Chapter 9

Immunometabolism in Obesity

Efrain Chavarria-Avila,
Rosa-Elena Navarro-Hernández,
Milton-Omar Guzmán-Ornelas,
Fernanda-Isadora Corona-Meraz,
Sandra-Luz Ruíz-Quezada and
Mónica Vázquez-Del Mercado

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Abstract

Immunometabolism is a current issue that has shown relevance in recent years, because the way we understand the adipose tissue has shifted from simply being a site of energy storage to a very active endocrine organ, which dysregulation has a major impact on other systems, especially on the immune one. Understanding the molecular basis of the regulation of adipose tissue is essential to look for alternatives in the treatment and prognosis of obesity in future generations. In this regard, it is described that the immune system has great importance in physiological processes of adipose tissue and vice versa. The main objective of this chapter is to describe the relationship between the immune system and metabolism, emphasizing dysregulation when obesity is present. Upon completion of this chapter, the reader will be able to understand the relationship between the immune system and metabolism, in normal and obesity states; also, will identify the chronic state of low-grade inflammation as the main etiological factor of obesity co-morbidities, such as insulin resistance, diabetes mellitus, osteoarthritis and susceptibility to some kinds of cancer, among others.

Keywords: immunometabolism, cytokines, adipokines, chemokines, low-grade inflammation, miRNAs

1. Introduction

Inmmunometabolism has been defined as the interphase between metabolism and immune response [1, 2], in which, adipose tissue plays a key role. Leptin was the first molecule described linking the immune system with metabolism. The first leptin function described was appetite control; however, nowadays it has been described with multiple permissive functions, like immune homeostasis, among others (**Figure 1**) [3]. On innate and adaptive immune response cells, leptin can mainly increase cytokine expression, cell surface adhesion molecules and chemokine receptors [3, 4]. Additional to leptin, free fatty acid receptors family (FFARs) has been reported to be expressed on key cell types regulating both: energy homoeostasis and inflammatory responses [2]. Obesity induces changes in gut microbiota, it has been reported that *Bacteroidetes* and *Firmicutes* phyla produce high levels of the short chain free fatty acids (SCFAs) C2–C4 which are the main agonists for FFAR2 [5]; at the same time, microbiota can influence innate and adaptive immune responses [6, 7]. These examples make clear the inter-relation between metabolism and the immune system. We will focus along this chapter in alterations of this interphase due to the presence of obesity.

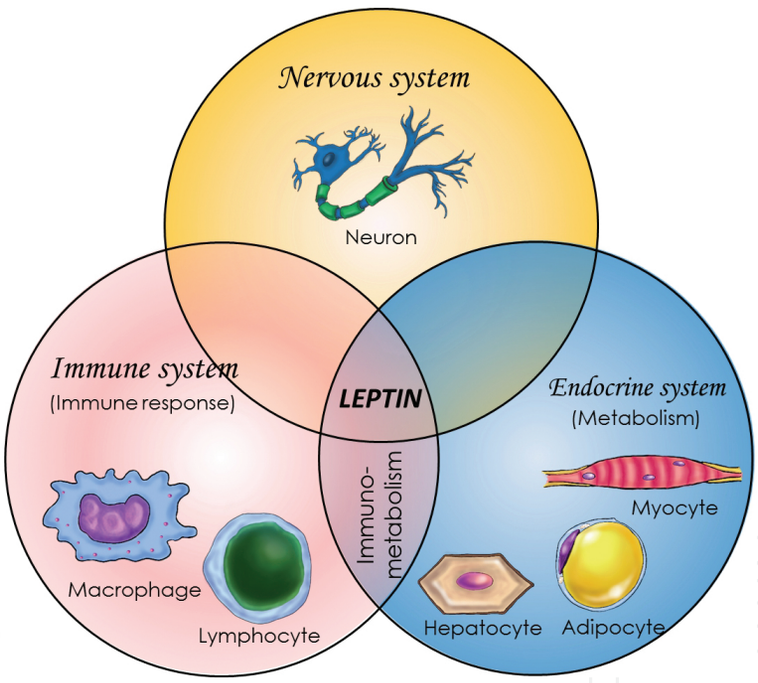

Figure 1. Neuroendocrine immune system.

2. Immunometabolism characteristics on normal weight range and obese individuals

Immune cell status in normal weight range (lean) individuals is mostly anti-inflammatory; this environment needs a continual production of type 2 cytokines (i.e. interleukin (IL)-5 and IL-13).

On the other hand, the presence of obesity is associated with a low-grade inflammation state characterized by increased pro-inflammatory cytokine production (i.e. tumour necrosis factor (TNF)-α, IL-1β and IL-6) (**Table 1**) [10, 24].

Component	Changes respect to normal weight range
Cells	
White adipocytes [8]	↑↑↑
Brown adipocytes [8]	↓
M1 [9]	↑↑
M2 [9]	↓↓
Mast cells [10]	↑
Eosinophils [10]	↓
Non-cytotoxic ILCs (NK) [11]	↑↑
ILC1s [11]	↑
ILC2s [12]	↓
Molecules	
Th-1 cytokines [13]	↑
Th-2 cytokines [13]	↑
Leptin [14, 15]	↑↑
Adiponectin [16]	↓↓
Resistin [17]	↑
Chemerin [18]	↑
CCL2 [19]	↑
FFA [20]	↑
Glucose [21]	↑↑
Insulin [20]	↑/↓↓↓
Cholesterol [21]	↑
Microbiote	
Bacteroidetes sp. [22]	↓
Actinobacteria sp. [22]	↑
Faecalibacterium prausnitzii [23]	↓

M1: macrophages associated with Th-1 cytokines; M2: macrophages associated with Th-2 cytokines; ILC1s: innate lymphoid cells type 1; CCL2: C-C motif ligand 2 and FFA: free fatty acids.

*During obesity onset, an insulin increase occurs (insulin resistance), after a time it frequently progress to exhaustion of β-cells.

Table 1. Changes on immunometabolic components due to obesity.

2.1. Adipose tissue-resident cells

Adipose tissue is a specialized connective one, white adipose tissue (WAT) is the most abundant in adult human (~85%) by its distribution, it can be classified in subcutaneous or visceral (omental, mesenteric and retroperitoneal accumulation). Besides WAT, there exist two more types: brown adipose tissue (BAT) and bone marrow adipose tissue (BMAT), each one

have singular cellular composition, anatomical location and pathophysiological properties [25].

In lean individuals, macrophages count for around 5% of WAT's cells depots, in obesity conditions, macrophages increase as much as 50% [1]; nevertheless, besides quantitative changes, there also occurs qualitative ones. The main function of macrophages (or initially described) have been phagocytosis, however, nowadays, they are recognized as a heterogeneous population with multiple functions [1, 2].

2.1.1. Adipocytes

Three types of adipocytes have been described: white, beige (brite, brownish) and brown adipocytes; these are different in structure and metabolism [26]. The terms white and brown came from the appearance of tissues when stained with immunohistochemistry against UCP-1 [27, 28].

White adipocytes are specialized cells, arising from a $Myf5^-$ preadipocyte lineage, which have a unilocular large lipid droplet and comprise predominantly the WAT [8, 26]. Many functions are attributed for these cells such as insulation and physical protection of the viscera, thermal insulation, reservoir of stored energy in form of triglycerides and regulation of fat release and storage. Beyond these functions, white adipocytes produce and secrete several molecules (adipokines), including leptin, resistin, retinol binding protein 4 (RBP4), fibroblast grown factor 21 (FGF21) and adiponectin mainly. Endocrine communication of adipose tissue is bidirectional, white adipocytes also respond to hormonal signals to induce lipolysis and release free fatty acids (FFAs) into the circulation, for oxidation or storage by other tissues [8, 26].

Brown adipocytes are multilocular with small lipid droplets and express uncoupling protein-1 (UCP-1) [26], emerge from $Myf5^+$ precursor lineage and are developmentally more related to skeletal muscle cells than to white adipocytes. The amount and location of brown adipocytes changes throughout life, in the early years, the number of reservoirs is higher and is mainly located in breastbone, interscapular space and retroperitoneal level; whereas in adults, it decreases and can only find deposits in carotid bodies, aortic bodies and adrenal gland. The main function of these adipocytes is thermogenesis, keeping temperature homeostasis in cold. This is because brown adipocytes possess a large number of mitochondria, and these in turn, express UCP-1 in the inner membrane. This protein decouples the electron transport chain, making it more inefficient, thus the number of ATPs produced decreases and energy is dissipated as heat [29, 30].

The name brite is the result of combining brown (br) and white (ite), since this kind of adipocytes was described to be morphologically similar to white adipocytes but at the same time, they expressed minimum levels of UCP-1 [26]. The discovery of this kind of cells challenged the initial idea of WAT and BAT as two different tissues [31, 32], and opened the possibility of considering them as a single adipose organ [33]. Beige adipocytes arise in subcutaneous white adipose tissue from precursors expressing CD137 and transmembrane protein 26, under the condition of low temperatures or β-adrenergic-receptor stimulation. When the stimulus stops, the cells appear to return to white cells, however, upon re-stimulation

respond as beige adipocytes [10]. This phenomenon has encouraged the ability to control the differentiation of adipose tissue by increasing energy expenditure to reduce the accumulation of energy in the form of triglycerides, including WAT change to BAT (browning of WAT) under pathological conditions in humans was reported [34, 35]. We should learn more about browning control (on and off) to avoid presentation of undesired effects (i.e. cachexia, atherosclerosis and hepatic steatosis) [27, 36].

2.1.2. Macrophages

Macrophages were reported by Elie Metchnikoff in 1884. For many decades, they were considered to be a homogeneous lineage with a main function, phagocytosis. Now they are recognized as very plastic immune cells with multiple functions, because of this, their population is highly heterogeneous and difficult to classify, but two main phenotypes (subtypes) are generally accepted: classically (M1) or alternatively (M2) activated [37, 38].

The M1 phenotype is promoted by T-helper 1 (Th1) cytokines (i.e. interferon (IFN)-γ) or by pathogen-associated molecular patterns (PAMPs) (i.e. LPS) and is characterized by the production of pro-inflammatory cytokines (IL-6, TNF-α, IFN-γ, IL-1β, IL-12 and IL-23), chemokines promoting inflammatory infiltrate (CXCL9,10,11,13, CCL8, 15, 19, 20), and expressed on surface high levels of MHCII, CD80, CD86 and CD11c, among other markers (i.e. Ly6C, CD11b, CD62L, CCR2, CX3CR1 and CCR5). In nucleus, STAT1 and IRF5 are the consensus transcription factors [37, 38].

In contrast, T-helper 2 (Th2) cytokines (i.e. IL-4, IL-10 and IL-13) drive the M2 phenotype, with high phagocytic capacity, and secrete extracellular matrix components, angiogenic and chemotactic factors (CCL17, 18, 22, 24), anti-inflammatory cytokines (IL-10) and the transforming growth factor β (TGF-β), then M2 activates expression of immunosuppressive factors and the peroxisome proliferator-activated receptor gamma (PPARγ) that promotes tissue remodelling and helps to resolve inflammation [37, 38].

Generally, in lean individuals, M2 exists in the WAT; however, the accumulation of adipose tissue leads to increased number of macrophages, besides, the macrophages display M1 phenotype. There are two possible explanations for this phenomenon: (1) environmental factors present in adipose tissue of obese individuals causes a switch in phenotype from M2 to M1; (2) on the other hand, the increase in chemokines (such as CCL2) promotes the recruitment of circulating monocytes and due to the low-grade inflammation state they differentiate to M1 [10, 37].

2.1.3. Eosinophils and mast cells

To maintain the M2 polarization of WAT- residents macrophages, a constant production of IL-4 is necessary. It is speculated that the eosinophils present in the WAT are the main source [8, 10, 12]. Studies in normal weight mice showed that there are a lot of infiltrated eosinophils, which are a major source of IL-4. Moreover, their amount decreased in obese mice no matter what the origin is (genetic as ob/ob or high fat diet) [8, 10, 12], however, there are no reports in humans. Mast cells unlike eosinophils, increases their number in WAT of humans with

diabetes mellitus type 2 or obesity, there are reports in fed mice with high fat diet, linking these cells with an increasing adiposity and insulin resistance [8, 10].

2.1.4. Innate lymphoid cells

In recent years, studies have been published to identify innate lymphoid cells (ILCs), all members of this new family are characterized by a similar lymphocyte morphology, however, lack markers on its surface that identifies them as another immune cell type, because this is defined as lacking cells lineage markers (Lin^-) [39]. The ILCs come from two development pathways: (1) the first called cytotoxic ILCs, integrated by classic NK; (2) on the other hand, we have non-cytotoxical ILCs. The last group is subdivided into three types: ILC1s, ILC2s and ILC3s; they express T-bet, GATA-3 and ROR-γT, respectively, which is the main difference between them. ILCs can directly communicate with several varieties of cells and regulate immunity, inflammation and homeostasis in different tissues [8, 39].

ILC2s plays an important role in the regulation of glucose metabolism, lipid storage and redox balance in lean individuals. It accomplishes these by communicating with other immune cells associated with the type 2 immune axis (i.e. M2, eosinophils and invariant natural killer T) and participates in cross-talk with adipocytes [8, 39]. These cells produce cytokines associated with lymphocyte T-helper 2, cytokines that are required for immunity against helminths, allergic inflammation and tissue repair [8].

In contrast, it was found that cytotoxic ILCs (individuals and mice) and non-cytotoxic ILC1s (mice) are increased in visceral adipose tissue when obesity is present, accompanied by an increase in the production of interferon gamma, the latter contributes to change of the phenotype of macrophages to M1, thus, favours an inflammatory environment and increased recruitment of immune cells type 1 axis [8, 10, 12].

2.2. Adipokines

Adipose tissue was considered just an energy (triglycerides) storage site until obesity arises as a health problem worldwide. Adipose tissue came to the fore as an active secretory organ involved in various physiological and pathophysiological processes. Adipokines is a term used to identify molecules released from adipose tissue; some of them are secreted by others tissues (i.e. TNF-α, IL-1A, -1β, -5, -6, -8, 10, -15, -18) and certain are mainly or exclusively synthetized by adipocytes (i.e. leptin, adiponectin, resistin), these adipokines deserve that proposed term adipokinome [40, 41].

2.2.1. Leptin

Leptin is an adipokine secreted principally by white adipose tissue that regulates food intake and energy expenditure; furthermore, it also plays an important role in glucose homeostasis, immunity and fertility among others [42, 43]. Leptin exerts its action through leptin receptors, which are transmembrane proteins, members of the class I cytokine receptor superfamily, their pathway involves JAK/STAT, PI3K, MAPK/ERK systems, and PKC [42, 43]. There are six (a–f) isoforms of the leptin receptor generated by alternative splicing, b-isoform is the longest one,

and have all the signalling motifs; moreover, it is the most expressed in diverse cell lineages (i.e. adipocytes, myocytes, immune cells, neurons) permitting to leptin act in autocrine, paracrine and endocrine ways [44, 45].

Leptin production is proportional to the amount of adipose tissue; so, in subjects within a normal range weight, increasing leptin levels suppresses the need to eat by inhibiting the release of orexigenic neuropeptides (e.g. neuropeptide Y and Agouti-related protein) in the arcuate nucleus of hypothalamus, while obese individuals do not have this physiological response, a state called 'leptin resistance' [46].

It has been proposed that the establishment of this condition is a consequence of the combination of three main mechanisms: diminished intracellular leptin-receptor signalling, abnormal transport of leptin across the blood-brain barrier and development programming disorders; however, the molecular mechanisms by which lesser sensitivity to leptin is present in obesity have not yet been defined [46, 47].

2.2.2. Adiponectin

Adiponectin is a multifunctional and multi-named adipokine (adipocyte complement-related protein of 30 kDa, Acrp30; gelatin binding protein of 28 kDa, GBP-28; adipose most abundant gene transcript 1, apM1), coded by *ADIPOQ* gene, is a major adipocyte-secreted protein and is down-regulated in obesity and its co-morbidities. Adiponectin regulates metabolic homeostasis by acting on organs such as the brain, kidney, liver, pancreas and skeletal muscle by exerting potent insulin-sensitizing, anti-atherogenic and anti-inflammatory activities [8, 48].

Adiponectin is synthesized as a monomer, however, suffers extensive post-translational modifications to form trimers, hexamers and high molecular weight species (HMW, 12–18 monomers) before being secreted by adipocytes. Recent evidence suggests that depending on the degree of multimerization, different biological effects have been obtained [49, 50].

Biological activity of adiponectin is mainly mediated by binding to one of its two adiponectin receptors: AdipoR1 and AdipoR2. These receptors are differentially expressed, and adiponectin shows distinct affinity to them according to its multimerization degree [51]. AdipoR1 is most commonly found in skeletal muscle and binds preferably to low molecular weight species (trimers and hexamers), whereas AdipoR2 is abundant in liver and binds easily to HMW adiponectin [49, 51]. Liver and skeletal muscle have a crucial role in the IR process, therapeutic effect of thiazolidinediones is in part due to the enhanced expression of adiponectin and its receptors through PPAR-γ activation [52].

2.2.3. Resistin

Resistin was described in mice as the responsible molecule of IR; however in humans, results were not conclusive, in part because its specific receptor has not been identified yet. It is an adipokine that stimulates the synthesis of pro-inflammatory cytokines among which are: TNF-α, IL-1, IL-6 and IL-12; in various types of cells through pathway-dependent signalling nuclear factor (NF)-κB [17, 53]. It also induces increased expression of adhesion molecules (i.e. VCAM-1, ICAM-1) and chemokines (i.e. CX3CL1, CX3CR1) in human endothelial cells [17, 53].

Various studies report positive correlations of serum resistin levels with the amount of body fat, however, other studies have found no correlation [53–55]. The most important association of circulating resistin levels reported is with C-reactive protein, which could be a marker of systemic inflammation [53].

2.2.4. Chemerin

It is secreted by adipocytes; it is closely associated with amount and distribution of adipose tissue. As a chemoattractant protein, chemerin acts as a ligand for the coupled G-receptor protein (ChemR23) and participates in both adaptive and innate immunity [56]. In humans, chemerin gene (*RRARES2*) is highly expressed in WAT and to a lesser extent in liver and lungs. On immune cells, chemerin is known to stimulate chemotaxis of dendritic cells, macrophages and natural killer (NK) cells. Meanwhile, its receptor, ChemR23 gene (*CMKLR1*), is expressed in dendritic cells, monocyte/macrophages and endothelial cells [18, 56, 57]. ChemR23 is involved in the differentiation of adipocytes and increased intracellular glucose or lipids promote its expression [18].

The interaction of chemerin/ChemR23 has been shown to reduce cytokines, chemokines and phagocytosis, proving to be important in the inflammatory process associated with obesity [18, 57]. In this context, chemerin/ChemR23 axis has been shown to impact IR development, which influences the clinical course and severity of obesity-related diseases.

As has been exposed, dysregulation of adipokinome due to accumulation of adipose tissue in obesity establishes and perpetuates a vicious circle from which emerges the chronic low-grade inflammation state.

2.3. Low-grade inflammation state in obesity

Inflammation is a physiological response to a stimulus (i.e. injury or infection) described by Celsus and Galen and is characterized by five classical signs: pain, heat, redness, swelling and loss of function [58, 59]. The inflammation resolution is an active process influenced, in part, by the time and especially regulated by the formation of a group of lipid mediators, which are identified as LXs, protectins and resolvins [60].

The low-grade inflammation state is a term used to define the activation of the vascular endothelium and presence of inflammatory cells in the absence of the five classical signs (subclinical) [61]. This state is due, at least initially, to adipose tissue hypertrophy present in obese individuals because different pathological processes occurs (i.e. fatty acids in excess, hypoxia, cell infiltration and activation of the inflammasome), this pro-inflammatory state is chronic in obesity and now is considered the etiologic agent of its co-morbidities [58, 62]. Effects of this 'unresolved' inflammation state can be appreciated in other context not explored here, but in which we cannot ignore the nervous system [63, 64]; these three: metabolism, immunity and nervous system are so interdependent that now they are considered as branches of a higher hierarchical level, the neuroendocrine-immune system.

2.3.1. Recruitment of immune cells to adipose tissue

The corresponding number of immune cells in adipose tissue is increased in obesity, mainly due to circulating cells' recruitment, when compared to lean individuals. [10, 65]. The main infiltrating cells are monocytes, however, other cell types such as NK, LB and LT may also migrate principally [10, 65, 66]. The infiltrated cells promote a positive feedback loop for a chronic low-grade inflammation state.

A key molecule for this recruitment of macrophages is CCL2 chemokine (formerly MCP-1), its expression displays positive correlation with the amount of adipose tissue [10, 19, 67], which is produced by macrophages and other cell types after stimulation. *In vitro* studies have shown that free fatty acids and TNF-α can stimulate production in chemotactic molecules of adipocytes [68].

CCL2 is the most important chemoattractant in the recruitment of monocytes, but possibly not the only one, mice fed with high-fat diets also showed an increase in expression of leukotriene B4 (LTB4) in muscle, liver and adipose tissue [10].

3. Insulin resistance: a direct consequence of immunometabolic imbalance

Insulin resistance (IR) is a condition characterized by the inability of cells to appropriately respond to insulin, which results in prolonged systemic hyperglycemia. It was considered a pathology since the 1930s, however, it was the development of insulin quantification assays and methodologies to estimate its biological action, as well as large epidemiology studies, which allowed to define the magnitude of the problem and the clinical implications.

3.1. Insulin resistance classification

The gold standard for IR assessment is the 'Hyperinsulinaemic-euglycaemic clamp' described by DeFronzo et al. [69]. However, this technique is hard to perform, time consuming, invasive and expensive; therefore, it is prohibitive for large studies. Because of this, numerous indexes have been developed and validated as surrogates, one of the most used is the 'Homeostasis Model Assessment of Insulin Resistance' (HOMA-IR) described by Matthews et al. that kept a correlation of 69 and 88% with euglycaemic and hyperglycaemic clamp, respectively [70].

Cut-offs and conditions has been tested to improve IR individuals classification; Stern et al. compiled demographics, clinical, laboratory and anthropometrics data of 2321 subjects studied with the euglycaemic insulin clamp technique and determined that with a combination of two simple rules: (1) HOMA-IR > 3.60 and (2) BMI > 27.5 kg/m^2; individuals can be classified as insulin-resistant with a sensitivity and specificity of 84.9 and 78.7%, respectively [71].

3.2. Insulin resistance aetiology

Establishment of IR arises from the interaction between environmental factors (principally obesity), and predisposition genes that confer susceptibility.

At the cellular level, there are two main mechanisms responsible of IR development: (1) cellular stress in the endoplasmic reticulum, and in the mitochondria of adipocytes, hepatocytes and myocytes; and (2) release of pro-inflammatory cytokines, principally, TNF-α and IL-6 by activation of the Toll-like receptor 4 (TLR-4) on the surface of infiltrated macrophages of white adipose tissue and liver [72, 73]. Obtained data point towards multiple triggering paths for this processes to be started, however, it is the obesity-associated chronic low-grade inflammation the most linked one [72, 73].

Moreover, in obesity, the amount and the size of adipocytes increase (hyperplasia and hypertrophy); furthermore, macrophage infiltration in white adipose tissue is higher and these processes together deregulate the secretion of adipokines. One of them, adiponectin, is negatively correlated with WAT accumulation (as mentioned before), this diminishes insulin signalling, already affected by the pro-inflammatory milieu. To add complexity to the IR phenomenon, they have been recently described novel mechanisms of immunometabolic regulation, the miRNAs.

4. Novel mechanisms involved on immunometabolic regulation: miRNAs

The microRNAs (miRNAs) were discovered in 1993 by Lee, Feinbaum and Ambros, when it was shown that their expression involves negative regulation at the post-transcriptional level and their biogenesis was a result of two unrelated molecular routes.

About miRNAs biogenesis, the non-coding region into the genes is transcribed, hence a small non-coding RNA is obtained, these molecules synchronized the downregulation of protein expression both at the transcriptional and translational levels. However, upregulation of translation has also been reported. In the negative regulation processes, the miRNAs bind to their complementary sites within the 3′-untranslated regions (UTRs) of target mRNA throughout sequence recognition, resulting in mRNA translational repression or degradation of the mRNA transcripts [74, 75].

The miRNAs are a kind of non-coding RNA of specific genes, whose products are single-stranded RNA molecules between 19 and 25 nucleotides, their sequences were identified by Northern blot analysis, microarrays or the real-time PCR method. Nucleotide sequences of miRNAs are reported in miRBase registry (http://mirbase.org/), and the correct nomenclature is discussed by several authors [76, 77].

4.1. microRNAs biogenesis

The molecular biosynthesis process of microRNAs involving multiple pathways however is possible to characterize a general mechanism, in which sequential routes of a particular method are identified (**Figure 2**):

1. miRNAs are transcribed in the cell nucleus by the RNA polymerase II, based on three main gene sequences: intronic regions, polycistronic clusters or from intergenic areas; the molecules obtained are called pri-miRNAs.

2. pri-miRNAs are improved by RNasa type III (Drosha) to become pre-miRNA, which is recognized by the XPO5 and RanGTP complex and transported to the cytoplasm through a nuclear pores [78].

3. The pre-miRNA, once it reaches in the cell cytoplasm, Dicer cleaves the double-stranded fragment and releases the loop, the miRNA duplex is unrolled and loaded into the complex miRISC [78].

4. Once it is loaded into the RISC complex, the mature miRNA is capable of associating with the mRNA target.

Subsequently when the mature miRNA is formed, their function will be to recognize by 3'UTR complementary sequences in the target mRNA. The level of coincidence in these sequences determines the degree of regulation of transcription, with one of two options, when the sequence of the complex is 100% complementary to the sequence in the region of the target (perfect complementarity), leading to denaturation and degradation of mRNA, while incomplete complementarity triggers silencing of mRNA through different molecular mechanisms, as repression of translation, degradation and/or sequestration of target [78, 79].

Since its deregulation has been related to different illnesses and it is estimated that more than 60% of the human genes expression are regulated by microRNAs [80].

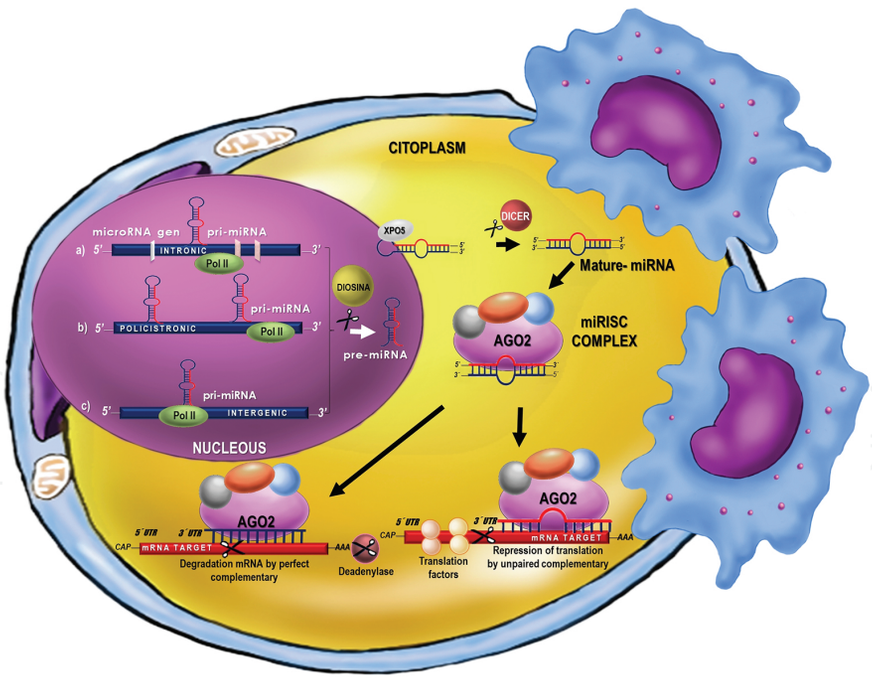

Figure 2. miRNA biogenesis. MicroRNA is transcribed by the polymerase II from (a) genes, (b) polycistronic clusters and (c) intronic regions. These pri-miRNAs are processed by the RNase Drosha which shortens them. The pre-miRNA formed is transported to cytoplasm by the XPO5 and then Dicer cleaves the loop leaving a double chain fragment that is recognized for the miRISC complex and targets the mRNA. POL II: polymerase II; XPO5: exportin 5; AGO2: argonaute protein 2; UTR: untranslated region; CAP: caperuse and AAAA: poly A chain.

4.2. microRNAs structural forms

For the reason that miRNAs are produced from the differential gene expression, heterogeneous structures are obtained with different length and nucleotide sequences. Sequences of two biochemically stable forms with asymmetrical structural motifs, the immature form (pre-miRNA) and the mature form (miRNA) have been reported.

The pre-miRNAs have a length of more 60 nucleotides, obtained from pri-miRNAs by the division in the union on the opposite side of the double chain loop. The recurrent structure is a double-helix that ends in a loop that joins both chains, the chain that goes from 5′ to 3′ and the chain of the direction from 3′ to 5′, which are 100% non-complementary and the length varies according to the mature stage due to the cuts made by the enzymes [81]. This double chain contains a monophosphate group in the end 5′, and a free OH group in end 3′.

The mature microRNAs are approximately of 18–25 nucleotides in length, and differ from one another in their nucleotide sequence and length. Finally, two chains are generated in sense (5') and antisense (3') directions, emerging from the 5' arm of the hairpin microRNA or 3' arm of the microRNA hairpin, these sequences are called -5p or -3p, respectively. Generally, only one of them is dominant while the other is considered as a minor product, due to the intracellular concentration. However, both sequences are functional, as this depends on the tissue and species in which they are located [76, 82].

4.3. The functional diversity of miRNAs

The study of miRNAs is relevant because it has been involved in diverse functions and their expression levels have been associated with different diseases, such as insulin resistance, diabetes, atherosclerosis or cancer. While the new miRNAs sequence is identified, their participation in biological processes at the molecular level is to be defined. The mechanisms described include adipocyte differentiation, metabolic integration and appetite regulation [80, 83].

Among the metabolic diseases associated with the expression of miRNAs, their role in diabetes mellitus type 2 has been described, where it was shown that the miR-375 is directly involved in the regulation of insulin secretion. These data suggest their participation in the metabolic pathways and the possible association with inflammatory markers and their expression in individuals with phenotypic characteristics associated with obesity inflammation [84].

On the other hand, the miRNAs can also be found in the soluble form and it is important to remark that they are stable in serum for long periods of time due to two mechanisms, formation of a complex of ribonucleoprotein with argonaut proteins and the addition of exosomes and micro-particles. Besides, it has been discovered that HDLc can transport miRNAs in the plasma and take them to the target cells where they will be captured [85].

Related to their location in the serum of the miRNAs mature form, Slack has proposed two hypotheses: first, the miRNAs of tissues can be present in the circulation as a result of cell death and lysis, and second, the tissue cells actively secrete miRNAs in their microenvironment,

where they get into the blood vessels so that they get their way up the circulation, so that it has been also suggested their potential usage as markers for different diseases [86].

Due to the strong correlation between the expression patterns of miRNAs and the state of the diseases, that has been showed not only in animal models but also in human patients, the miRNAs have been considered as promising candidates for the next generation of biomarkers.

4.4. miRNAs and immunometabolism

It is important to recognize that metabolic diseases have a strong immune component derived from inflammation and oxidative stress in which microRNAs regulate different ways (**Figure 3**).

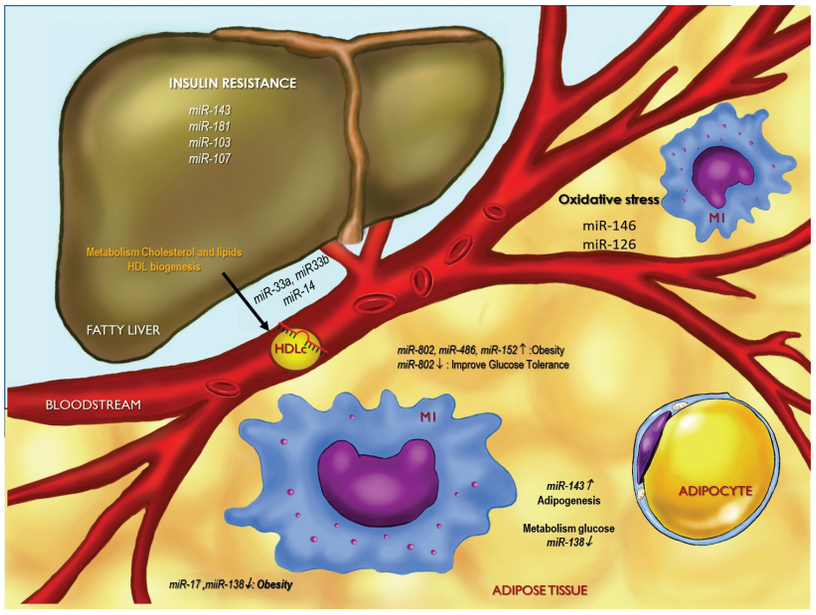

Figure 3. miRNA and immunometabolism. In immunometabolism, the microRNAs have an important role in regulation of different levels of metabolism. Further, the oxidative stress is the link between inflammation and obesity, since the accumulation of adipose tissue stimulates the expression of inflammation markers allowing the establishment of pathologies like DM2 and insulin resistance. Otherwise, there are microRNAs that improve glucose tolerance and lipid metabolism. M1: macrophages phenotype 1; HDLc: high-density lipoprotein cholesterol and miR: microRNA.

In diabetes mellitus type 2 patients, associations of miR-146 and miR-126 with markers of endoplasmic reticulum stress and inflammation as well as decreased miR-20b, miR-21, miR-24, miR-15a, miR-126, miR-191, miR-197, miR-223, miR-320 and miR-486 have been reported. Evenly, in obesity, the miR-152 increases, whereas miR-17 and miR-138 decreases [75, 87, 88].

With regard to metabolic functions in the skeletal muscle and the deregulation of the miRNA species, it is known that it can lead to deep alterations of glucose and the lipid metabolism in the adipose tissue, which is an important metabolic regulator. Regarding this, it has been shown that the expression of miR-14 decreases the levels of triacylglycerol and diacylclycerol, so that miR-14 could be an important lipid regulator in this level, besides other involved miRNAs such as miR-143 [89], miR-181a [90], miR-103 and miR-107 [91] have all been shown

to affect hepatic insulin sensitivity, and more recently, miR-802 has been shown to be increased with obesity and that its reduction improves glucose tolerance and insulin action [92].

According to the association between obesity and the fatty acids different microRNAs have been associated, among them miR33a and miR33b, which are found in the intronic regions of the genes *SREBF2* and *SREBF1* that code for the transcription factors SREBP2 and SREBP1 and control the expression of the genes involved on the synthesis of cholesterol and fatty acids [93].

miR33a/b act as suppressing genes that oppose the functions of SREBP, for instance, the cholesterol efflux and the oxidation of fatty acids; so that under low-cholesterol conditions the transcription of SREBP2 and the regulation of the genes involved in the synthesis of cholesterol and absorption is activated, therefore, co-transcription of miR-33a acts in exporting cell cholesterol inhibiting the transcription of ABCA1 [93].

On the other hand, it is known that high levels of free fatty acids (FFA) and different adipokines, such as leptin and resistin, have a strong regulator role in other microRNAs; while expression of miR-143 can modulate the differentiation of the preadipocytes by increasing the storing of lipids, likewise, inhibition of miR-143 blocks differentiation of adipocytes through the kinase 5 regulator of extracellular signals (ERK5) [94].

5. Perspectives

At present, it seeks to make the value of clinical information available further effective, favouring a comprehensive approach to identify novel early biomarkers in the development of obesity and its co-morbidities.

In obesity, the biologic representative hallmark is the establishment of a subclinical chronic low-grade inflammatory process, promoted by the dysregulation of the immune system cells resident of white adipose tissue. Antagonistically, an underlying molecular mechanism induced by BAT control (hypermetabolism) can be developed. The understanding of both processes may allow the identification of early biomarkers with therapeutic aim of mitigate or eliminate the associated immunometabolic effects.

Researchers have focused their efforts on finding new biomarkers in obesity, based on the concept that a biomarker is identified as a qualitatively and/or quantitatively measurable biological parameter, which can be characterized as an indicator of health status versus disease, and also it serves as a marker for susceptibility or to stratify the relative risk in the general population.

The importance of a novel early biomarker is that it can have a high diagnostic or prognostic value in the context of development, establishment and progression of obesity and its co-morbidities. Because cut-off values are established in the biomarker validation process, it can be identified as a 'distorted indicator and differentiated predecessor' of clinical manifestations, with the possibility of aid at establishment, a classification in the progression of co-morbidities and severity of obesity.

As a consequence, from discovery to clinical application, an ideal early biomarker need run into the following characteristic: be easily accessible by using a sampling procedure minimally invasive, therefore, samples of blood, urine and saliva are excellent sources of choice.

In this contextual group of ideas, miRNAs that are transported to target cells through the bloodstream, are relatively stable and easily removed from blood serum have been identified. They are associated with metabolic risk and dysregulation of the immune system when white adipose tissue increases, and they are postulated as candidates of 'novel early biomarkers', due to their ability to become acquainted with the progression of the pathogenic process of obesity.

Author details

Efrain Chavarria-Avila[1,2,4,5], Rosa-Elena Navarro-Hernández[1,2,3,5], Milton-Omar Guzmán-Ornelas[1,2], Fernanda-Isadora Corona-Meraz[1,2], Sandra-Luz Ruíz-Quezada[2,3] and Mónica Vázquez-Del Mercado[1,5,6,7*]

*Address all correspondence to: dravme@hotmail.com

1 Institute for Rheumatology Research and MuscleSkeletal System, CUCS, University of Guadalajara, Guadalajara, Jalisco, Mexico

2 UDG-CA-701, Research Group on Immunometabolism and Emerging Diseases, Health Sciences School, Guadalajara, Jalisco, Mexico

3 UDG-CA-817, Research Group on Genomics and Biomedicine, Department of Farmacy and Biology, University of Guadalajara, Exact Sciences and Engineering School, Marcelino García Barragán Boulevard, Guadalajara, Jalisco, Mexico

4 Deparment of Philosophical, Methodological, and Instrumental Disciplines, University of Guadalajara, Health Sciences School, Guadalajara, Jalisco, Mexico

5 Department of Molecular Biology and Genomics, University of Guadalajara, Health Sciences School, Guadalajara, Jalisco, Mexico

6 Rheumatology Service PNPC 004086, CONACyT, Internal Medicine Division, Civil Hospital Dr. Juan I. Menchaca, Guadalajara, Jalisco, Mexico

7 UDG-CA-703, Research Group on Immunology and Rheumatology, University of Guadalajara, Health Sciences School, Guadalajara, Jalisco, Mexico

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