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AUTHOR-INITIATED REVIEW - UNSOLICITED

The regulatory role of insulin in energy metabolism and leukocyte functions

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Abstract

Insulin is the hormone responsible for maintaining glucose homeostasis in the body, in addition to participating in lipid metabolism, protein synthesis, and the inhibition of gluconeogenesis. These functions are well characterized in the classic organ target cells that are responsible for general energy regulation: the liver, skeletal muscle, and adipose tissue. However, these actions are not restricted to these tissues because insulin has been shown to affect most cells in the body. This review describes the role of insulin in leukocyte signaling pathways, metabolism and functions, and how insulin resistance could affect this signaling and deteriorate leukocyte metabolism and function, in addition to showing evidence that suggests leukocytes may substantially contribute to the development of systemic insulin resistance.

KEYWORDS
insulin resistance, insulin signaling, insulin, leukocytes

Abbreviations: AGE, advanced glycation end products; AKT, protein kinase B; AP1, activator protein 1; AS160, AKT substrate of 160 kDa; ATP, adenosine triphosphate; CCL3 C-C, motif chemokine ligand 3; CD16, cluster of differentiation 16; CD36, cluster of differentiation 36; cel/μL, cells/microliter; c-Myc, c-Myc transcription factor; CRP, C-reactive protein; DAG, diacylglycerol; ERK, 1/2 extracelular signal-regulated kinases 1/2; ETC, electron transport chain; FADH2, flavin adenine dinucleotide; FAO, fatty acids oxidation; FAT, fatty acids translocase; FcRI, high affinity IgE receptor; FFA, free fatty acids; FMLP, N-Formyl-metionyl-leucyl-phenylalanine; FOXO1, forkhead box protein O1; GLUT, glucosa transporter; GLUT1, glucose transporter 1; GLUT3, glucose transporter 3; GLUT4, glucose transporter 4; Grb2/SOS, growth factor receptor-bound protein 2/ son of sevenless complex; GSK3, glycogen synthase kinase 3; H_2O_2, hydrogen peroxide; HIF-1α, hypoxia-inducible factor 1-alpha; HK, hexokinase; IFN-γ, interferón gamma; IGF-1, insulin-like growth factor 1; IKK, IkB kinase; IL-10, interleukin-10; IL1A, interleukin-1 alpha; IL-2, interleukin-2; IL-4, interleukin-4; IL-5, interleukin-5; IL-5, interleukin-5; IL-6, interleukin-6; IL-8, interleukin-8; IRS-1/2, insulin receptor substrate 1/2; JNK, c-Jun N-terminal kinase; kDa, kilodaltons; LDH, lactate dehydrogenase; LDL, low-density lipoprotein; LPS, lipopolysaccharide; Mac-1, macrophage-1 antigen; MAPK, mitogen-activated protein kinase; MCP-1, monocyte chemoattractant; MEK, mitogen-activated protein kinase kinase; MHC II, major histocompatibility complex; MMP-9, matrix metalloproteinase 9; mTOR, mammalian target of rapamycin; mTORC1, mTOR complex 1; mTORC2, mTOR complex 2; NAD+, oxidized nicotinamide adenine dinucleotide; NADH, reduced nicotinamide adenine dinucleotide; NADPH, nicotinamide adenine dinucleotide phosphate; NE, neutrophil elastase; NET, neutrophil extracelular trap; NFKBIA, NFκB inhibitor alpha; NFκB, nuclear factor kappa B; O_2-, superoxide radical; OP, oxidative phosphorylation; PDK1, 3-phosphoinositide-dependent protein kinase 1; PFK, posphofructokinase; PI3K, phosphatidylinositol 3-kinase; PIP_3, phosphatidylinositol (3, 4, 5)-trisphosphate; PKC, protein kinase C; PPP, pentose phosphate pathway; PTB, phosphotyrosine-binding domains; Rac-1, Rac family small GTPase 1; Raf-1, RAF proto-oncogene serine/treonine-protein kinase; RAGE, advanced glycation end products receptor; Ras, small GTP-binding protein Ras; RAW, 264.7 RAW 264.7 cell line; ROS, rective oxygen species; RTKs, receptor tirosine kinases; S6K1, ribosomal protein S6 kinase beta-1; SERCA2b, sarco/endoplasmatic reticulum Ca^{2+}-ATPase; Shc, adapter protein Shc; SRA, scavenger receptor class A; TCA, tricarboxylic acid cycle; THP-1, THP-1 cell line; TLR4, toll-like receptor 4; TNFα, tumor necrosis factor alpha; TNF-β, tumor necrosis factor beta

1 | INTRODUCTION

Homeostatic regulation of glucose is primarily governed by the action of insulin in adipose, muscle, and liver tissue, which responds by activating signaling pathways involved in the metabolism of glucose and lipids, energy storage, and cell growth. Insulin is a highly effective anabolic hormone whose molecular weight of 5.8 kDa is composed of 2 peptide chains: the A chain, which contains 21 amino acids, and the B chain, which contains 30 amino acids. Both peptide chains are linked by disulfide bridges.[1] Insulin is produced and stored in granules within the beta cells of the islets of Langerhans in the pancreas, from which it is secreted primarily in response to increased plasma glucose concentrations.[2] Its main function is to promote the intake of circulating glucose into skeletal muscle and adipose tissue cells, where it also promotes the formation of glycogen and lipogenesis, respectively, whereas in liver tissue, it inhibits the production of glucose.[3] Other actions of insulin include regulation of growth, cell survival, and protein synthesis.[4] The actions of insulin are governed by the activation of 2 canonical signal transduction pathways that are ubiquitous in practically all cells and that begin once it binds to the insulin receptor present in the plasma membrane.[5–7] Although studies on the actions of insulin have focused on the tissues responsible for energy homeostasis in the body, there is evidence that the hormone participates in the metabolic and functional regulation of other cells, called nonclassical insulin targets, among which are cells of the central nervous system,[8,9] epithelial cells,[10,11] and even bone cells.[12–14] However, recent evidence describes the relationship between the effects of insulin and the regulation of metabolism and/or the functions of immune cells.

Leukocytes, also known as white blood cells, are the effector cells of the immune system, and their main function is to defend the host against pathogenic microorganisms or against any type of tissue damage. These cells are produced in the bone marrow from hematopoietic stem cells that give rise to 2 cell lineages, the myeloid line and the lymphoid line, from which the mature leukocytes that enter into circulation are derived.[15,16]

In the bloodstream, leukocytes are present at a concentration of 4,000–10,000 cells/μl under normal conditions.[17] In a routine hematologic analysis, 5 types of leukocytes are morphologically distinguished using conventional histologic stains: neutrophils, eosinophils, basophils, monocytes (belonging to the myeloid line), and lymphocytes (from the lymphoid line). Neutrophils represent 75% of total leukocytes,[18] eosinophils and basophils represent 8% and 2%, respectively (these are known as granulocytes), monocytes represent 5%, whereas lymphocytes make up 20–45% of total leukocytes in circulation.[17]

This review describes the role of insulin in the metabolism and functions of leukocytes and how the state of insulin resistance affects its molecular signaling as well as its metabolic and immune function. Similarly, evidence has suggested that leukocytes substantially contribute to the development of systemic insulin resistance. Therefore, the primary objective of this review was to address the molecular and signaling mechanisms of insulin in leukocytes and their relationship with metabolic and functional control, proposing leukocytes as cells with the potential for consideration in the analysis of the molecular processes involved in the development of insulin resistance.

2 | INSULIN SIGNALING PATHWAYS IN LEUKOCYTES

Initiation of insulin signal transduction occurs from binding to its receptor. The insulin receptor is a heterotetrameric protein of the receptor tyrosine kinase family that is autophosphorylated in the intracellular region at tyrosine residues, which are recognized by cytoplasmic proteins that serve as scaffolds for the recruitment of other molecules that participate in signaling.[19]

One of the pathways activated by insulin in leukocytes is MAPKs, in which insulin receptor substrate proteins 1/2 (IRS-1/2) or the Shc protein recognize tyrosine residues phosphorylated on the receptor β chain through its phosphotyrosine binding domains, which are also phosphorylated. Subsequently, the protein complex growth factor receptor-bound protein 2/Son of sevenless (Grb2/SOS) recognizes the phosphorylated residues of IRS-1/2 or Shc and in turn activates a cascade of Ras, Raf-1, and MEK kinases. The final result of the pathway is the translocation of ERK 1/2 to the nucleus, regulating migration, proliferation, and cellular growth functions.[20]

The other signaling pathway that is activated is PI3K, in which IRS-1/2 recognizes phosphorylated tyrosine residues in the receptor, which in turn are recognized by the regulatory subunit of PI3K that activates the catalytic subunit, which is capable of phosphorylating membrane phospholipids, such as phosphatidylinositol-4,5 bisphosphate, to generate phosphoinositol-3,4,5, triphosphate, allowing the recruitment of protein kinase B (AKT), which is phosphorylated by the 3-phosphoinositide-dependent kinase 1 at threonine 308 and by the mTORC2 complex at serine 473, achieving its activation. AKT phosphorylates different substrates that regulate metabolic processes in cells, including the protein glycogen synthase kinase 3, favoring an increase in glycogen synthesis,[21] the mammalian target of rapamycin (mTOR), which regulates protein synthesis through ribosomal protein S6 kinase beta 1[22] and AKT substrate of 160 kDa protein that favor the translocation of GLUT4 transporters from cytosolic vesicles to the plasma membrane to allow glucose entry into cells,[23,24] which is the primary effect of insulin in adipose and muscle tissues that contributes to the maintenance of plasma normoglycemia.

Comparative results about the effects of insulin on glucose metabolism in leukocytes were contradictory,[25–29] as glucose uptake by leukocytes may be influenced by different factors, such as interindividual variability, analysis strategy due to leukocyte half-life, but importantly, because the duration of insulin interaction with the receptor is not known, so there is no clear evidence that such factors directly impact glucose uptake or metabolism. In recent years, the role of insulin in the regulation of metabolism in immune cells has begun to be analyzed with respect to the signaling pathways and metabolic processes involved in energy production, highlighting the different requirements in the quiescent and activated state of leukocytes, as well as the direct impacts on their functions.

3 | ENERGY METABOLISM OF LEUKOCYTES

Glucose is the primary energy-producing molecule. Once it enters cells, 3 main interconnected metabolic pathways are responsible for the generation of ATP from glucose: glycolysis, the tricarboxylic acid cycle (TCA) and oxidative phosphorylation (OP). Glycolysis occurs in the cytoplasm and begins with the generation of glucose-6-phosphate, a reaction mediated by hexokinase (HK), which prevents glucose from leaving the cell. Subsequent enzymatic reactions generate 2 molecules of NADH, 2 ATP, and 2 pyruvates as final products.[30] The availability of oxygen determines the next step in the metabolic pathways, under hypoxic conditions, hypoxia-inducible factor 1α (HIF-1α) is the main driver of glycolysis where pyruvate is converted into lactate by the enzyme lactate dehydrogenase (LDH), which produces oxidized NAD+ molecules from NADH[31] that favor the continuity of glycolysis. Under normoxic conditions, pyruvate is converted to acetyl coA, which enters the TCA in the mitochondria, which, through NADH and reduced flavin adenine dinucleotide, maintains the electron transport chain and, finally, OP, which ultimately produces ATP.[32] A pathway derived from glycolysis and glucose-6-phosphate is the pentose phosphate pathway, which is necessary for the production of ribose or NADPH,[33] whereas fatty acid oxidation (FAO) and glutaminolysis replace the acetyl coA and α-ketoglutarate metabolites, respectively, which can be used during the anabolism of proliferating cells.[34,35]

Once activated, leukocytes migrate through diapedesis to different sites inflammation, where the availability of oxygen is variable, but they have the ability to rewire their metabolism (aerobic or anaerobic) to carry out their function once they are activated. This phenomenon is known as metabolic plasticity, determined by the measurement of oxygen consumption rate, which is a reflection of mitochondrial energy production via OP, whereas the extracellular acidification rate determines energy production through glycolysis and the generation of lactate.[36]

The basal energy metabolism of neutrophils is derived from glycolysis.[37] Once activated, they increase their glucose consumption anaerobically and produce lactate, even when oxygen availability is not limiting, an effect known as Warburg metabolism. This process is mediated by HIF-1α, a transcription factor that is activated under oxygen-limited conditions.[38] However, HIF-1α can also be activated under normoxic conditions by signaling through TLR4 present on leukocytes through the activation of NFκB.[39] As neutrophils depend preferentially on anaerobic glycolysis, the mitochondria present in them do not participate in the generation of ATP but do participate in the production of mitochondrial reactive oxygen species (mtROS) through the NADPH oxidase system,[40] that helps in maintaining mitochondrial membrane potential by importing NAD+ produced by glycerol 3-phosphate shuttle, which is active in neutrophils.[41]

In the case of eosinophils, there are few studies regarding their metabolic requirements, likely due to their low number in circulation and the technical limitations of their isolation; however, it has been discovered that they contain a glycolytic metabolism "similar" to that of neutrophils but with a higher oxygen consumption and greater flexibility toward the use of OP.[42] Finally, with respect to basophils, their metabolic requirements are not yet known.

Circulating monocytes satisfy their basal energetic requirements by producing ATP via OP.[36] Once they migrate to tissues, they become macrophages, where the microenvironment generates local signals that allow them to acquire 2 possible phenotypes: classically activated macrophages (M1 or proinflammatory) or alternatively activated macrophages (M2 or anti-inflammatory).[43] M1 macrophages have a preferentially anaerobic glycolytic metabolism with the formation of lactate, very similar to that shown by neutrophils[43]; they also have a low number of mitochondria that do not participate in the generation of energy but are necessary for the production of mtROS.[44] M2 macrophages, on the other hand, have an energy metabolism that depends on both OP[45] and FAO.[46]

Lymphocytes are normally under resting conditions, satisfying their energy requirements through OP.[47] When activated, they increase glucose consumption using aerobic glycolysis, but they also maintain active TCA and OP that are necessary to supply the molecules required for proliferation during clonal expansion, a process supported by glutaminolysis that replaces the intermediaries for OP[47] (Fig. 1).

4 | THE INVOLVEMENT OF INSULIN IN GLUCOSE METABOLISM

The insulin receptor is constitutively expressed on the plasma membrane of both monocytes and neutrophils, and its expression does not change as a function of insulin concentration.[48] There is no evidence to date that had directly evaluated expression of the receptor in eosinophils and basophils, although as they are granulocytes, it is very likely that their expression is similar to that of neutrophils. However, in lymphocytes, expression of the insulin receptor is not constitutive but is positively regulated in response to insulin,[49] and the effect is potentiated during its activation.[50–52] These findings suggest the probable participation of insulin in the regulation of metabolism in these immune cells.

In monocytes, insulin increases the transport and utilization of glucose,[53–55] whereas in neutrophils, insulin does not seem to regulate glucose uptake; however, it shows an influence on glucose metabolism once transported to the cytoplasm, regulating molecules that participate in glycolysis.[56,57] Glucose enters the cell by facilitated diffusion through the glucose transporter protein GLUT. Expression of GLUT in the membrane is crucial for increased glucose uptake in activated leukocytes. Fourteen isoforms expressed in humans have been described, of which GLUT1-4 are the most studied, and leukocytes express the GLUT1 and GLUT3 isoforms independent of insulin stimulation, as well as GLUT4, which has been shown to be dependent of insulin-activated signaling.[58,59]

In inactive monocytes, the presence of the insulin receptor appears to be relevant in glucose transport, as it has been shown that insulin stimulation is directly proportional to glucose uptake.[55,59] It has been described that in cells isolated from humans, the GLUT3 isoform is the most highly expressed compared with the GLUT1 and GLUT4 isoforms,[55,60] and this finding coincides with studies in the monocytic cell lines THP-1 and RAW 264.7.[60,61]

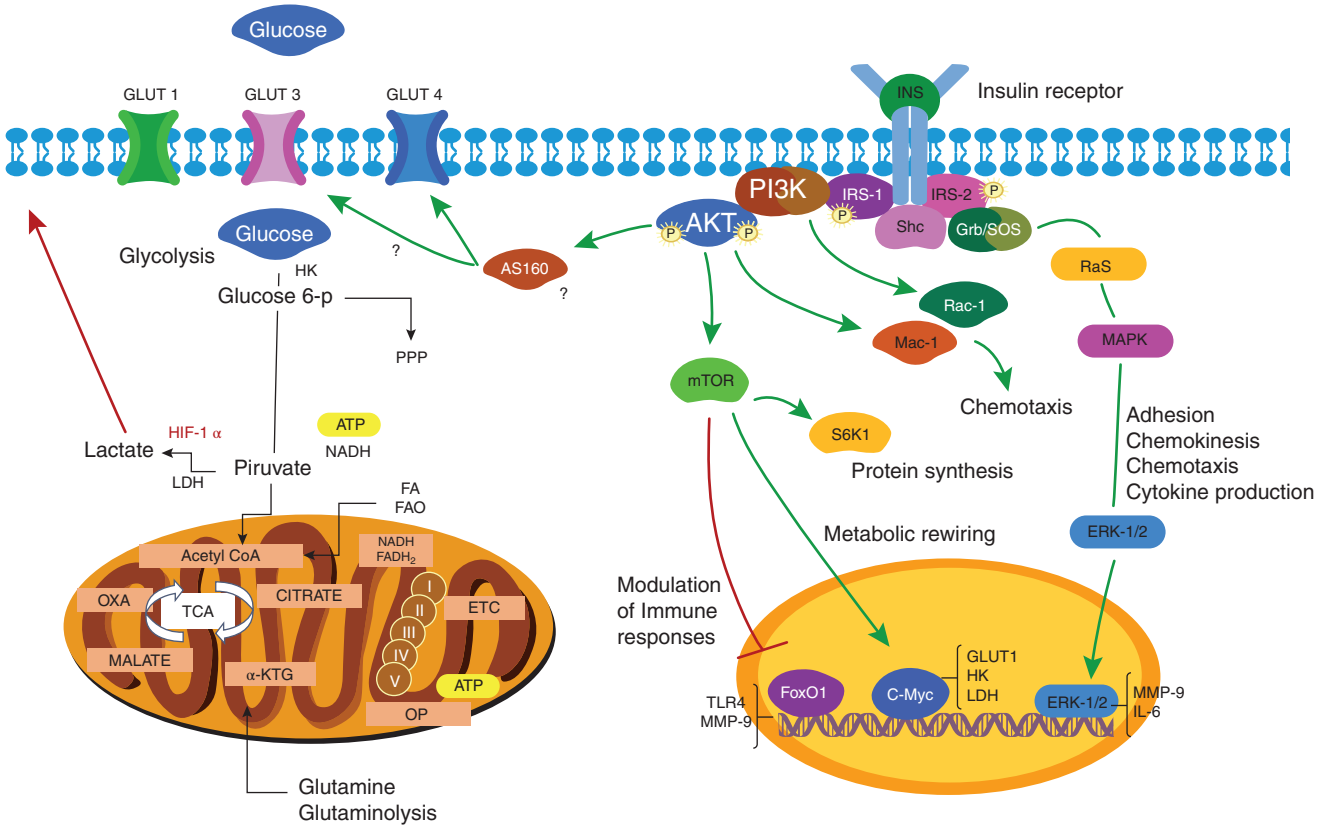

FIGURE 1 **Regulation of metabolism and leukocytes functions by insulin.** Leukocytes express the insulin receptor. Once insulin binds to its receptor, it activates two signal transduction pathways. The MAPK pathway results in ERK-1/2 activation, in leukocytes it participates in adhesion, chemokinesis, chemotaxis and cytokine production. PI3K pathway, activating AKT and phosphoryling different substrates downstream of the signaling cascade in functions such as protein synthesis, inflammatory response, metabolic rewiring, and chemotaxis

Once activated, monocytes differentiate into macrophages; their activation results in an increase in the 3 isoforms, but in the presence of insulin, the translocation of the GLUT3 and GLUT4 isoforms to the plasma membrane is increased.[59] In vitro differentiation of monocytic cell lines toward macrophages has also been shown to increase the expression of GLUT1 and, primarily, GLUT3.[60,61]

Because monocytes have the ability to increase membrane GLUT4 expression and glucose uptake in the presence of insulin, similar to what occurs in tissues such as adipose and skeletal muscle, they can likely be considered cells for evaluation of systemic insulin sensitivity; however, their probable clinical utility in metabolic pathophysiology, such as insulin resistance, must be further examined.[62]

In the inactive state, neutrophils express GLUT1 and GLUT3 transporters, and glucose internalization occurs primarily through GLUT1.[63] Activation of neutrophils has been shown to increase glucose consumption,[64] coinciding with the increase in GLUT1, GLUT3 and GLUT4 transporters in the membrane, and expression of GLUT3 and GLUT4 is observed as a function of insulin concentration via PI3K.[59]

Eosinophils differ in their ability to uptake glucose from the medium, being less effective than neutrophils; however, it was also shown that GLUT1, GLUT3, and GLUT4 transporters are involved in transporting glucose into eosinophils.[65] The presence of GLUT4 is interesting as there are no studies related to the effect of insulin on this type of leukocyte. IL-5 is a factor that stimulates the activation of eosinophils.[66] It was observed that the incubation of eosinophils with IL-5 substantially increased glucose internalization, and this effect was attenuated when the GLUT 4 transporter, tyrosine kinase activity in general and the specific activity of PI3K were inhibited.[65] Although there is no further evidence in this regard, the results support the idea that glucose uptake in activated eosinophils is regulated by insulin signaling.

Lymphocytes show differential expression of the insulin receptor as inactive lymphocytes do not express the insulin receptor on their membrane, and the GLUT1 isoform is the prevalent isoform in this cell lineage.[60] In B lymphocytes, there are no direct studies on the presence or absence of the insulin receptor when cells are inactive; however, insulin stimulates both glucose uptake and increases the expression of GLUT3 and GLUT4 in the plasma membrane. This effect was suppressed with inhibition of the PI3K pathway, suggesting expression of the insulin receptor in the quiescent state.[59] Inactive T lymphocytes do not express the insulin receptor on their membrane, so in this state, they are insensitive to insulin,[50] but once activated, both subtypes of lymphocytes (B and T) express the receptor and are able to increase glucose uptake.[59,67] The influence of insulin on the metabolic regulation of lymphocytes was demonstrated when analyzing diabetic patients, in whom immune cells are mostly activated, demonstrating that treatment with insulin increased glucose uptake in lymphocytes, implicating hormone-sensitive transporter translocation to the membrane.[68]

Pathogenic signals alone can influence the metabolic regulation of lymphocytes; however, they also require costimulatory signals that direct changes in glucose uptake and glucose metabolism necessary to perform their effector function.[69] In this sense, there is evidence that insulin is the extracellular molecule that regulates this process, as elimination of the insulin receptor in these cells leads to impaired glucose transport and aerobic glycolysis.[67,70] In activated lymphocytes, glucose and glutamine metabolism are regulated by the transcription factor c-Myc, which promotes the transcription of glucose transporters and the enzymes that participate in glycolysis,[71] through the PI3K and AKT-mTOR signaling axes.[72] Lymphocytes lacking the insulin receptor exhibit low levels of c-Myc expression and decreased glucose absorption and glycolytic capacity, which is related to decreased expression of GLUT1 and the glycolytic enzymes HK, phosphofructokinase and LDH.[73] Therefore, accumulating evidence indicates that insulin plays an important role in the metabolic regulation of leukocytes (Fig. 1).

5 | THE ROLE OF INSULIN IN LEUKOCYTE IMMUNE FUNCTION

In leukocytes, insulin not only influences glucose uptake and metabolism but also participates in the regulation of some of its immune functions. Neutrophils are the first leukocytes to reach the site of an infection, and their dynamic capacity is extensive as they migrate from the bloodstream to the sites of injury or inflammation, where their primary function is to phagocytose microorganisms and destroy them intracellularly with the generation of ROS and the fusion of cytoplasmic azurophilic granules; however, neutrophils also have the ability to destroy pathogens extracellularly through secretion of proteolytic enzymes released during degranulation and generation of neutrophil extracellular traps (NETs).[74,75]

Although insulin does not regulate the transport of glucose in neutrophils, it does mediate effects on the regulation of some of its functions. Insulin *per se*, in the presence of normal physiologic glucose concentrations, has been shown to stimulate chemokinesis of neutrophils in healthy individuals[76] and to increase migration toward a positive gradient of chemotactic substances.[77] Although the signaling pathways that regulate chemotaxis can be diverse, it has been shown that the PI3K pathway associated with the insulin receptor participates in the regulation of this function.[78] Activation of the insulin-dependent MAPK pathway produces an increase in the adhesion capacity of neutrophils after stimulation with chemoattractants, such as FMLP.[79] On the other hand, it was shown that insulin is capable of modulating the inflammatory response of neutrophils through the phosphorylation of FoxO1, which remains inactive, preventing the expression of TLR4 and extracellular matrix metalloproteinase 9 (MMP-9) in response to LPS.[80] Insulin also participates in the regulation of phagocytic and bactericidal capacities of neutrophils.[81] These data show that insulin directly influences some functions of neutrophils, although the role of this hormone in the uptake of glucose by these cells remains to be fully elucidated.

Monocytes are phagocytes and sentinel cells found in circulation, and they have a powerful capacity to migrate toward sites of infection or inflamed tissues in response to chemotactic stimuli. In these cells, insulin-dependent activation of ERK-1/2 promotes expression of MMP-9, which facilitates monocyte chemotaxis in response to MCP-1.[82] Insulin also stimulates the adhesion and migration capacity of monocytes through the PI3K-AKT pathway, favoring expression of macrophage antigen 1 in the membrane,[83] in addition to the activation of Rac-1, an important molecule in the regulation of cell migration.[84] Once monocytes reach the site of infection or injury, they are able to modulate the inflammatory response through cytokine production, and indeed, insulin-dependent ERK1/2 activation synergistically enhances IL-6 production induced by saturated fatty acids[85]; therefore, insulin influences the functions of monocytes, promoting and improving their migration through the secretion of proteolytic enzymes and the production of adhesion molecules and cytokines.

Lymphocytes are the effector cells of the adaptive immune system. There are 2 primary types of lymphocytes, B lymphocytes (or B cells), which produce antibodies, and T lymphocytes (also called T cells), which mediate the destruction of infected or tumoral cells and modulate immune responses. Both types of lymphocytes are capable of proliferating and can give rise to 2 subpopulations, effector and memory lymphocytes.[86]

There is limited evidence about the effect of insulin on B cells; however, its effect on the regulation of T lymphocytes is known. Inactive T cells require less energy consumption for their survival and functions, but once activated, become effector T cells, and their activation leads to an increase in the consumption of ATP, which is generated by rewiring their oxidative metabolism to glycolysis.[69] It has been shown that activation and metabolic turnover are accompanied by positive regulation of the insulin receptor, which allows lymphocytes to have a greater capacity for proliferation, cytokine production, and survival, translating into the correct activation of these cells.[70] Similarly, insulin signaling mediated by its receptor exerts a stimulatory effect on T cells, activated by antigenic recognition, enhancing not only metabolism but also proliferation and cytokine production.[73]

Eosinophils, a type of immune cell typically associated with allergies and defense against parasitic infections, also regulate the activation state of macrophages in mammalian adipose tissue and may play an important role in metabolic homeostasis.[87] To date, no studies have been reported that directly associate insulin regulation with eosinophil functions.

Basophils are the least abundant leukocytes in the circulation and are known to play an important role in the body's defense against parasitic infections, as well as in allergic reactions. Activation of basophils leads to an increase in the release of histamine and IL-4.[88] Histamine release has been shown to be regulated by growth factors, such as IGF-1, as well as by insulin.[89] Basophils share high structural and functional similarity with mast cells, although the latter are not found in circulation but are present in connective tissue and near blood vessels. Insulin in mast cells has been shown not only to promote survival in a PI3K-dependent manner but also to enhance their degranulation more

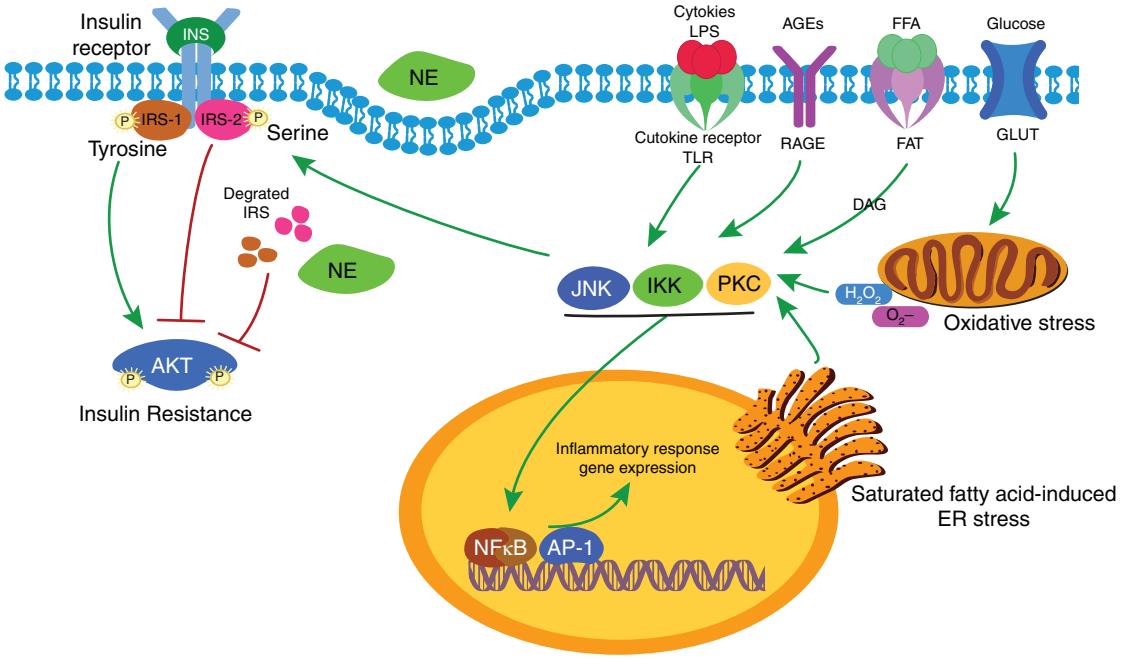

FIGURE 2 **Molecular mechanisms involved in insulin resistance.** Insulin signaling requires IRS-1/2 phosphorylation at tyrosine residues and subsequent AKT phosphorylation at Thr308 and Ser473. Kinases such as JNK, IKK and PKC phosphorylate IRS in serine residues activating transcription factors wich improving pro-inflammatory genes expression, deteriorating insulin signaling and producing insulin resistance. These kinases are activated by molecular mechanisms involving hyperglycemia, AGEs production, pro-inflammatory cytokines, LPS, the increasing of free fatty acids and endoplasmic reticulum stress. Another mechanism described involve NE, wich can intake into the cell and degrade IRS, producing insulin resistance

than activation mediated by the high affinity receptor for IgE (FcRI) alone.[90,91]

An adequate immune response requires the production and secretion of a large number of proteins, such as cytokines. Insulin also regulates the production of proteins on cells of different tissues, and in this sense, it was observed that in mononuclear cells and neutrophils of adult and elderly individuals, it significantly stimulated the rate of protein synthesis.[92] Therefore, insulin is not only responsible for the homeostatic maintenance of energy in leukocytes but also influences their functional capacities.

6 | INSULIN RESISTANCE

When cells are unable to respond to the transduction of signals derived from the interaction of insulin with its receptor, a pathophysiologic state known as insulin resistance occurs.[93] As a consequence, glucose cannot be transported into cells, causing an increase in plasma levels both when fasting and postprandial.[94] The compensatory mechanism of the pancreas is beta cell hyperplasia and overproduction and hypersecretion of insulin as an attempt to maintain normoglycemia.[95] Constant overexpression of insulin leads to dysfunction of pancreatic beta cells with the consequent appearance of type 2 diabetes, which is considered the primary pathology of systemic insulin resistance.[96]

In the state of insulin resistance, cells exhibit alterations in signaling pathways at the level of their receptor or of the proteins involved in the signal transduction cascade. The molecular mechanisms described in the generation of insulin resistance involve chronic low-grade inflammation,[97] hyperglycemia and advanced glycation end products formation,[98,99] increased free fatty acids,[100] oxidative stress and mitochondrial dysfunction,[101–103] endoplasmic reticulum stress,[104–106] and the presence of xenobiotic agents[107,108] (Fig. 2)

7 | INSULIN RESISTANCE IN LEUKOCYTE FUNCTION

The deterioration of intracellular signaling in leukocytes during insulin resistance and its effects on the response mechanisms of the host immune system are poorly understood, and analyzing the leukocyte dysfunction present in the state of insulin resistance as an independent pathophysiology has been complicated. The information obtained is associated with morbidities, such as obesity[109] and diabetes,[110] where the deterioration of immune function has been demonstrated.

Free fatty acids present in circulation during obesity induce insulin resistance; monocytes can capture and internalize these fatty acids through CD36 scavenger receptors.[111,112] It has been observed that in obese individuals with insulin resistance, the increase in CD36 receptors on monocytes induces a greater uptake of oxidized LDL, which

is associated with the development of atherogenesis linked to insulin resistance.[113,114] In addition to these factors, these individuals not only have a higher number of circulating monocytes compared with lean individuals but also exhibit a higher number of CD16+ monocytes, which are considered potent inducers of inflammation.[115]

Endoplasmic reticulum stress, in addition to being involved in the development of insulin resistance, also activates signaling pathways that lead to apoptosis.[116] In insulin-resistant macrophages, reduced AKT phosphorylation interferes with retention of the FOXO1 factor in the cytoplasm, causing it to translocate to the nucleus, where it promotes apoptosis due to cholesterol-induced stress.[117] It has also been shown that macrophages with deletion of the insulin receptor present decreased AKT phosphorylation and an increase in endoplasmic reticulum stress markers, favoring expression of the class A scavenger receptor and apoptosis when they are exposed to oxidized LDL.[118] Deterioration in MEK–ERK pathway signaling in insulin resistance leads to a decrease in the activity of the SERCA2b pump. This dysfunction causes calcium to be released from the lumen of the endoplasmic reticulum, favoring the increase in apoptosis associated with stress.[119] The direct effects on macrophage apoptosis may also be associated with the defective efferocytosis, a decrease in the ability to phagocytose apoptotic cells, seen in obesity, suggesting that insulin resistance plays an important role in atherogenic pathophysiology.[120]

Insulin resistance in macrophages not only leads to reduced phosphorylation of AKT but also favors sustained activation of the mTORC1 protein complex, which causes insulin-resistant macrophages to elevate glycolysis levels, inducing a phenotype similar to M2 with a decreased bactericidal capacity.[121] In fact, insulin resistance is a condition that favors what is known as trained immunity in macrophages, a form of memory of the cells of the innate immune system that involves changes at the metabolic and epigenetic level and leads to an altered response to damage or pathogens in diseases, such as obesity and diabetes.[122]

The molecular and functional effects of insulin on neutrophils are poorly understood; however, it is evident that its function is deregulated as, in diabetic individuals, a decrease in its bactericidal[123] and chemotactic[124] capacity has been observed, whereas in obese individuals, neutrophils show a primed state determined by increased production of superoxide, both in basal and activated states, as well as its chemotactic activity.[125,126] These controversial results can be explained because during obesity, the state of low-grade chronic inflammation determined by the increase in proinflammatory cytokines leads to a priming state in leukocytes, whereas in diabetes, hyperglycemia, or impairment in insulin signaling leads to neutrophil immune dysfunction.

In activated T lymphocytes, elimination of the insulin receptor decreases expression of GLUT 1 and several genes involved in glycolysis, causing a decrease in their ability to internalize glucose and in the inability to rewire their metabolism toward aerobic glycolysis.[73] This attenuates T lymphocytes' proinflammatory function and in the same way, decreases the proliferative capacity and increases apoptosis, suggesting that the lack of signaling by the insulin receptor affects both the metabolism and immune function of lymphocytes.[70]

During insulin resistance, chronic hyperinsulinemia occurs, which may or may not be accompanied by hyperglycemia.[127] It has been difficult to evaluate the effect of chronic exposure of leukocytes to high concentrations of insulin, glucose or both in vitro, as the half-life of these cells is short, and the results have been contradictory. Acute exposure to hyperinsulinemia has been observed to improve leukocyte function; however, under hyperglycemic conditions, monocytes and neutrophils show a deterioration in functions, such as chemotaxis, phagocytosis and bactericidal capacity.[128]

It has been observed in vivo that under conditions of hyperglycemia, neutrophils decrease expression of inflammatory genes, such as NFKBA, IL1A, and CCL3, whereas under conditions of hyperglycemia and hyperinsulinemia, the opposite effect occurs but does not affect the chemotactic, phagocytic, or respiratory burst functions of neutrophils.[129] However, chronic exposure of neutrophils from individuals with diabetes results in reduced chemotaxis.[124] The controversial effects derived from acute or chronic exposure to hyperglycemia, hyperinsulinemia, or both is clear; it seems that acute or short-term exposure to leukocytes leads to an "improvement" in some of their functions, whereas when exposure is chronic, their functions decrease, probably due to the influence of other metabolic disorders, such as inflammation, dyslipidemia, or arterial hypertension, which can induce expression of various genes that affect the function of leukocytes.[129]

8 | INSULIN THERAPY IMPROVES LEUKOCYTE FUNCTION

Diabetes is the primary disease caused by altered insulin signaling in insulin-dependent tissues, and it is clear that there is a dysfunction of the immune system reflected by the increase in infections in diabetic individuals.[130,131] The evidence that leukocytes are involved in both the initiation and progression of various diseases, such as diabetes and cardiac complications, has been analyzed[132,133]; however, it has also been shown that insulin treatment in diabetic patients has positive effects, such as longer survival of critically ill patients[134] and improved wound healing.[135] This suggests that insulin may play an immunoregulatory role in these conditions.

Insulin treatment of patients with type 2 diabetes is used to improve glycemic control and reduce complications derived from this pathology.[136] There is evidence that insulin, in addition to improving glycemic control in patients, may have direct effects on the attenuated immune functions of leukocytes, as insulin treatment restores defective chemotaxis,[137] bactericidal function,[123] and the phagocytic activity of neutrophils both in vivo and ex vivo, suggesting that insulin exerts a direct effect on these cells, regardless of its action on glycemic control.[138]

Type 1 and type 2 diabetes are associated with the presence of low-grade inflammation as shown by the high concentration of biomarkers, such as TNFα, IL-6, and CRP,[139–141] and the immune system plays a crucial role in the incidence and progression of these diseases, especially given its participation in inflammation of pancreatic and adipose tissue, where immune cells with proinflammatory characteristics, such as M1

macrophages and Th1 and Th17 cells, prevail.[142,143] Insulin has been shown to exert anti-inflammatory effects, favoring the polarization of macrophages toward the M2 phenotype[144] and of T cells toward the Th2 phenotype,[67] suggesting that insulin restores the dysregulated inflammatory response in diabetic individuals.

Although glycemic control has been shown to reduce the risk of infections, the precise mechanisms through which diseases, such as type 2 diabetes, lead to secondary diseases that deteriorate the prognosis of patients are not known, so additional studies are needed. Exploring how insulin treatment could reverse these complications by improving the prognosis of patients is vital.

9 | ROLE OF LEUKOCYTES IN SYSTEMIC INSULIN RESISTANCE

In recent years, foundations have been established due to the discovery of immunometabolism as a field of science that has 2 aspects: the first is to understand how changes in cellular metabolism contribute to regulation of the functions of immune cells, such as their activation, polarization, differentiation, and proliferation, whereas the second focuses on understanding how leukocytes modulate cellular processes in tissues to drive the necessary changes in the body in response to environmental stimuli.[145]

Initially, it was considered that in adipose tissue, proinflammatory molecules had a central role in the development of insulin resistance[146]; however, it was determined that the cells of the immune system infiltrated in these tissues were the ones that significantly modulated the generation.[147] In fact, many of the immune cells, including macrophages, B and T cells,[148] neutrophils and eosinophils, are involved in the production of cytokines during chronic inflammation.[149]

Proinflammatory cytokines, such as TNFα and IL-6, are capable of activating serine/threonine kinases that can impair the insulin signaling pathway through serine phosphorylation of IRS.[150] In this sense, the relationship of leukocytes with insulin resistance has been widely established. The increase in the availability of nutrients generates chemotactic molecules, such as MCP-1 in adipocytes,[151] that lead to the recruitment of macrophages in adipose tissue, which additionally contributes to potentiating inflammation by releasing additional cytokines and chemoattractants[152]; therefore, genetic inhibition as the endogenous function of MCP-1 favors insulin sensitivity, suggesting that the development of insulin resistance related to obesity is promoted through an increase in macrophage infiltration, inducing inflammation in response to chemoattractant stimuli in adipose tissue.[152] In addition, vitamin D deficiency promotes the differentiation of macrophages toward the M2 phenotype through endoplasmic reticulum stress; however, these macrophages exhibit production of proinflammatory cytokines that contributes to the development of insulin resistance.[153] These results suggest that in response to specific signals, leukocytes may exert deleterious effects.

Lymphocytes also participate as mediators of insulin resistance in the presence of obesity, as adipocytes express the major MHC II that activates CD4+ T cells and promotes inflammation.[154] As a consequence, lymphocytes acquire the Th1 phenotype, which produces proinflammatory molecules, such as IFN-γ, IL-2, and TNF-β, which mediate the cellular response and inflammation through the activation of phagocytes.[155] An increase in the number of Th1 cells in adipose tissue and peripheral blood is associated with the presence of insulin resistance.[142] Synergy between T and B lymphocytes can modulate the inflammatory response, as B lymphocytes from individuals with type 2 diabetes secrete reduced IL-10 than is necessary to modulate Th1 cell differentiation. This imbalance could affect tissue metabolism, increasing inflammation.[156] In fact, during obesity, B cells secrete proinflammatory chemokines, such as IL-8; they present antigens to T cells that lead to IFN-γ production, which polarizes M1 macrophages and, in the latter, induces TNFα secretion,[157] suggesting the involvement of lymphocytes in the development of insulin resistance.

Finally, neutrophils play a relevant role in modulating inflammation through the production of various molecules. An enzyme produced by these cells is neutrophil elastase, a protease responsible for the destruction of phagocytosed pathogens, which can be secreted into the environment during degranulation and NET production. Neutrophil elastase is able to enter the cell where it degrades IRS1, which is key in the insulin signaling pathway in adipose and liver tissue cells; therefore, elastase released by neutrophils impairs insulin signaling, directly contributing to insulin resistance[158,159] (Fig. 2).

10 | CONCLUDING REMARKS

The emergence of immunometabolism as a field of science that relates metabolic regulation and its effect on the cells of the immune system has reoriented the focus of the actions of insulin on leukocytes, proposing insulin as a hormone that regulates the metabolism and function of virtually all cells in the body. Knowledge regarding the molecular mechanisms involved in insulin signaling that guide the control of metabolism and the functions of leukocytes is gradually being uncovered. During this process, insulin resistance, as a condition that affects the mechanisms of signal transduction, is still in the early stages of study, representing a new field to explore. Additionally, the discovery that some leukocytes exhibit insulin sensitivity comparable to that of muscle or adipose cells raises the possibility of considering cells of the immune system as possible targets for the identification for early markers of pathophysiology, such as insulin resistance.

AUTHORSHIP

W.D.C.P. and O.L.G.C. wrote the manuscript and prepared the figures; H.A.R.R. and I.M.G. helped to design the figures and edited the paper; B.I.A. helped to review and design the manuscript; O.L.G.C. and I.P.R. contributed with style correction and review of the manuscript.

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DISCLOSURES

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