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REVIEW

Molecular Mechanisms of Regulation of Functional Activity of Mononuclear Phagocytes by Leptin

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Abstract—Leptin is a peptide hormone synthesized by adipocytes. The main function of leptin is associated with regulation of the body energetic balance and restriction of excess accumulation of fat. This review considers in detail the involvement of leptin in regulation of fundamental effector functions of mononuclear phagocytes, which express receptors for this hormone. Possible molecular mechanisms of modulation by leptin of phagocytic activity, oxygen-dependent microbicidity, and nitric oxide generation by mononuclear phagocytes are analyzed, as well as the role of leptin in the formation of the produced cytokine pattern. The data presented suggest that the regulation of mononuclear phagocytes by leptin is associated with activation of the JAK/STAT signaling pathway, which leads to stimulation of phagocytosis, production of oxygen and nitrogen reactive species, and also to increase in secretion of pro-inflammatory cytokines.

Key words: leptin, mononuclear phagocytes, phagocytosis, cytokines, production of oxygen and nitrogen reactive species

Leptin is a 16-kD peptide hormone that is mainly synthesized by adipocytes and circulates in blood in both bound and free form [1]. The major biological role of leptin is associated with regulation of energetic balance of the body, food consumption, and restriction of excess fat accumulation. Blood plasma content of leptin directly correlates with the amount of fatty tissue and also varies depending on specific features of metabolism and changes in the contents of some hormones and cytokines [1-5].

The physiological role of leptin has been mainly studied on an obesity model of homozygous mouse strains *ob/ob* (with leptin production deficiency) and *db/db* (with leptin receptor mutation). In addition to hyperphagia and obesity, these mice are found to have hormonal imbalance and suppressed reproductive function, hemopoiesis, and cell-mediated immunity [1-6]. Similar disorders are also found in human homozygous carriers of the *ob* gene mutation, and exogenous injection of recombinant leptin prevented the development of the abovementioned dysfunctions [1-6].

Modulation by leptin of functional activity of cells is mediated through specific membrane receptors (Ob-R) [7-12]. Leptin regulates the body's energetic balance mainly by suppression of neuropeptide Y production and activation of synthesis of melanocyte-stimulating hormone through the interaction with the hypothalamic Ob-R. This lowers appetite, increases tonicity of the sympathetic nervous system, and as a result, enhances the basal metabolism in peripheral organs and tissues [1, 4, 5, 12]. Moreover, on the level of the hypothalamus, leptin stimulates the production of thyrotropin and gonadotropins that increases the secretion of thyroid and steroid hormones, which are active regulators of metabolism [1, 4, 12]. Concurrently, increased levels of insulin, sex steroids, and glucocorticoids suppress the secretion of leptin by adipocytes and thus lock negative feedback [1, 4, 12].

Involvement of leptin in regulation of immune reactions is determined by its immediate influence on

Abbreviations: EPR) endoplasmic reticulum; AP-1) activating protein-1; CD) membrane molecules of lymphomyeloid cells; CaN) calcineurine; CR) receptor to complement components; DAG) diacylglycerol; ERK) extracellularly regulated protein kinase; FcR) Fc-receptors; G-CSF) granulocytic colony-stimulating factor; GM-CSF) granulocytic-macrophagal colony-stimulating factor; IL) interleukins; IP_3) inositol 1,4,5-triphosphate; JNK) c-Jun-NH_2-terminal protein kinase; MAPK) mitogen-activated protein kinase; MARCKS) myristoylated alanine-rich C kinase substrate, or actinogelin; MLCK) myosin light-chain kinase, or ERKII-induced MLC kinase; MR) mannose receptor; NFAT) nuclear factor of activated T-cells; NF-*k*B) nuclear factor *k*B; Ob-R) leptin receptor; PKC) protein kinase C; PLC) phospholipase C; PLA_2) phospholipase A_2; PIP_2) phosphatidylinositol 4,5-diphosphate; PtdIns(3,4,5)P_3) phosphatidylinositide 3,4,5-triphosphate; PI_3-K) phosphatidylinositol-3 kinase; STAT) proteins of signal transduction and activation of transcription; Sam68) RNA-binding protein; SOCS-3) suppressor of cytokine signal transduction; Th) T-helpers; TNF-α) tumor necrosis factor α.

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immune system cells that express Ob-R. In addition to cells of the central nervous system and cells of lungs, liver, kidneys, pancreas, and gonads [1, 5], Ob-R is expressed by splenocytes [8-11], monocytes/macrophages [2, 13], lymphocytes [10], neutrophils [3], and natural killers [6]. Such a wide variety of Ob-R representation in immunocompetent and effector cells is explained by its structural similarity with members of class I cytokine receptors (long-chain helical cytokine family) [1, 7]. This structural likeness is favorable for leptin regulation of immune response, inflammation, and hemopoiesis [5, 8, 11-13], where mononuclear phagocytes and macrophages play a key role. The literature data convincingly show that leptin is a pro-inflammatory hormone that promotes the prevalence of cell-mediated immune reactions due to T-helper-1 (Th1) type shifting the balance of the produced cytokines [1, 5, 13]. To understand the regulatory mechanisms of immune reactions under various physiological and pathological conditions, it is necessary to systematize knowledge on molecular principles of the immunomodulating effects of leptin.

STRUCTURE OF Ob-R, MOLECULAR MECHANISMS OF SIGNAL TRANSDUCTION

The structure and functions of Ob-R are like those of the class I cytokine receptors, which have a common signaling component gp130 [5, 7, 14-16]. This family also includes interleukin receptors IL-2, IL-6, IL-11, and IL-12, and receptors to oncostatin M, granulocytic colony-stimulating factor (G-CSF), growth hormone, prolactin, etc. [5, 16]. Ob-R produces homodimers, each of which binds a leptin molecule with the formation of a tetrameric complex consisting of two Ob-R and two leptin molecules [7, 16]. Non-activated monocytes/macrophages express on the surface only 5-25% of Ob-R molecules, whereas the others make an intracellular pool [7, 16].

There are several Ob-R isoforms generated as a result of alternative splicing that differ in structure of the intracellular signaling domain [16]. The extracellular domain of Ob-R consists of 816 amino acid residues and has two cytokine-like binding motifs [7]. Based on the structure of the intracellular domain, Ob-Rs are classified as short (32-97 amino acid residues), long (302 amino acid residues), and soluble form [7, 16, 17]. Interaction of leptin with the short form of Ob-R does not activate the transcription factors [7, 16, 17], and this form is thought to transfer the leptin across the blood–brain barrier, whereas in blood serum the hormone is transported with the involvement of the soluble form of Ob-R [18].

As in the case of other gp130-containing cytokine receptors, ligation of the long form of Ob-R initiates an intracellular signaling cascade that is like that for IL-6 [19]. During the first stage in the leptin-induced signal transduction Ob-R-associated tyrosine protein kinases of the Janus family (JAK kinases) are activated [4, 7, 16, 20]. JAK kinases phosphorylate tyrosine residues in the intracellular domain of Ob-R and also proteins of signal transduction and activation of transcription (STAT proteins) [4, 7, 16, 17]. Activated STAT proteins, in their turn, interact with phosphorylated tyrosine residues of Ob-R. Depending on the cell type, the ligation of Ob-R activates different STAT proteins [4, 7, 16, 17]. In mononuclear leukocytes JAK kinases mainly phosphorylate STAT-3 [20-22]. Activated STAT-3 form dimers in the cytoplasm by binding the SH2-domain of one molecule with phosphorylated tyrosine residues of another [18, 21, 22]. Dimeric STAT-3 molecules acquire the ability for penetrating into the nucleus, where they bind to DNA promoter sites and regulate gene transcription in different chromosomes [7, 19, 20]. Moreover, phosphorylated STAT-3 proteins can interact with the RNA-binding protein (Sam68) which, in turn, activates SH2-containing signal molecules, such as phospholipase C (PLC), phosphatidylinositol-3 kinase (PI_3-K), and protein kinase C (PKC) [4, 7, 21, 23-25].

The interaction of Sam68 with the membrane enzyme PLC stimulates hydrolysis of phosphatidylinositol 4,5-diphosphate (PIP_2) resulting in two major secondary messengers, diacylglycerol (DAG) and inositol 1,4,5-triphosphate (IP_3). The first of these stimulates PKC and the other activates IP_3-dependent Ca^{2+}-channels of the endoplasmic reticulum (EPR) and thus causes the influx of intracellular Ca^{2+}, which is accepted by calcineurin (CaN) [6, 13, 26-28]. Activated PKC triggers a sequential cascade of mitogen-activated protein kinases (MAPK): RAS, RAF-1, MEK, and ERK-I/II [6, 13, 20, 26-28].

The interaction of Sam68 with PI_3-K results in PI_3-K-induced phosphorylation of PIP_2, with the generation of some products stimulating GTPases of the RHO family, and, as a consequence, p38 MAP kinase and c-Jun-NH_2-terminal protein kinase (JNK kinase) [7, 20-23].

At the final stage of signal transduction the transcription factors are activated, which include nuclear factor of activated T-lymphocytes (NFAT), nuclear factor *k*B (NF-*k*B), and the activating protein-1 (AP-1) as the most important [9, 24, 29-31]. PKC is a principal activator of NF-*k*B, CaN is responsible for activation of NFAT, and ERK and JNK induce the transcription of the oncogenes *c-fos* and *c-jun*, respectively, whose products are constituents of AP-1 [6, 9, 13, 31]. The activated transcription factors penetrate into the nucleus, and interacting with promoter and/or enhancer sequences of certain genes control their transcription [6, 9, 13, 30, 31].

Similarly to other gp130-containing cytokine receptors, signal transmission during the ligation of Ob-R is regulated negatively by the suppressor of cytokine signal transduction (SOCS-3) [7]. SOCS-3 contains an SH2-domain and can interact with phosphorylated JAK proteins suppressing signal transmission on the leptin binding to Ob-R [7].

Thus, STAT proteins and Sam68 are major intracellular mediators which realize leptin effects on the molecular level and immediately and/or through specific protein kinases determine the phosphorylation of appropriate transcription factors and, therefore, are responsible for its biological effects. Homology in the structure of leptin, its receptors, and the induced signaling cascade with cytokines suggests a possibility of a cytokine-like effect of leptin in regulation of immune reactions.

MOLECULAR MECHANISMS OF REGULATION OF PHAGOCYTIC ACTIVITY OF MONONUCLEAR PHAGOCYTES BY LEPTIN

Monocytes and macrophages are major effectors of the body's nonspecific resistance. Their contributions to immune reactions depend on engulfment and destruction of a pathogen and on its antigenic determinants, as well as on the production of biologically active compounds, including cytokines, which modulate the development of the immune response [26].

Monocytes and macrophages express both the short and long form of Ob-R [21, 25, 32]. Among leukocytes, mononuclear phagocytes display the highest density of Ob-R expression [25, 32]. The activation of monocytes/macrophages is associated with a significant increase in the number of Ob-Rs on their membranes [14, 25].

The binding of leptin to Ob-R of mononuclear phagocytes induces the expression of activating molecules, such as CD25 (an IL-2 receptor), CD38 (an adhesive molecule), CD69 (an inducer of activation), CD71 (a transferrin receptor), and also HLA-DR (molecule of the major histocompatibility complex II class), CD11b (CR3, or a receptor for complement components), and CD11c (CR4) [2, 15, 21]. In an *in vitro* system, leptin stimulates the proliferation of monocytes, increasing the production of granulocyte-macrophage colony-stimulating factor (GM-CSF) [2, 11, 15, 20, 25]. In addition, leptin prevents apoptosis of monocytes, acting with the involvement of STAT-3 and MAPK [14, 21].

Leptin stimulates the ability of monocytes and macrophages for the engulfment of various objects of phagocytosis [5, 11, 13]. The phagocytic activity of macrophages toward *Klebsiella pneumoniae* was decreased in *ob/ob* mice but could be restored by the addition of leptin [33]. In other works the inability of macrophages of *ob/ob* and *db/db* mice for engulfing and digesting the fungus *Candida* was shown [13]. The introduction of recombinant leptin restored the engulfment function of phagocytes in *ob/ob* but not in *db/db* mice [13]. Thus, leptin regulation of the phagocytic activity of the cell was associated with the immediate interaction of the hormone with the membrane Ob-R of phagocytes [13].

Studies on molecular mechanisms responsible for leptin regulation of the engulfing activity of monocytes/macrophages have revealed that the hormone effects are mediated, on one hand, by the increase in expression of receptors necessary for adhesion of the object of phagocytosis, e.g., CR1, CR3, CR4 [2, 11] and, on the other hand, by activation of molecules which control actin polymerization on submerging the adhered particle and the formation of a phagosome [11, 23].

In all eucaryotic cells the reorganization of the cytoskeleton is controlled by GTPases of the RHO family, and Rac and Cdc-42 are the most important of these [27, 29, 34]. It has been mentioned that the leptin binding to Ob-R results in interaction of Sam68 with PI_3-K and PI_3-K-induced phosphorylation of PIP_2 with generation of some products, including phosphatidylinositol 3,4,5-triphosphate ($PtdIns(3,4,5)P_3$) as the most significant [26, 27]. $PtdIns(3,4,5)P_3$ is the major activator for the RHO family GTPases Rac1 and Cdc42, which immediately trigger polymerization of low-molecular-weight G-actin to F-actin [26, 27, 29, 34, 35]. Moreover, PKC activates actinogelin (MARCKS), a protein responsible for cross-binding of actin [27, 28, 34, 35]. This complex is contracted with the involvement of myosin, and the process is controlled by ERKII-induced MLC kinase (MLCK), which activates myosin and Ca^{2+} [26, 27]. As a result, the object of phagocytosis together with the part of the phagocyte membrane is submerged into the cell as a phagosome [26, 27].

Thus, the increase in the engulfing activity of phagocytes caused by the interaction of leptin with Ob-R seems to be associated with the activation of RHO GTPases and MARCKS, which modulate the assemblage and contractibility of actomyosin, because SH2-containing molecules of PI_3-K, protein kinase C, and phosphokinase C are targets for the stimulating effect of Sam68 [21-23]. Figure 1 presents a possible mechanism of the modulation of phagocytic activity of cells by leptin. Because both FcR-mediated phagocytosis and engulfment of particles with the involvement of CR, mannose receptors (MR) need RHO GTPases to be involved in reorganization of the cytoskeleton [27], the regulation by leptin of phagocytic activity of monocytes and macrophages on the molecular level seems to be independent of the kind of receptors interacting with the object.

MOLECULAR MECHANISMS OF LEPTIN REGULATION OF GENERATION OF REACTIVE OXYGEN AND NITROGEN SPECIES BY PHAGOCYTES

Phagocytosis is the initial stage in degradation of a pathogen. To destroy the engulfed object, the oxygen-dependent mechanisms of microbicidity are required, which are realized under the influence of reactive oxygen and nitrogen species [36, 37].

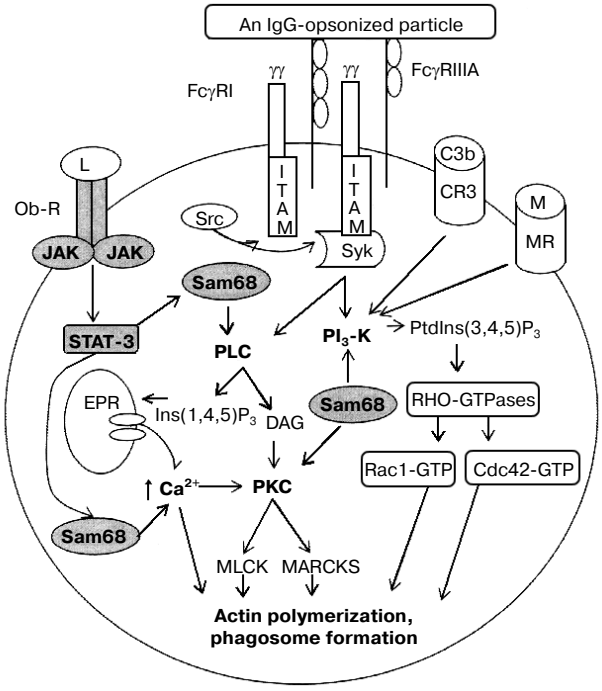

Fig. 1. Modulations of phagocytic activity of mononuclear phagocytes by leptin. Cross-binding of two FcγRs with an IgG-opsonized particle on the phagocyte membrane activates tyrosine kinases of the Src and Syk families. Syk kinase phosphorylates PLC and PI_3-K. PLC hydrolyzes PIP_2 whose metabolites DAG and IP_3 activate PKC and mobilization of Ca^{2+} from the EPR, respectively. PKC activates MARCKS and MLCK. PI_3-K phosphorylates PIP_2 with the formation of some products, including PtdIns(3,4,5)P_3, which activates the RHO GTPases Rac1 and Cdc42. These events result in reorganization of cell cytoskeleton. Interactions of microbial carbohydrate components (M) with MR and of C3b-opsonized particles (C3b) with CR3 also initiate actin polymerization with the involvement of RHO GTPases. The binding of leptin (L) to Ob-R increases the engulfing activity of the cell due to the activation of RHO GTPases, MLCK, and MARCKS because PI_3, PKC, and PLC are targets for the stimulating effect of Sam68.

Stimulation of phagocytes during phagocytosis induces oxygen burst which leads to generation of reactive oxygen species with high oxidative activity. The production of these metabolites potentially dangerous for the cell is controlled by NADPH oxidase, which is involved in their generation and is produced only on activation of the cell [37, 38].

NADPH oxidase consists of the membrane cytochrome b558 (subunits $gp21^{phox}$ and $gp91^{phox}$) and cytosolic protein components p47 and p67 [37]. During phagocytosis, NADPH oxidase is activated through phosphorylation of its cytosolic components p67 and p47, and PI_3-K phosphorylates the RHO GTPase Rac-2 and, as a result, p67, whereas PKC phosphorylates p47 [37]. Phosphorylated cytoplasmic components are translocated to the membrane and associated with cytochrome b558 creating a functional NADPH oxidase complex that provides electron transfer by the cytochrome from NADPH onto O_2 with the production of O_2^- [37, 38].

Data on the leptin effect on microbicidal potential of phagocytes are scarce. Nevertheless, leptin is established to increase the production of reactive oxygen species by peripheral blood mononuclear cells [39]. Similar effects of leptin are also described for other cell types [9]. As to molecular mechanisms of leptin effects, it was suggested that the increase in the production of oxygen metabolites during the hormone interaction with Ob-R should be caused by the Sam68-induced stimulation of PKC and PI_3-K and also by an increase in the Ca^{2+} content in the cell and the resulting activation of NADPH oxidase [7, 9, 23, 37, 39]. Moreover, leptin activates in mononuclear phagocytes phospholipase A_2 (PLA_2), which is also involved in the induction of NADPH oxidase [40, 41].

The regulation by leptin of microbicidal potential of phagocytes is also provided by its involvement in the control of nitrogen metabolism. Nitric oxide (NO) plays the principle role in the destruction of intracellular pathogens which leave the phagosome and can reproduce in the cytoplasm where the effect of reactive oxygen species is

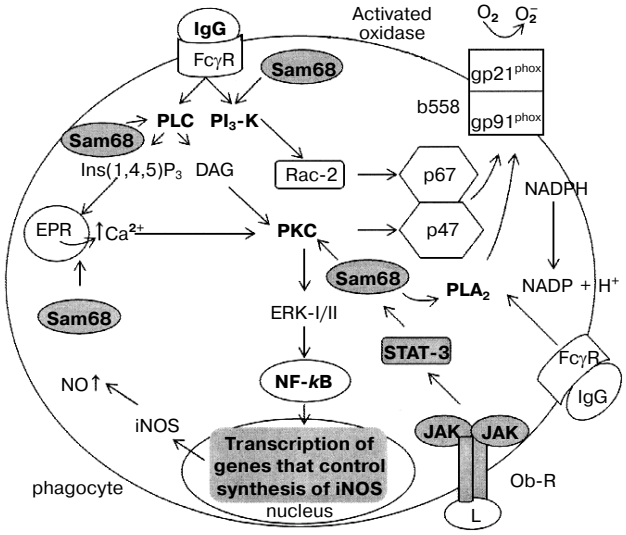

Fig. 2. Mechanism of leptin regulation of microbicidal activity of phagocytes. During phagocytosis, NADPH oxidase is activated through the phosphorylation of its cytosolic components p67 and p47, which afterwards are incorporated into the cell membrane and interact with the membrane cytochrome b558. The RHO family GTPase Rac-2 activates p67, and PKC phosphorylates p47. During phagocytosis, PKC triggers a sequential cascade MAPK, which finally activates NF-*k*B responsible for the control of iNOS expression and production of NO. The increase in the microbicidal activity of phagocytes during the interaction of leptin (L) with Ob-R is caused by the Sam68-induced stimulation of PKC and PI_3-K, PLA_2, and also by the increase in the Ca^{2+} concentration in the cell and, as a result, the activation of NADPH oxidase. The increase in the NO production by phagocytes on the ligation of Ob-R is caused by the increased expression of iNOS due to the PKC-dependent activation of NF-*k*B.

weak. NO is generated in the cell during the oxidation of arginine under the influence of inducible (i) or constitutive NO synthase (NOS). The production of NO by phagocytes is mainly regulated on the level of iNOS transcription, which is controlled by NF-*k*B [7, 32, 42, 43]. Leptin has been shown to increase the NO production by peripheral blood mononuclear leukocytes through the initiation of iNOS expression [7, 32, 43], and inhibition of the OB-R signaling cascade with the specific inhibitors of JAK kinases abolishes the activating effect of leptin on the NO production [44]. Leptin deficiency in *ob/ob* mice is accompanied by disorders in NO production, and this indicates the essential role of the hormone in the control of nitrogen metabolism [45].

The leptin-induced production of NO by mononuclear cells was suppressed by inhibitors of PKC [32], which is a major activator of NF-*k*B responsible for the control of iNOS expression [7, 9]. The interaction of leptin with Ob-R of phagocytes initiates the Sam68-induced stimulation of PKC, which triggers a sequential cascade MAPK finally activating NF-*k*B. Thus, the leptin-induced increase in the production of NO by phagocytes is caused by the increased expression of iNOS due to the activation of NF-*k*B with protein kinase C. Figure 2 shows a possible mechanism of leptin regulation of the microbicidal activity of phagocytes.

Thus, leptin plays a significant role in the modulation of the oxygen-dependent microbicidity of phagocytes through the regulation of expression and functional activities of NADPH oxidase and iNOS.

MOLECULAR MECHANISMS OF LEPTIN REGULATION OF CYTOKINE PRODUCTION BY PHAGOCYTIZING CELLS

The contribution of phagocytes to the immune response is determined not only by engulfment and destruction of a pathogen, but also by the production of cytokines which modulate the development of the immune response [26]. The spectrum of cytokines produced by mononuclear phagocytes can vary depending on the object of phagocytosis and receptors involved in the engulfment. Thus, the interaction of monocytes and macrophages with FcγR, MR, and CR stimulates the production of pro-inflammatory cytokines IL-1β, tumor necrosis factor α (TNF-α), IL-6, and IL-12 [46, 47]. The engulfment by macrophages of cells subjected to apoptosis with the involvement mainly of scavenger receptors, fibronectin receptors, and CD14 is accompanied by a decrease in the synthesis of pro-inflammatory cytokines IL-1β, TNF-α, and IL-8 [48].

Leptin has been shown to increase the production by monocytes and macrophages of pro-inflammatory cytokines IL-1, IL-6, IL-12, TNF-α, G-CSF, and GM-CSF, which belong to the Th-1 cytokine profile [2, 5, 11, 13, 15, 22, 25, 49]. Disorders in the synthesis or reception of leptin in *ob/ob* or *db/db* mice are accompanied by the suppression of production of pro-inflammatory cytokines and increase in the secretion of anti-inflammatory cytokines, respectively [5, 13, 15, 22, 49, 50]. Pro-inflammatory cytokines IL-1, TNF-α, and IL-6 induce the secretion of leptin by mononuclear phagocytes, and the level of pro-inflammatory cytokines is in strong positive correlation with the leptin content in blood serum [25, 49, 51]. The production of leptin increases in inflammatory and infectious diseases, and just this seems to result in anorexia and weight loss during the progress of these conditions [5, 25, 49].

Thus, on one hand, the increase in the level of leptin is a result of development of inflammatory reaction in response to increase in the level of pro-inflammatory cytokines, and, on the other hand, the hormone itself acts as a factor enhancing the inflammation by Th-1-like shifting of the balance of cytokines produced by immunocompetent and effector cells and stimulating the cell-mediated immune response [5, 13, 15, 22, 25].

Overall, protein synthesis in all eucaryotic cells is controlled by a number of transcription factors, among which NF-*k*B, AP-1, and NFAT are the most important [4, 9, 25, 29]. Sites binding these transcription factors have been identified in promoter and enhancer regions of genes encoding the synthesis of pro-inflammatory cytokines [4, 9, 29, 31, 52]. In resting cells transcription factors are inactive. They are activated on stimulation of various membrane proteins in response to the interaction with cytokines, bacterial products, phorbol esters, viruses, and hormones, including the cross-binding of FCγR [26]. As mentioned earlier, PKC is the main stimulator for NF-*k*B. PKC triggers an activating cascade which involves molecules of various signaling pathways: ERKI/II, JNK, and some others, which results in the destruction of the NF-*k*B complex with its inhibitor and the following translocation of NF-*k*B into the nucleus and the initiation of transcription of the genes, including those of pro-inflammatory cytokines [6, 9]. Activation of NFAT requires mobilization of intracellular Ca^{2+} and CaN [30]. The latter binds to the regulatory subunit of NFAT and dephosphorylates this protein initiating its penetration into the nucleus [30]. The binding of NFAT to DNA requires cooperation with AP-1, which is produced by homo- or heterodimerization of the proteins Jun and Fos and induced at the initiation of an intracellular signal which includes ERK and JNK [9, 30].

On the contrary, the transcription of anti-inflammatory (Th-2) cytokines is under negative control of transcription factors, which leads to suppression of humoral immune response [30].

Thus, leptin regulation of production of Th-1 and Th-2 cytokines through Ob-R seems to be associated with a differential modulation of activities of transcription fac-

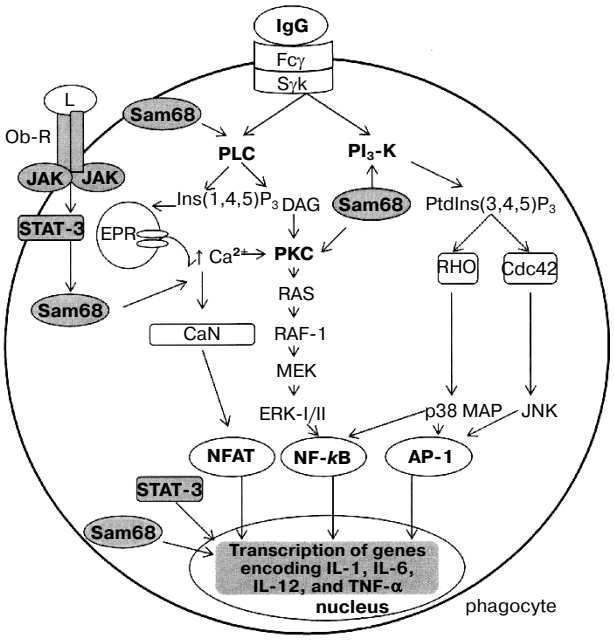

Fig. 3. Mechanism of leptin regulation of pro-inflammatory cytokine production by phagocytes. The interaction of FcγR with an IgG-opsonized particle on the membrane of phagocyte initiates MAPK cascades (ERK-I/II, JNK, p38 MAP) and activates CaN necessary for induction of nuclear transcription factors, including NF-*k*B, NFAT, and AP-1. The leptin (L) regulation of the balance of produced cytokines is mediated by the predominant interaction of STAT-3 and Sam68 with promoter and/or enhancer sequences of the genes of pro-inflammatory cytokines. In addition, the leptin binding to Ob-R induces the Sam68-dependent activation of molecules such as PLC, PKC, and PI_3-K, which initiates activation of corresponding MAPK (ERK-I/II, JNK, and p38 MAP) and, consequently, of transcription factors NF-*k*B, NFAT, and AP-1, which control the synthesis and the production of pro-inflammatory cytokines.

tors controlling the synthesis of pro- and anti-inflammatory cytokines.

The interaction of leptin with Ob-R of phagocytes triggers a signaling cascade which predominantly activates transcription factors controlling production of pro-inflammatory cytokines. The ligation of Ob-R activates STAT-3 and Sam68, which can directly interact with promoter sites of DNA and activate transcription of the pro-inflammatory cytokine genes [7, 21, 53]. In parallel, STAT-3 and Sam68 trigger some signaling pathways, molecules of which, first of all, protein kinase C, calcineurine, p38 MAP, ERK, and JNK are effective inducers for transcription factors NF-*k*B, NFAT, and AP-1 [7, 9, 21, 24, 25, 29, 50]. In particular, the leptin-stimulated production of TNF-α by monocytes has been shown to be due to the activation by p38 MAP of transcription factors NF-*k*B and AP-1 [25]. Moreover, in some cell types STAT-3 immediately induces destruction of the NF-*k*B complex with its inhibitor [54]. Activated transcription factors NF-*k*B, NFAT, and AP-1 mainly interact with promoter and/or enhancer regions of the pro-inflammatory cytokine genes and, as a result, competitively suppress transcription of anti-inflammatory cytokines [7, 9, 24]. A possible mechanism of leptin regulation of the pro-inflammatory cytokine production by monocytes and macrophages is shown in Fig. 3. Overall, leptin stimulates the production of pro-inflammatory cytokines by monocytes/macrophages and in this way enhances the cell-mediated immune response.

Leptin is a hormone which regulates the intensity of energetic metabolism and, thus, determines functioning of immune system cells. Regulation of the leptin level and Ob-R expression in various organs and tissues seems to be an important mechanism of maintaining homeostasis. Being like cytokines in structure and mechanisms of signal transduction, leptin acts as a pro-inflammatory factor which stimulates the functional activity of mononuclear phagocytes, increases the production of pro-inflammatory cytokines, and thus promotes the prevalence of the cell-mediated immune response.

The regulation by leptin of functional activity of mononuclear phagocytes is determined by an immediate interaction of the hormone with membrane Ob-R. Ligation of Ob-R triggers the JAK/STAT signaling pathway. The activation of STAT-3 and Sam68 is a key stage in realization of regulatory effects of leptin on the molecular level. Sam68 binds to SH2-containing signaling molecules, such as PLC, PKC, and PI_3-K, initiates other messenger cascades, and this changes the functional activity of the cell. Thus, the activation of PI_3-K during the interaction with Sam68 stimulates the RHO family GTPases which control changes in the cytoskeleton, and this results in increase in the engulfing function of the cell. Sam68 binding to PLC triggers the PIP_2 metabolism, with the proteinase C activation as a key stage. Protein kinase C and PI_3-K induce the expression and functional activity of NADPH oxidase during phagocytosis, and this increases the microbicidal potential of phagocytes during their interaction with leptin. Concurrently, protein kinase C together with PI_3-K initiates the MAPK cascades ERK, JNK, and p38 MAP, which, in turn, mainly activate the transcription factors. These factors determine the synthesis of pro-inflammatory cytokines, first of all NF-*k*B, NFAT, and AP-1 that increases the production of Th-1 cytokines by mononuclear phagocytes during interaction with leptin. At the same time, the protein kinase C-dependent activation of NF-*k*B also promotes an increase in the iNOS expression and resulting increase in the NO production by phagocytes under the influence of leptin.

Thus, leptin acts as a fundamental regulator of non-specific resistance of the body. Modulation of the leptin concentration and Ob-R expression by pharmaceuticals seems to be an important approach to regulate the activation of monocytes/macrophages and the development of immune reactions.

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