for lipedema could be triggered by a selective accumulation of bacterial lipopolysaccharides (LPS; also known as endotoxin) in gluteofemoral WAT. Together with a malfunctioning complement system, this induces low-grade inflammation in the depot and raises its uncontrollable expansion. Correspondingly, more attention should be paid in future research to the endotoxemia prevalent in patients with lipedema. We would like to propose that proper management of endotoxemia can reduce the progression and even improve the state of disease in patients with lipedema.

Highlights

Lipedema is an adipose tissue disease with a massive expansion of subcutaneous fat tissue. Its etiology is poorly understood.

We suggest that a major driving force of the disease is an enrichment of bacterial lipopolysaccharide (endotoxin) in lower-body fat depots (gluteofemoral white adipose tissue; gfWAT).

Endotoxin, combined with a malfunctioning complement system, can induce low-grade inflammation in gfWAT and prompt massive depot expansion.

The components of the terminal stage of the alternative complement pathway (ACP) are widely expressed in adipocytes and characterized by the production of the membrane attack complex that can lyse host cells as well, including adipocytes.

In lipedema patients, a suppression of the ACP is observed, ultimately leading to an expansion of that fat mass.

Lipedema – a disorder of adipose tissue with unknown pathophysiology

Lipedema is a disorder of adipose tissue (AT) characterized by abnormal but symmetrical deposition of subcutaneous white adipose tissue (sWAT) in proximal extremities. This disorder affects almost exclusively females and has a genetic component [1]. The prevalence of lipedema is uncertain and was estimated by different authors to vary between 7 and 18% among the female population, thus affecting a large number of people [2]. Lipedema does not necessarily correlate with BMI and is considered to be unrelated to cellulite [3].

Surprisingly, histological descriptions of WAT in lipedema do not report major abnormalities. Whereas some authors found a hypertrophic phenotype of WAT with a moderate secondary hyperplasia and reduced fibrotic content in the extracellular matrix (ECM) of patients with lipedema [4], others described a hypertrophic phenotype and increased fibrotic depositions [5]. Remarkably, no hypertrophy was apparent in patients with early stages of lipedema [6]. An additional observation was that a pronounced hypertrophic phenotype of adipocytes in lipedema was found only in nonobese individuals compared to their matched controls, whereas the patients with obesity and lipedema demonstrated no additional hypertrophy compared to obese controls [7]. The description of the skin morphology also seems contradictory. While some authors described a roughly 50% thickening of the epidermis in patients with lipedema [5], others were unable to find any differences between skin biopsies in controls versus affected patients [6]. These variable results could be caused by differential disease stages in the various cohorts investigated. Some other morphological modifications in lipedema appear less controversial, such as increased permeability and fragility of blood vessels, leading to accumulation of fluids and proteins in the affected WAT areas, as well as malfunctioning of the lymphatic system, leading to secondary lymphedema that is an established comorbidity for lipedema.

The pathophysiology of lipedema remains largely unknown. Genetic and metabolic analysis is normally provided on small cohorts and predominantly on the more advanced stages of the

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disease when samples are more readily available. This strongly restricts the possibility of identifying the initial pathophysiological steps of the condition. Gene expression analysis of ten patients with lipedema and matched controls revealed a significant upregulation of cyclin D1 (*CCND1*), as well as a downregulation of the CCAAT/enhancer-binding protein delta (*CEBPD*), complement factor D (*CFD*), nuclear receptor co-repressor 2 (*NCOR2*), and Krüppel-like factor 4 (*KLF4*) as the most prominent gene modifications in lipedema compared to controls [5]. Analysis of 13 patients with lipedema compared to matched controls demonstrated that gluteofemoral WAT (gfWAT) in lipedema can be characterized by a chronic low-grade inflammation with substantially increased levels of oxidative stress [2,7]. These and some other findings support the existence of a low-grade inflammation in gfWAT that may be a primary pathophysiological factor triggering the development of lipedema. However, the answer to the question as to what the main reasons for this inflammation are remains open. Here, we propose that the underlying cause can be a selective accumulation of bacterial lipopolysaccharides (LPS also known as endotoxin) in gfWAT that, together with a malfunctioning complement system, can induce low-grade inflammation in this adipose tissue depots and cause its uncontrollable expansion.

Some special physiological features of gfWAT connecting lipedema to pregnancy

Gluteofemoral WAT is the predominant adipose tissue depot in females that is mainly under development during childhood and puberty. During the first and second trimesters of pregnancy, females accumulate ~3.5 kg of fat in this body area, demonstrating a significant expansion of gfWAT; this adipose tissue is meant to be mobilized to cover the needs of the growing fetus in the third trimester [8]. Women undergoing lactation also preferentially utilize this normally highly protected fat from hips and thighs, demonstrating an average loss of ~0.8 kg gfWAT per month [8].

While there is clearly an important role of gfWAT in pregnancy and lactation, there are negative aspects associated with gfWAT as well. Low-grade inflammation in gfWAT and its hypertrophy, both in cellulite and lipedema, may be caused by the increased presence of LPS [9]. To connect the two phenomena, we should consider whether intestinal permeability is modified in pregnancy. Pregnant women have generally higher intestinal permeability compared to nonpregnant controls, as judged by a dual sugar lactulose/rhamnose ratio test [10]. Additionally, intestinal permeability can be assessed by plasma markers, such as zonulin, which increases in overweight and obese women from early to late pregnancy and positively correlates with LPS activity in circulation [11]. Of note, such an increase in zonulin in overweight and obese pregnant women demonstrated a broad distribution and showed a four-fold variation among individuals [12]. This suggests that just a subset of pregnant individuals really develop increased intestinal permeability known as 'leaky gut', leading to higher endotoxemia. Whereas the reasons for the development of a leaky gut during pregnancy are unknown, these changes may be caused by continuously increasing estrogen production, reaching its maximum value in the third trimester, associated with the estrogen-induced destruction of intestinal tight junctions [13].

In this context, we could argue that lipedema could be viewed from the perspective of the gfWAT fat pad as a 'pseudo-pregnant' physiological state.

Low-level inflammation as a hallmark for morphological modifications of adipose tissue

Adipose tissue can undergo different morphological modifications, such as generalized/partial expansion of WAT (as in obesity/lipedema), as well as its generalized or partial atrophy (as in different types of lipodystrophy). Obesity and lipedema on the one hand, and different types of lipodystrophy on the other hand, reflect opposite modifications of WAT volume, but both of

these states are characterized by a low-grade inflammatory component in the affected tissue areas. These states even share similar cytokine profiles, implicating in both extremes the involvement of the immune response.

Low-level inflammation in hypertrophic WAT was long considered to be mainly caused by activation of Toll-like receptor (TLR)4 through direct interaction with long-chain saturated fatty acids [14]. However, LPS is a well-known canonical TLR4 agonist as well. This means that WAT inflammation induced by LPS and saturated fatty acids are mechanistically connected to each other. The proinflammatory effects of palmitate are critically dependent on the TLR4-induced priming of macrophages [15]. This finding leads to the hypothesis that metabolic endotoxemia (increased concentration of circulating LPS and its differential accumulation in tissues), further exasperated by associated increased intestinal permeability, may be the main driver for the presence of low-grade inflammation in hypertrophic WAT [15]. This model postulates that beyond saturated fatty acids, additional factors are involved in pathological changes in WAT, and one of these critical factors is likely to be endotoxin -related.

Lipedema is connected with different comorbidities; some of them are morbid obesity (with estimated prevalence of 37% and even 87% in stage 3), hypothyroidism (31%), asthma (18%), and polycystic ovary syndrome (PCOS; 3–6%) [16,17]. Most of these comorbidities are connected with enhanced endotoxemia (Box 1).

Box 1. Role of LPS in comorbidities for lipedema

Morbid obesity

Zonulin and LPS are significantly increased in serum of individuals with morbid obesity [18,19]. The prevalence of morbid obesity in adult females is 4–5% and thus much lower than the prevalence of morbid obesity of up to 87% amongst individuals with late stages of lipedema. This large differential in the prevalence of morbid obesity in the absence versus the presence of lipedema suggests a tight link between the pathophysiological mechanisms leading to these conditions.

Hypothyroidism

Serum concentrations of zonulin and LPS are significantly increased in patients with Hashimoto's thyroiditis [20,21]. Hypothyroidism affects about 5% of the general population, whereas another 5% are assumed to be unrecognized. This is much less than the 31% prevalence observed in the lipedema subpopulation, indicating an enrichment in this group.

Allergies/asthma

Increased intestinal permeability is typical in different allergic diseases, including asthma [22]. Zonulin is more than threefold increased in patients with asthma and demonstrates a strong correlation with its severity [23]. Prevalence of asthma among adult German females ranges in different studies between 3.0% and 9.7%, which is lower than 18% observed in the lipedema subpopulation, again arguing for an enrichment.

PCOS

Women with PCOS demonstrate increased serum concentrations of zonulin and LPS compared to controls [24,25]. Such increases are typical only for obese individuals. Lean individuals do not display increased serum LPS, regardless of whether they have PCOS or not [26]. The prevalence of PCOS strongly varies in accordance with diagnostic criteria – for Caucasian women it is reported to be between 6% and 19.9% [27]. Based on incidence rate, the question whether PCOS is really a comorbidity driving the lipedema phenotype will need further investigation.

Lymphedema

Lymphedema is a recognized comorbidity for late stages of lipedema. There are various indications that lymphedema is connected to endotoxemia. Lymphatic endothelial cells express different TLRs including TLR4. These endothelial cells react to an LPS challenge with the induction of various pro-inflammatory cytokines, promoting inflammation in lymphatic vessels and increasing their permeability [28,29]. In addition, administration of LPS increases albumin extravasation and decreases interstitial fluid pressure [30,31]. Thus, the most important comorbidities for lipedema can be connected to endotoxemia with increased accumulation of LPS in serum as well as highly likely in gfWAT depot of affected individuals.

In gfWAT, palmitoleic acid may compensate for the proinflammatory effects of palmitic acid, and its enhanced production in the tissue points to the existence of a persistent low-grade inflammation in this depot, even under normal physiological conditions. Expression of the *CYP19A1* gene encoding aromatase that is responsible for the conversion of androgens to estrogens is increased in gfWAT in early stages of lipedema compared to controls; it is also locally higher in gfWAT than in abdominal WAT in individuals with lipedema [6]. Such an increased presence of aromatase in adipocytes and associated vascular cells was previously linked to inflamed adipose tissue [32].

Recently, we connected the low-grade inflammatory state in cellulite to a selective accumulation of LPS and the development of a local endotoxemia in gfWAT [9]. There is no direct correlation between lipedema and cellulite (probably because of the high overlapping prevalence of cellulite in female population that some studies put over 85% in those over the age of 20 years). However, low-grade inflammation in gfWAT is seen under both conditions and suggests that cellulite and lipedema may represent consecutive stages of one and the same processes: cellulite presents an early stage and has high prevalence, whereas lipedema with its lower prevalence represents the progressive stages of some chronic gfWAT modifications. Consistent with this suggested pathophysiology, gfWAT is especially sensitive to LPS (Box 2).

Role of dysregulation of complement system in morphological WAT modifications

Both expansion of WAT (as seen in obesity and lipedema) and its reduction (as seen in different types of lipodystrophies) reflect substantial but distinct dysregulations of the complement pathway. This is also related to the development of low-grade inflammation in WAT [43]. An important role in this process is played by the alternative complement pathway (ACP). LPS can activate both the TLR4 and the alternative complement pathway, and there is extensive crosstalk between the complement and TLR signaling pathways, mediating their mutual regulation [44].

WAT is a well-known safe haven and a long-term reservoir for the accumulation of pathogens [45,46]. This explains the established phenomenon that many important components of the complement cascade are produced in adipose tissue. Adipocytes synthesize different components of the alternative complement pathway (ACP), including complement factor C3, complement factor (CF)B and complement factor D (CFD). CFD (also referred to as adipsin) is a key and rate-limiting factor playing

Box 2. Tissue sensitivity to LPS

We appreciate that there are significant inter-individual differences in the response to LPS exposure. This phenomenon is well-established in patients with sepsis, mainly suffering from severe bacterial infections, where the disease course can range from a mild sepsis syndrome to septic shock. Individuals stratified with a high versus low response to LPS (as judged by the level of LPS-induced cytokine production) can be determined by different sets of upregulated genes before and after LPS exposure [33]. Two genes that are upregulated in high LPS-responders are directly connected to adipose tissue function: *PLIN2* encoding perilipin-2 (adipophilin) (4.26-fold change) and *OLR1* encoding lectin-like oxidized low density lipoprotein receptor 1 (3.71-fold change). Additionally, two genes related to adipose tissue – *CSF3* encoding granulocyte colony stimulating factor (4.15-fold change) and *CXCL3* encoding chemokine C-X-C motif ligand 3 (3.01-fold change), are upregulated in the high responder group upon LPS stimulation. Both *CSF3* and *CXCL3* are downstream targets of *OLR1* [34]. *PLIN2* is LPS sensitive and demonstrates a significant dose-dependent upregulation upon LPS exposure [35]. The upregulation of *PLIN2* in high LPS responders should be indicative of a shift from mature adipocytes to immature cells in affected adipose tissue or can be a reflection of ectopic fat accumulation. *OLR1* (also known as *LOX1*), encoding the receptor for oxLDL, is involved in the development of oxidative stress and inflammation [36]. Activation of *OLR1* enhances uptake of cholesterol and fatty acids in adipocytes [37]. Application of LPS significantly upregulates *OLR1*; this upregulation is time dependent [38] and strongly dose dependent, and a considerable *OLR1* stimulation can be observed even with a challenge as low as 1 ng/ml LPS [39]. As it was shown in primary macrophages, OLR1, similar to PLIN2, plays a critical role in NLRP3 inflammasome activation [40]. OLR1 is colocalized with MMP14 and produces a functional ORL1/LOX1/MMP14 axis that is essential for RhoA and Rac1 activation [41]. Rac1 directly interacts with CAV1 [42]. Additionally, LPS stimulation induces a shift of TLR4 into lipid rafts where it mechanistically interacts with CAV1. The deletion of CAV1 reduces inflammatory response mediated by the LPS/CD14/TLR4/NF-κB pathway [42]. All these established connections lead to a model that possibly involves the MMP14/CAV1 axis as a player in the pathophysiology of lipedema.

a special role in the activation of ACP: it cleaves CFB and thus catalyzes the formation of C3 convertase which is important for development of complement-dependent cytotoxicity through production of the membrane attack complex (MAC or C5b-9).

Adiponectin is an adipose-specific peptide secreted by adipocytes that has a positive effect on insulin sensitivity and demonstrates an anti-inflammatory effect, potently suppressing the LPS-induced NF-kB activation and IL-6 expression in WAT [47]. Adiponectin demonstrates a high structural similarity with some factors from the complement protein family. While this does not reflect the main physiological function, it can activate the classical complement pathway, leading to the activation of component C3 and the formation of the MAC [48]. This potential ability of adiponectin to induce the production of MACs is inhibited by its binding to complement factor H [49]. Whereas adiponectin demonstrates an anti-inflammatory effect against LPS applied in high doses, chronic application of LPS in low doses induces a significant reduction of adiponectin expression selectively in sWAT, but not in other fat depots [50]. Remarkably, adiponectin release by subcutaneous adipocytes was previously found to be almost the same in lean and obese individuals [51] and thus should theoretically be unchanged in lipedema. However, the adipose-derived stem cells and mature adipocytes isolated from lipoaspirates obtained from patients with lipedema and cultured *in vitro* indeed demonstrated a reduced expression of adiponectin and leptin compared to their counterparts obtained from non-lipedema patients [52]. This supports the presence of LPS in gfWAT and its involvement in a dysregulation of the complement system in the disease.

While a reduced expression of CFD was reported in serum and in adipose tissue in different rodent models of obesity [53], the opposite behavior was found in obese humans [54]. Also, increased CFD mRNA in sWAT was reported in individuals with body mass index (BMI) >30 compared to controls (BMI <30) [55]. Analysis of monozygotic twin pairs with acquired obesity but a different BMI revealed an upregulation of early and a downregulation of terminal pathway complement genes in sWAT and adipocytes. In contrast, downregulation of the terminal pathway was connected to the activation of clusterin, inhibiting formation of the MAC [56]. Moreover, adipocyte volumes demonstrate positive/negative correlation with early/terminal complement pathways and it was proposed that the complement pathway is involved in the clearance of apoptotic debris in WAT [56]. In agreement with these findings, plasma CFD was reported to be significantly reduced in various forms of lipodystrophy compared to control patients. In fact, CFD levels are the lowest in congenital generalized lipodystrophy characterized by an almost total loss of adipose tissue in the whole body [57]. Two exceptions from this rule are presented by lipedema and Barraquer–Simons syndrome (Box 3).

The ACP is mainly directed against pathogens or their products, such as LPS; however, this pathway can also induce an autoimmune reaction in WAT [59]. The key component of the alternative complement pathway, the C5b-9 protein complex (MAC), normally induces the assembly of transmembrane channels, causing cell lysis. However, if not properly regulated, such lysis can affect both the pathogens and normal tissue cells, as seen in Barraquer–Simons syndrome [58]. Importantly, eukaryotic cells have different lines of defense against autolysis induced by

Box 3. Expression of complement factor D in lipedema and Barraquer–Simons syndrome

The generalized obesity and different forms of lipodystrophy are normally connected with enhanced/reduced expression of CFD in adipocytes. However, there are at least two exceptions this rule. Contrary to other forms of lipodystrophy, CFD levels in serum were found to be significantly increased in Barraquer–Simons syndrome, which is a type of acquired partial lipodystrophy with a cephalocaudal loss of subcutaneous adipose tissue [58]. Also surprisingly, transcriptional analysis revealed a 1.88-fold downregulation of the gene for *CFD* in lipedema compared to controls [5], which clearly points to a suppression of the complement pathway in this disease.

activation of ACP. They include the expression of inhibitors that block early stages of pore assembly in the plasma membrane (the main components are CD46, CD55, and CD59) as well as the removal of MAC complexes from membranes through shedding or endocytosis [60]. Endocytosis of MAC is regulated through the expression of the signaling protein caveolin (CAV)1 and microtubule-associated protein dynamin-2. Suppression of CAV1 inhibits MAC endocytosis and makes the cells much more sensitive to complement-dependent cytotoxicity [61]. This mandates elevated expression levels of CAV1 in inflamed adipose tissue that can potentially develop a complement-dependent cytotoxic reaction to exert cytoprotective functions.

Whereas a strong activation of ACP induces cell death, its sublytic activation induces adipogenesis [59]. These processes are connected to each other, assuming that ACP can play a part not only in the pathological but also in the physiological death of adipocytes, thereby regulating physiological turnover of these cells and maintaining the cellular balance in WAT. Internalization of sublytic amounts of MAC provides activation of the NLR family pyrin domain containing 3 (NLRP3) inflammasomes, thus inducing the production of cytokines and the development of inflammation [62]. Activation of these inflammasomes was shown to dysregulate the inflammatory responses under different metabolic conditions, such as obesity, type 2 diabetes, and atherosclerosis. Highly hypertrophic adipocytes *in vivo* degenerate and die before the formation of the typical crown-like structures containing macrophages, and NLRP3-dependent caspase-1 activation in such adipocytes induces a pro-inflammatory programmed cell death by pyroptosis [63].

As we know from macrophages, the activation of the NLRP3 inflammasomes requires two sequential steps: a priming signal (normally LPS) triggering the NF-κB signaling pathway and a secondary signal (such as microbial toxin or a saturated fatty acid such as palmitic acid) [64]. Moreover, peroxisome proliferator-activated receptor (PPAR)γ – the master regulator of adipogenesis – can directly interact with NLRP3 attenuating the activation of inflammasomes by blocking caspase-1 and interleukin (IL)-1β maturation and thus inducing anti-inflammatory effects. It can be inferred that endocytosis of a small number of MACs in adipocytes controlled by expression levels and phosphorylation of CAV1 in their cell membranes will induce similar effects. Conversely, low levels of ACP activation are able to initiate the PPARγ pathway, which eventually can stimulate adipogenesis. In contrast, strongly activated ACP connected with the overexpression of CFD in gfWAT can cause pyroptosis of adipocytes; a condition that would not be desirable over the course of pregnancy. The observed reduction of CFD expression in lipedema [5] suggests that gfWAT manifests a reduced ability of this WAT to activate the alternative complement pathway as well, thereby preventing the overproduction of MAC and reducing the probability of adipocyte death. The role of complement-induced adipocyte autolysis mediated through induction of MAC under normal physiological conditions, obesity and under extreme conditions, such as lipodystrophies and lipedema, is a phenomenon that needs to be carefully studied in future research.

Some specific transcriptional modifications in lipedema and their relation to the alternative complement pathway

Beyond the reduction of *CFD*, transcriptomic analysis revealed a 2.16-fold upregulation of *CCND1* as well as 2.7-fold, 1.81-fold, and 3.57-fold downregulation of *CEBPD*, *NCOR2*, and *KFL4* in lipedema compared to controls [5]. Can these transcriptional changes be driven by a dysregulated alternative complement pathway in gfWAT?

Increased expression of *CCND1* was reported in patients with lipedema, mostly in stage 3, which means that these individuals already had a significant expansion of WAT [5]. Such expression is characteristic for cells in the G1 phase of their cycle. An increase in *CCND1* in early stages of

adipogenesis is essential for the clonal expansion phase of human mesenchymal stem cells before these cells differentiate into preadipocytes. In contrast, *CCND1* expression is decreased in progressive adipogenesis to reduce the proliferation and increase PPARγ synthesis [65]. Adipose-derived stem cells in females generally have a substantially higher expression of the proliferation marker Ki67 than in males [66]. Indeed, the number of $Ki67^+$ cells in lipedema was reported to be higher than in control tissues [4]. Recently, a remarkable sexual dimorphism has been reported for adipose stem cells with respect to LPS responses in female mice, displaying 2–3 times higher gene expression for IL-1β, IL-6, and IL-8, up to four times more CCL2 transcripts, and at least an order of magnitude more granulocyte–macrophage colony-stimulating factor in females relative to males of similar age and BMI [66]. Importantly, cyclin (CCN)D1 is a downstream target for the NLRP3 inflammasome as NLRP3 can promote CCND1 transcription [67]. Consequently, such enhanced transcription of *CCND1* can be induced by the activation of sublytic levels of ACP in adipocytes, highly likely initiated by LPS.

CCAAT/enhancer-binding protein δ (CEBPD) encoded by the *CEBPD* gene is a transcriptional factor operating as a suppressor of proliferation and a crucial mediator of proapoptotic gene expression; its expression is inversely related to the expression of *CCND1* that induces proliferation and suppresses apoptosis [68]. Additionally, *CEBPD* amplifies the LPS-mediated enhanced NF-κB transcriptional activity and discriminates between transient and persistent TLR4 signals [69]. Overexpression of *CEBPD* sufficiently potentiates cytokine production induced by LPS in a dose- and cell-type-dependent manner [70]. CEBPD can facilitate pyroptosis by activating the NLRP3/caspase-1 signal axis [71]. The suppression of *CEBPD* in lipedema may be a mechanism to reduce the chronically amplified inflammatory reaction to LPS and NLRP3 inflammasome-induced pyroptosis in gfWAT. Overexpression of *CCND1* combined with an overall lowered *CEBPD* in lipedema point to a proliferative and anti-apoptotic/anti-pyroptotic state of the affected gfWAT in lipedema.

Nuclear receptor corepressor (NCOR)2 inhibits adipogenic differentiation through repression of PPARγ target genes [72], thus reducing the ability of adipocytes to counteract the pro-inflammatory and pro-pyroptotic effect induced by internalized NLRP3. NCOR2 is involved in the development of hypertrophic phenotypes in adipose tissue [73], and LPS significantly suppresses the expression of NCOR2 [74]. The lipedema-associated downregulation of NCOR2 is, therefore, consistent with the hypertrophic and anti-pyroptotic phenotype observed.

Krüppel-like factor (KLF)4 is known as a master transcription factor regulating anti-inflammatory responses that is involved in transition of macrophages from M1 to M2 phenotype. KLF4 binds to the NLRP3 promoter and promotes pyroptosis, whereas KLF4 inhibition reduces the level of NLRP3 and promotes cleavage of caspase-1, IL-1β, and IL-18 [75]. Palmitic acid (which is abundantly present in gfWAT) promotes the expression of DNA methyltransferases and inhibits KLF4 in 3T3-L1 adipocytes [76]. Thus, the reduced expression of KLF4 in lipedema should be considered as a defense mechanism against NLRP3-induced pyroptosis in adipocytes.

The observed changes in gene expression in lipedema gfWAT are consistent with LPS-induced activation of ACP leading to a low-level formation of MAC on the membranes of adipocytes followed by their internalization accompanied by production of NLRP3 inflammasomes in the affected gfWAT.

The MMP14/CAV1 axis in lipedema and its LPS dependence

In mouse models of obesity, expression of matrix metalloproteinase (MMP)14 is strongly increased in hypertrophic WAT [77]. MMP14 is the main pericellular collagenase in adipose tissue

and the only MMP that directly promotes cellular invasion in collagen-rich matrices [9]. MMP14 is co-localized within caveolae and physically interacts with CAV1. Intact macrophages express low levels of MMP14, and upon LPS stimulation, they display a dramatic (up to ten times) increase in MMP14 expression, both at the mRNA and protein levels, as well as the translocation of MMP14 to the plasma membrane [78]. In contrast, MMP14 levels in serum are significantly reduced in severe endotoxemia, especially in patients with sepsis [79].

Less attention has been given to MMP14 as a regulator of the ACP. However, C3b (an essential component of the ACP) is a substrate for MMP14 that can effectively shed this opsonin from the cell surface, thus suppressing the development of the terminal stages of the complement pathway [80]. In addition to the protective mechanisms connected with the expression of inhibitors (such as CD46, CD55, and CD59), which block early pore assembly in plasma membranes and CAV1-dependent endocytosis, shedding of complement factors provides the third defense line of eukaryotic cells against MAC production on their plasma membranes. High expression of MMP14 in hypertrophic WAT reduces the probability of MAC formation on plasma membranes of adipocytes. The presence of LPS in WAT activates the non-canonical caspase-4/5 inflammasomes that can, in turn, activate the canonical NLRP3 inflammasomes, and this process is connected with the secretion of two MMPs – ADAM10 and MMP14 [81]. Low-level stimulation of ACP with high expression of MMP14 in WAT will reduce the internalization of MAC, preventing the intracellular production of NLRP3 inflammasomes as well as PPARγ expression that should neutralize these inflammasomes. However, when MMP14 is reduced and is unable to shed the MAC from adipocyte membranes, the morphological changes in WAT will be different. In such case, low-level stimulation of ACP will cause intracellular production of NLRP3 and activation of PPARγ, consequently inducing adipogenesis and lipid storage in affected adipose tissue leading to its expansion.

CAV1 is also significantly upregulated in obesity, and its expression correlates with different inflammatory markers [82]. Expression of CAV1 and/or its phosphorylation are substantially upregulated in the presence of LPS in different tissues [83,84]. The overexpression of CAV1 in LPS-challenged adipocytes should cause increased adipogenesis with the development of predominantly mature adipocytes in WAT. Indeed, CAV1 expression in adipocytes is strongly dependent on the differentiation state of these cells, being much higher in mature adipocytes than in preadipocytes [85]. In the preceding text, we have described the important role of CAV1-dependent endocytosis in the internalization of MAC complexes from the surface of adipocytes and the corresponding development of the NLRP3–PPARγ axis that is able to stimulate adipogenesis.

Therefore, a low-level LPS challenge of adipose tissue should both stimulate the expression of MMP14 and CAV1. An increase of MMP14 and CAV1 expression should also occur in progressive stages of lipedema, characterized by progressive hypertrophy of adipocytes. However, under normal physiological conditions, MMP14 and CAV1 are negatively coupled to each other: MMP14 induces CAV1, and CAV1, in turn, can negatively regulate MMP14 activity through its internalization from the cell surface [86]. One can speculate that these negative correlations may be at least partly caused by competition between MMP14 and CAV1 for the MAC complexes on the surface of adipocytes. Also, a possible feedback mechanism for the MMP14–CAV1 axis could involve the extracellular signal-regulated kinases (ERK): whereas ERK1/2 is needed to support the CAV1 expression in hypertrophic adipocytes, its own expression can be stimulated by MMP14 that induces downstream phosphorylation of ERK1/2 through the MAPK pathway [86]. To activate this pathway, some optimal level of active MMP14, but not the total level of MMP14 in the tissue, is needed to induce the accumulation of phospho-ERK1/2 [87]. These results collectively suggest that the MMP14–CAV1 axis can be implicated in the activation of ACP, inflammation and adipogenesis.

Recently, we proposed that lipedema is an estrogen-dependent disorder of adipose tissue, which is triggered by the uncoupling of a feedback mechanism involving CAV1 and MMP14, as well as between CAV1 and estrogen receptor α, which is also colocalized with CAV1 in different types of cells [3]. The MMP14–CAV1 axis should therefore be carefully analyzed in progressive stages of lipedema compared to healthy control adipose tissue in future research.

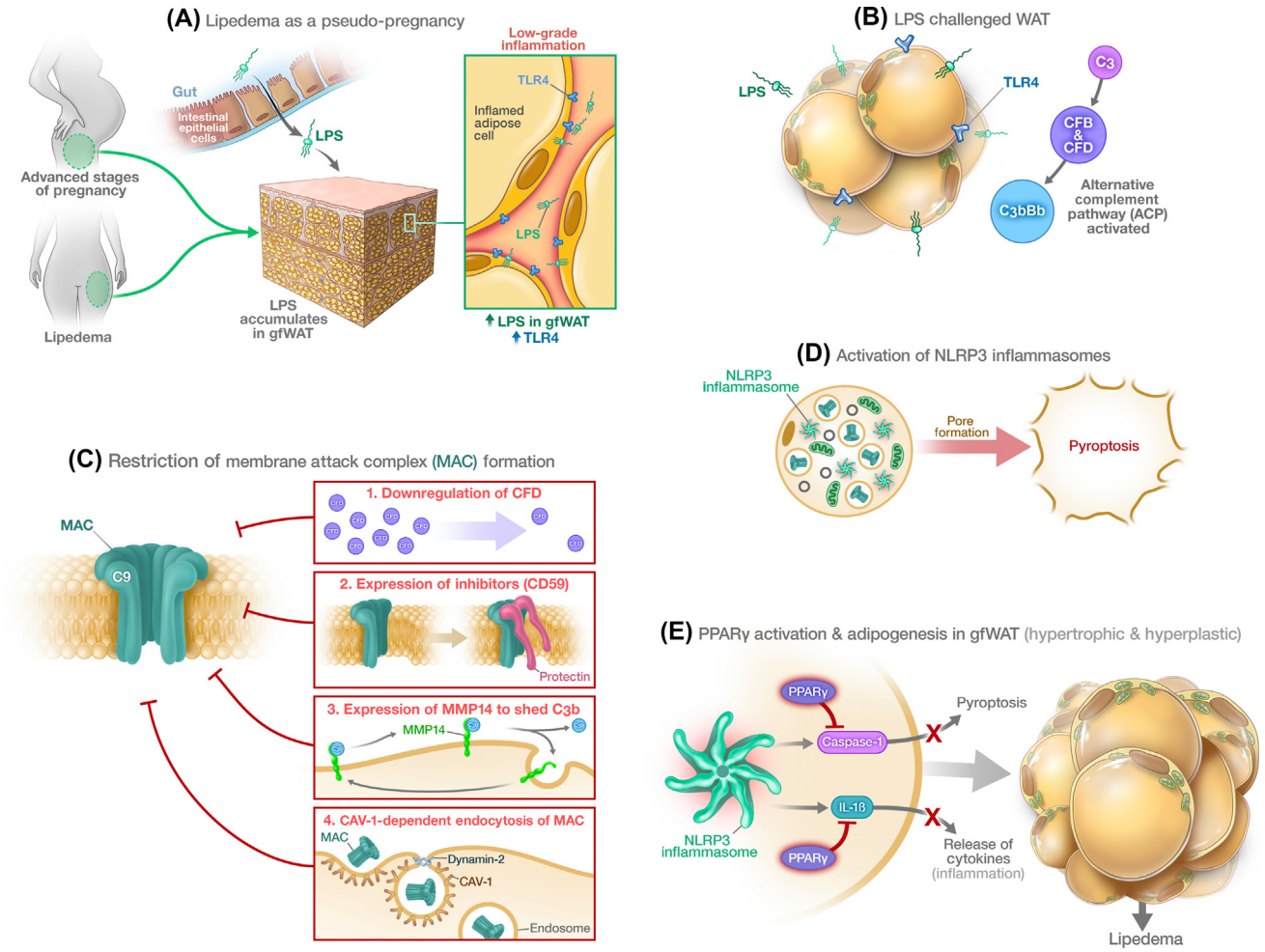

Figure 1. Lipedema as a phenotypic manifestation of a 'pseudopregnancy'. (A) Gluteofemoral white adipose tissue (gfWAT) plays an important role during pregnancy and lactation and is characterized by an increased sensitivity to lipopolysaccharides (LPS). The appearance of LPS in the circulation is typical over the course of pregnancy and elicits a strong response in gfWAT. Even in a non-pregnant individual, from a gfWAT perspective, increased LPS levels could be viewed as a state of 'pseudopregnancy'. Low-level accumulation of LPS in gfWAT produces a corresponding low-level inflammation in this fat depot. (B) Accumulation of LPS in gfWAT stimulates the innate immune response through an activation of the alternative complement pathway (ACP). The terminal stage of ACP is characterized by production of the membrane attack complex (MAC) that is able to induce lysis not only of pathogens, but also of host cells themselves, including adipocytes. (C) Production of MAC has to be avoided over the course of pregnancy as it would trigger a proinflammatory programmed death of adipocytes through the mechanism of pyroptosis. To prevent the reduction of gfWAT also in the 'pseudopregnant' state, the terminal stages of ACP must be suppressed. This suppression is achieved via reduction of the rate-limiting complement factor D in gfWAT of patients with lipedema, which restricts the activation of ACP to a sublytic level. To further reduce the probability that even a single MAC complexe is able to induce cell lysis in adipocytes, the precursors of MAC are shed from the cell surface by matrix metalloproteinase 14 (MMP14), or the produced MACs removed by endocytosis via caveolae. (D) Dysregulation of the MMP14–caveolin 1 (CAV1) axis through reduced expression of MMP14 will shift the protection of adipocytes towards CAV1-regulated endocytosis of MAC complexes. Such endocytosis of MACs in sublytic doses leads to an induction of the NLR family pyrin domain containing 3 (NLRP3) inflammasomes in adipocytes. (E) Production of NLRP3 inflammasomes in adipocytes is counteracted by the induction of the peroxisome proliferator-activated receptor γ (PPARγ) activity in these cells, which in turn can induce adipogenesis in the affected areas of gfWAT manifested as lipedema. Figure adapted, with permission, from [59].

Concluding remarks and future perspectives

The observations discussed in this Opinion Article suggest the following model in which we refer to lipedema as a phenotypic manifestation of a 'pseudopregnancy' (Figure 1). gfWAT exerts an important role during pregnancy, both as a source of energy and as a protective tissue, and is characterized by an increased sensitivity to LPS. The presence of LPS in circulation and its accumulation in WAT is primarily caused by an enhanced intestinal permeability known as the 'leaky gut syndrome'. The development of enhanced intestinal permeability is typical over the course of pregnancy and finds a strong response in gfWAT. Even in a non-pregnant individual, from a gfWAT perspective, increased endotoxin levels could be viewed as a 'pseudopregnancy'. Low level accumulation of LPS in gfWAT produces a corresponding low level of inflammation in this fat depot. This stimulates the innate immune response through activation of the ACP. The terminal ACP is characterized by the production of the MAC, and is able to induce lysis of pathogens and host cells, including adipocytes. Production of MAC has to be avoided over the course of pregnancy, as it would trigger a pro-inflammatory programmed death of adipocytes through the mechanism of pyroptosis. To prevent the reduction of gfWAT in this 'pseudopregnant' state, the terminal stages of ACP are suppressed. This suppression is achieved via reduction of the rate-limiting CFD in gfWAT of patients with lipedema, which restricts the activation of ACP to a sublytic level, characterized by a low-level production of the MAC in the plasma membranes of adipocytes. To further reduce the probability that MAC complexes induce cell lysis in adipocytes, the precursors of the MAC are shed from the cell surface by MMP14 or removed by internalization via caveolae. This requires sufficient levels of CAV1 and dynamin-2 in the plasma membranes of adipocytes and invokes the MMP14–CAV1 axis, which, under physiological conditions, is characterized by a negative correlation between MMP14 and CAV1, at least partly achieved with a competition between MMP14 and CAV1 for direct interaction with the MAC complexes. However, the MMP14–CAV1 axis can be dysregulated, for example, through reduced MMP14 expression in lipedema tissue. Under these circumstances, the protection of adipocytes from MAC-induced cell lysis will be shifted towards CAV1-regulated endocytosis. Such endocytosis of MACs in sublytic doses leads to an induction of the NLRP3 inflammasomes in affected adipocytes that is counteracted by the induction of the PPARγ activity, which in turn induces adipogenesis in the affected areas of gfWAT.

What makes this model particularly attractive is that most physiological and pathophysiological states of adipose tissue (including opposing phenomena such as obesity and lipodystrophy) lend themselves to modeling with this proposed mechanism. A connection between the sublytic activation of ACP and adipogenesis invokes the proposition that ACP can play a part in the physiological death of adipocytes by regulating the turnover of these cells and thus maintaining cellular homeostasis in WAT. Many aspects of this model lend themselves to *in vivo* testing in preclinical models.

We believe that this model (upon further refinement with studies in preclinical models) may substantially influence the treatment strategy in patients with lipedema. More attention should be paid in patients with lipedema to the management of endotoxemia, which can reduce the progression and even improve the state of disease in these patients (see Outstanding questions).

Outstanding questions

Components of the alternative complement cascade are expressed at high levels in adipocytes. Does this complex lead to lysed adipocytes under other (patho) physiological conditions?

What are the key regulators of CAV1 in adipocytes?

Would inflammasome inhibitors be beneficial for lipedema patients?

Can the activity of ACP in gfWAT be reduced through stimulation of the inhibitors such as CD46, CD55, or CD59 that block early stages of pore assembly in the plasma membrane?

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